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Approches et considérations innovatrices reliées à l'équivalence pharmacocinétique (PK) et pharmacodynamique (PD) des médicaments

New and alternative approaches to the assessment of pharmacokinetic and pharmacodynamic equivalence

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Résumé

La bioéquivalence, une mesure de substitution de l'innocuité et de l'efficacité à différents stades du processus de développement des médicaments, est tout particulièrement importante lors du développement d'un médicament générique. Entre autres critères, la bioéquivalence garantit que les médicaments génériques sont équivalents aux produits innovateurs ou de références approuvés en termes d'efficacité clinique et d'innocuité tout en contournant le long cours et le coût élevé des essais chez les animaux et des essais cliniques chez les patients exigés pour les médicaments innovants. Malgré les avancées dans le développement d'approches robustes au cours des dernières décennies, la pratique actuelle de la bioéquivalence fait toujours l'objet de controverses. Le but de cette thèse est d'explorer certaines de ces controverses et de les aborder en proposant des approches nouvelles et alternatives.

L'une des questions les plus controversées dans la pratique actuelle de la bioéquivalence est l'extrapolation des résultats d'études de bioéquivalence d'une population à une autre. La majorité des études de bioéquivalence portant sur des formes pharmaceutiques orales efficaces par voie systémique reposent sur les critères de pharmacocinétique obtenus chez des sujets sains, alors que la population cible est constituée de patients. Ceci est basé sur l'hypothèse que si deux produits sont bioéquivalents dans une population, ils devraient l'être dans une autre.

L'extrapolation des résultats des études de bioéquivalence ne se limite pas à celle des sujets sains aux patients. Depuis 2007, une proportion croissante d'études de bioéquivalence pharmacocinétique portant sur des soumissions génériques nord-américaines ou européennes a été réalisée auprès de populations géographiques/ethniques autres que celles visées, en raison du coût moins élevé de ces études en dehors de l'Amérique du Nord et de l'Europe.

Dans le premier volet de cette thèse, nous avons examiné si les résultats de la bioéquivalence obtenus dans une population géographique ou ethnique pouvaient être extrapolés à une autre. À cette fin, nous avons extrait les résultats des études de bioéquivalence pharmacocinétique disponibles publiquement et provenant de soumissions

génériques à Santé Canada et à la Food and Drug Administration des États-Unis. Pour dix médicaments différents, nous avons calculé l'effet d'un repas normalisé sur le produit de référence et comparé les résultats obtenus chez deux populations ethniques, les indiens et les nord-américains. Cette approche novatrice est basée sur le raisonnement suivant: si l'effet d'un repas sur le produit de référence est le même chez les populations indienne et nord-américaine, le produit générique et sa référence qui se sont révélés bioéquivalents dans la population indienne devraient également l'être dans la population nord-américaine. Pour 90% des médicaments à l'étude, une différence statistiquement significative a été détectée entre les deux populations après un repas. Pour 30% de ces médicaments, la différence s'est révélée d'une pertinence clinique possible. Les résultats de cette étude ont mis en évidence que l'extrapolation des résultats de bioéquivalence d'une population à l'autre devrait possiblement être reconsidérée pour certains médicaments.

Les défis dans le contexte de la bioéquivalence ne se limitent pas toujours aux études pivots où la performance d'un produit générique est comparée à celle de la référence. En effet, une étude pilote peut être menée afin d'établir un protocole d'étude approprié pour cette étude pivot. Par conséquent, les résultats inexacts provenant d'une étude pilote, tels qu'une estimation imprécise du moment ou de la durée d'administration optimale de la dose lors de la comparaison du produit testé par rapport à la référence, pourront affecter négativement les résultats de l'étude de bioéquivalence. Ceci est particulièrement crucial pour les produits indiqués pour un usage topique dermatologique dont les corticostéroïdes constituent un cas d'espèce. En effet, leur bioéquivalence est démontrée par une mesure pharmacodynamique, le blanchiment cutané, à différents temps après application topique. L'intensité du blanchiment est comparée entre le produit générique et le produit de référence à une durée d'administration spécifique d'une dose donnée, la $DD_{50,}$ soit la durée associée à 50% de l'effet maximal observé. Par conséquent, cette durée d'administration de la dose doit d'abord être déterminée dans le cadre d'une étude pilote. L'agence réglementaire américaine recommande l'utilisation d'une approche populationnelle basée sur la modélisation non linéaire à effets mixtes pour l'estimation de la DD_{50} et ce, quelle que soit la méthode d'analyse. Étant donné qu'il existe différents types de méthodes d'analyse non linéaire à effets mixtes, chaque commanditaire peut en choisir une différente. Dans le deuxième volet de cette thèse, nous avons examiné si

les mêmes estimations de DD_{50} pouvaient être obtenues en utilisant différentes méthodes non linéaires à effets mixtes. À cette fin, nous avons ajusté les données de blanchiment de la peau d'onze études avec deux méthodes non linéaires à effets mixtes différentes : le maximum de vraisemblance avec maximisation de l'espérance (MLEM) et l'estimation conditionnelle de premier ordre (FOCE). Les résultats ont favorisé MLEM, compte tenu d'une meilleure puissance discriminative pour l'estimation de la DD_{50} de population et d'une meilleure minimisation de la variabilité interindividuelle.

Bien que l'approche de la bioéquivalence fondée sur la pharmacocinétique ait contribuée de manière significative au développement de versions génériques de haute qualité des formes pharmaceutiques orales indiquées pour un effet systémique, la disponibilité de versions génériques pour les produits dermatologiques topiques demeure limitée et ce, par manque de méthodes acceptées par les agences réglementaires pour l'évaluation de la bioéquivalence de ces produits. Dans le troisième volet de cette thèse, une nouvelle approche pour l'évaluation de la bioéquivalence de formulations de crème topique d'acyclovir a été développée en utilisant une analyse basée sur un modèle de données d'exposition locales récupérées à partir d'échantillons de peau abrasée prélevés à une seule durée d'administration de la dose, la DD_{50} à l'aide de bandes adhésives. Un seul échantillonnage de peau effectué à la DD_{50} a non seulement assuré que les données pharmacocinétiques étaient recueillies à la durée d'administration de la dose ayant le meilleur pouvoir discriminant pour détecter une différence au niveau des formulations, mais a également permis de diminuer considérablement le nombre d'échantillons à analyser. Et surtout, cette nouvelle approche a permis de générer un profil pharmacocinétique au niveau même de la peau. Ce faisant, nous avons pu utiliser l'analyse compartimentale populationnelle et contourner les nombreuses hypothèses et calculs sophistiqués requis par les méthodes précédentes. Notre approche a également permis de générer de nouveaux paramètres pharmacocinétiques permettant de décrire la vitesse et le degré d'exposition cutanée pour l'évaluation de la biodisponibilité et de la bioéquivalence topiques. Finalement, cette méthode a le potentiel de discerner une formulation bioéquivalente d'une autre qui ne l'est pas.

Mots-clés : Bioéquivalence, médicaments génériques, effet d'aliment, ethnicité, enzymes CYP, transporteurs de médicaments, analyse compartimentale de population, corticostéroïdes topiques, FOCE, MLEM

Abstract

Bioequivalence is a surrogate measure of safety and efficacy in different stages of drug development process with the most pronounced significance in the development of generic drugs. Bioequivalence, among other standards, ensures that generic drugs are equivalent to their approved innovator or reference products in terms of clinical efficacy and safety while circumventing the lengthy-time course and high cost of animal and clinical trials in patients required for innovator drugs. Despite the advancements in development of robust bioequivalence approaches over the past decades, there are still controversies in the current practice of bioequivalence. The aim of this thesis is to explore some of these controversies and address them by putting forward new and alternative approaches.

One of the most controversial issues in the current practice of bioequivalence is the extrapolation of bioequivalence study results from one population to another. The majority of bioequivalence studies for systemic effective oral dosage forms are conducted based on pharmacokinetic endpoints in healthy volunteers whilst the targeted population is patients. This is based on the assumption that if two products are bioequivalent in one population, they should be bioequivalent in another one.

The extrapolation of bioequivalence study results is not limited to that from healthy volunteers to patients. Since 2007, an ever-increasing proportion of pharmacokinetic bioequivalence studies for North American or European generic submissions have been performed in geographical/ethnic populations other than the intended ones, due to the lower cost of these studies outside North America and Europe.

In the first part of this thesis, we investigated whether the bioequivalence results obtained in one geographical or ethnic population can be extrapolated to another one. To this purpose, we extracted pharmacokinetic bioequivalence studies results from generic submissions to Health Canada and the US Food and Drug Administration. We calculated food effect for ten different reference drug products and compared the results for each product between two ethnic populations, Indians and North Americans. This is based on the reasoning that if food effect is found to be the same between the Indian and North American populations,

then the generic product and its reference that were found to be bioequivalent in the Indian population should also be bioequivalent in North American population. For 90% of the study drugs, statistically significant difference was detected in the food effect between two populations. For 30% of these drugs, the difference was found to be of possible clinical relevance. The results of this study raised a flag for extrapolating the bioequivalence results from one population to another.

Challenges in the context of bioequivalence are not always limited to the pivotal studies where the performance of a generic product is compared to that of Reference. Prior to pivotal bioequivalence studies, a pilot study may be conducted to establish an appropriate study design for the pivotal bioequivalence study. Therefore, inaccurate results from a pilot study, such as inaccurate estimation of time point or dose duration for comparison of test versus reference, can affect the bioequivalence outcomes adversely. An example to this case is the comparison of the extent of skin blanching, the pharmacological effect of generic versus reference products of topical dermatological corticosteroids at specific dose duration, DD_{50}, where the effect is half maximal. This dose duration should initially be determined in a pilot study. The US FDA 1995 Guidance document recommends the use of non-linear mixed effect population modeling for the estimation of DD_{50}, irrespective of the method of analysis. Given the availability of different types of non-linear mixed effect modeling methods, each sponsor could choose a different one. In the second part of this thesis we investigated whether the same DD_{50} estimates can be obtained when different non-linear mixed effect modeling methods are used. To this purpose, we fitted the skin blanching data from eleven studies with two different non-linear mixed effect modeling methods, the Maximum Likelihood Expectation Maximization (MLEM) and the First Order Conditional Estimation (FOCE). The results favored MLEM given its lower population DD_{50} estimates that would locate in a more discriminative portion of the E_{max} curve and better minimization of inter-individual variability.

Although the pharmacokinetic-based bioequivalence approach has contributed significantly to the development of high-quality generic versions of systemic effective oral dosage form, the availability of generic versions of topical dermatological products remains constrained due to the limited methods accepted for bioequivalence evaluation of these products. In the third part of this thesis, a novel approach for the bioequivalence assessment of

topical acyclovir cream formulations was developed based on the model-based analysis of local exposure data recovered from tape stripping of the skin at a single dose duration, DD_{50}. Conducting the stripping procedure only at DD_{50} not only ensured that the PK data was collected at the dose duration that is most discriminative of formulation differences, but it also decreased the number of samples to be analyzed significantly. More importantly, our novel approach in generating the local PK profile in the skin (dermatopharmacokinetic profile) and the implementation of population compartmental analysis circumvented the numerous assumptions and sophisticated calculations that were inherent to previous methods, while yielding the PK parameters relevant for topical bioavailability and bioequivalence assessment (rate and extent of exposure to the skin). This method successfully concluded bioequivalence and its absence.

Keywords : Bioequivalence, Generic drugs, Food effect, Ethnicity, CYP enzymes, Drug transporters, Population compartmental analysis, Topical corticosteroids, FOCE, MLEM

Table of contents

List of Tables

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

APPENDIX 1

List of Figures

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

APPENDIX 1

List of Abbreviations and Symbols

Abbreviation	Definition
A	Surface area
$+\delta$	Upper limit of equivalence interval
ΔC	Concentration difference
ABC	ATP-Binding Cassette
ABE	Average Bioequivalence
ACV	Acyclovir
AIC	Akaike Information Criterion
ANDA	Abbreviated New Drug Application
ANDS	Abbreviated New Drug Submission
ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
ATP	Adenosine TriPhosphate
AUC	Area under the concentration time curve
$AUC_{0\text{-}inf}$	Area under the concentration time curve from time 0 to infinity
$AUC_{0\text{-}\infty}$	Area under the concentration time curve from time 0 to infinity
$AUC_{0\text{-}t}$	Area under the concentration time curve from time 0 to the last measurable concentration, *t*
$AUC_{0\text{-}tau}$	Area under the concentration time curve from time 0 to the end of the dosing interval
$pAUC_{Ref,tmax}$	Partial area under the concentration time curve from time 0 to the population median of t_{max} of the Reference
$AUC_{t\text{-}inf}$	Area under the concentration time curve from the last measurable concentration, *t*, to infinity
AUEC	Area Under the Effect Curve
BA	Bioavailability
BCRP	Breast Cancer Resistance Protein
BCS	Biopharmaceutics Classification System
BDDCS	Biopharmaceutical Drug Disposition Classification System

BE	Bioequivalence
BIE	Bioinequivalence
BMSR	Biomedical Simulations Resource
C	Concentration
CI	Confidence Interval
CL	Clearance
C_{max}	Maximum (observed) concentration
$C_{obs,i}$	i^{th} observed concentration in individual
$C_{pred,i}$	i^{th} predicted concentration in individual
CPT	Compartment(al)
CR	Controlled-Release
C_t	Last measurable concentration
CV	Coefficient of Variation
CV%	Percent Coefficient of Variation
D/L^2	Drug diffusivity across the *stratum corneum* of thickness L
DD_{50}	Dose duration which produces 50% of maximal response
DDI	Drug-Drug Interaction
DV	Observation
E	Extraction ratio
E_A	Fraction of drug that is not absorbed further to drug release from dosage form
EC_{50}	Drug concentration which produces 50% of maximal response
ED_{50}	Dose which produces 50% of maximal response
E_G	Intestinal extraction ratio
E_H	Hepatic extraction ratio
EM	Expectation-Maximization
EMA	European Medicines Agency
Emax	Maximum effect
ER	Extended-Release
E-step	Expectation step
EXP	Exponential

ε_i	Epsilon: error term describing difference between predicted and observed concentrations
$\varepsilon_{i,j}$	Distribution of differences between the observed and predicted concentrations: residual variability
F	Bioavailability
f	Function
F_{abs}	Fraction of the drug dose absorbed
$F_{absolute}$	Absolute bioavailability
FDA	Food and drug administration
FE	Food Effect
F_G	Intestinal availability
F_H	Hepatic availability
FO	First Order
FOCE	First Order Conditional Estimation
FOCE-I	First Order Conditional Estimation with Interaction
F_{oral}	Oral bioavailability
F_{rel}	Relative bioavailability
FS	Extent of input into the skin
GI	Gastrointestinal
GMR	Geometric Mean Ratio
GOF	Goodness-of-Fit
GTS	Global Two-Stage
h	Hour
H_0	Null hypothesis
H_1	Alternative hypothesis
HC	Health Canada
HV	Healthy Volunteers
I^2	Indice of heterogeneity in terms of percentage
IMP	Importance Sampling Parametric Expectation Maximization
IND	Investigational New Drug Application

Inter-CV%	Inter-individual variability
IOV	Inter-Occasion Variability
IPRED	Individual-level predictions
IR	Immediate-Release
ITS	Iterative Two-Stage
IV	Intravenous(ly)
IVIVC	*In Vitro–In Vivo* Correlation
IVRT	*In Vitro* Release Test
J	Rate of passive diffusion or flux
K	Lipid–water partition coefficient of drug in the biologic membrane
k_a	Absorption rate constant
kcal	Kilocalorie
K_{diff}	Diffusion rate constant
K_{el}	Apparent terminal elimination rate constant estimated noncompartmentally
K_{ij}	Transfer rate constants from compartment "*i*" to compartment "*j*"
K_{in}	First-order input rate constant into the skin
K_{out}	Diffusion rate constant out from the last compartment
Kp	Permeability coefficient
LADME	Liberation, Absorption, Distribution, Metabolism. Excretion
LL	Log-likelihood
LLCI	Lower Limit of Confidence Interval
Ln	Natural logarithm; Logarithm to the base of e
Log	Logarithm
LSM	Least Squares Means
m	Mass
MAP	Maximum *a posteriori* Probability
MCPEM	Monte Carlo Parametric Expectation Maximization
MDR	Multidrug Resistance
min	Minute
ml	Milliliter
ML	Maximum likelihood

MLEM	Maximum likelihood expectation maximization
MOF	Minimum value of Objective Function
MR	Modified-Release
MRP	Multidrug Resistance Protein
M-step	Maximization step
n	Number of total observations
NCE	New Chemical Entity
NCPT	Non-compartmental
NDA	New Drug Application
NDS	New Drug Submission
NIH	National Institutes of Health
NLME	Non-Linear Mixed Effect
NME	New Molecular Entity
NONMEM	Non-Linear Mixed Effect Modeling
OAP	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
OF	Objective Function
OGD	Office of Generic Drug
OLS	Ordinary Least Square
OMAP	Maximum *a posteriori* probability objective function
OOLS	Ordinary least square objective function
OWLS	Weighted least squares objective function
pAUC	Partial Area Under the Curve
PD	Pharmacodynamic
PE	Point Estimate
PEG	Polyethylene Glycol
PEPT	Peptide Transporter
P-gp	Permeability glycoprotein
Pi	Vector of pharmacokinetic parameters for an individual

PK	Pharmacokinetic
PK_a	Measure of acidity
PM	Poor metabolizer
PRED	Population-level prediction
Q	Amount
Q_{max}	Maximum amount
QRPEM	Quasi-Random Parametric Expectation Maximization
$Q_{total(clearance)}$	Total amount of drug extracted from *stratum corneum* during the clearance phase
$Q_{total(uptake)}$	Total amount of drug extracted from *stratum corneum* during the uptake phase
Ref	Reference
RLD	Reference Listed Drug
SABE	Scaled Average Bioequivalence
SAEM	Stochastic Approximation Expectation Maximisation
SC	*Stratum corneum*
SD	Standard Deviation
shη	η-shrinkage
SLC	Solute Carrier
SLS	Sodium Lauryl Sulfate
SNP	Single Nucleotide Polymorphism
SR	Sustained Release
STS	Standard Two-Stage
SUPAC	Scale-Up and Post-Approval Change
t	Time
TE	Therapeutic Equivalence
TEWL	Trans Epidermal Water Loss
t_{max}	Time to reach maximum (observed) concentration
TOST	Two One-Sided Tests
TS	Tape stripping
TV	Typical value for population mean estimate

ULCI	Upper Limit of Confidence Interval
US	United States
V_d	Volume of distribution
Wi	Weighting factor
WLS	Weighted Least Squares
WRES	Weighted Residuals
α	Probability of committing a Type I error of a statistical test
β	Probability of a Type II error
γ	Hill coefficient
$-\delta$	Lower limit of equivalence interval
η_i	Eta: Difference between population and individual parameter value
θ	Theta: population (mean) model parameter estimate
λ_z	Terminal elimination rate constant estimated compartmentally
μ	Micro
μ_R	Mean bioavailability measure for reference
μ_T	Mean bioavailability measure for test
ρ	Density
Σ	Sigma variance covariance matrix
σ^2	Error variance
σ^2_{pooled}	Pooled error variance
τ^2	Indice of heterogeneity in terms of variance
Ω	Omega variance covariance matrix

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- *Winston Churchill*

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Preface

Reliance on bioequivalence studies has accelerated the development of high-quality generic drug products with extraordinary cost savings. The ultimate goal of bioequivalence studies in generic drug development is to ensure that the generic product has equivalent safety and efficacy to its reference, and therefore, the generic is interchangeable with the reference counterpart. Nevertheless, there are insufficiently studied areas that require further studies to ensure the safety and efficacy of approved generic products, along with the fields where the demonstration of bioequivalence is associated with difficulties. This thesis aimed to identify some of these areas with the purpose of answering outstanding question marks and proposing novel alternative approaches for demonstration of bioequivalence where needed.

One of the most controversial issues in the practice of bioequivalence is the reliability of bioequivalence study results obtained in populations other than the targeted ones. Another outstanding issue is the uncertainties associated with the estimated values of the dose duration at which the bioequivalence of topical dermatological corticosteroids must be evaluated. Eventually, lack of cost-effective, reproducible and surrogate-based approaches for bioequivalence assessment of topical dermatological products is another challenge that needs to be addressed in the context of bioequivalence.

To better illustrate the relevance and significance of the research presented in this thesis, basic principles of pharmacokinetics, pharmacodynamics, bioequivalence, and compartmental modeling are first discussed in the introduction of this thesis.

After describing the context in which the research presented herein has evolved, the following articles are presented individually:

1) Influence of Different Populations on Pharmacokinetic Bioequivalence Results: Can We Extrapolate Bioequivalence Results from One Population to Another?
2) Revisiting FDA's 1995 Guidance on Bioequivalence Establishment of Topical Dermatologic Corticosteroids: New Research Based Recommendations
3) Novel Approach for the Bioequivalence Assessment of Topical Cream Formulations: Model-Based Analysis of Tape Stripping Data Correctly Concludes BE and BIE

Chapter 1 – INTRODUCTION

1. Pharmacokinetics

Pharmacokinetics (PK) is the discipline that describes "what the body does to the drug" and it involves the study of the liberation, absorption, distribution, metabolism, and excretion of drugs and are often referred to collectively as "LADME"[1]. Whether or not a drug's PK includes all of these steps depends on its route of administration. Oral and topical routes of administration are the main focus of this thesis.

Oral administration is the most convenient route for access to the systemic circulation for systemic effective drug products. Topical routes of administration on the other hand are generally used for the purpose of local drug action. Topical administration is employed to deliver a drug substance at, or immediately beneath, the point of application, prior to reaching systemic circulation. A large number of topical medicaments are applied to the skin, although topical drugs are also applied to the eye, nose, throat, ear, vagina, and certain mucous membranes. In the context of this thesis, topical drugs refer to those administered to the skin [2].

In this chapter, each of the LADME steps will be discussed separately. Due to the relevance of absorption and metabolism to the focus of this thesis these topics are discussed more extensively.

1.1 Liberation

The liberation of a drug from its pharmaceutical dosage form is a critical first step in a drug's disposition pathway. Following oral dosing, the drug product is disintegrated, the active ingredient is released from the solid dosage form (tablet, capsule, etc.), and dissolves in gastrointestinal (GI) tract. These steps are referred to as "drug product performance" and are inherently determined by the formulation. Once the drug substance is dissolved, it will be available to be absorbed across intestinal cell membranes into the systemic circulation. Drug product performance is, therefore, one step before drug absorption and constitutes a key parameter in bioequivalence studies [3].

1.2 Absorption

When a drug is administered by an extravascular route (e.g., oral, intramuscular, transdermal, etc.), the drug must first be absorbed into the systemic circulation and then diffuse or be transported to the site of action before eliciting biological and therapeutic activity.

In order to be absorbed, drug molecules must pass through several biologic membranes before reaching the vascular system. If the drug is given orally, the drug molecules must pass through the GI tract wall into capillaries. For transdermal patches, the drug must penetrate the skin to enter the vascular system. Drugs administered intravenously bypass this step, since they are injected directly into the bloodstream, and therefore, will readily become available in systemic circulation without a need to pass biologic membranes.

In general, there are differences as well as similarities among the mechanisms and the various membranes through which a drug may pass to gain access to the systemic circulation. In the following subsections, the process of absorption and different factors influencing absorption will be discussed in more details. Because the drugs studied in the context of this thesis were administered orally and topically, their process of absorption for each route will be discussed in more details.

1.2.1 Oral Absorption

Orally administered drugs must be absorbed first from the GI tract. Absorption refers to the passage of drug molecules from the site of administration, the intestinal lumen, into the enterocytes [4]. The first major obstacle to cross in oral absorption is the intestinal epithelium which consists of enterocytes. Apical surface of enterocytes faces the lumen (apical membrane) while the basolateral surface faces the surrounding fluids which lead to the blood (basolateral or basal membrane) [5]. Transcellular absorption from lumen to blood requires uptake across the apical membrane, followed by transport across the cytosol, then exit across the basolateral membrane and into blood [6].

Lipophilic compounds may readily cross the enterocyte by a means of passive diffusion down the concentration gradient (from a high concentration in the luminal fluid to a low concentration in enterocyte, and then into the portal blood), in the absence of specialized transport systems. Passive diffusion is the most common mechanism of absorption across the

intestinal membrane which is based on the principles of Fick's First Law applied to membranes. In fact, classical explanations of the rate and extent of drug absorption have been based on the Fick's First Law [4]. However, not all compounds can cross enterocytes by passive diffusion. Highly hydrophilic and charged compounds often require specific carrier-mediated pathways to facilitate transcellular transport. During the past decades, many studies have revealed the significance of carrier-mediated processes in the passage of drugs through biological membranes and in regulating the absorption. Other mechanisms such as vesicular transport with relatively less contribution could also be involved in the absorption across biologic membrane. Vesicular transport, in different forms such as endocytosis and pinocytosis, is the process of engulfing particles or dissolved materials by the cell. This mechanism of transport is beyond the scope of this thesis and will not be described any further. In the following subsection, only the major mechanisms involved in drug absorption and their principles are discussed in more detail.

1.2.1.1 Mechanism of oral absorption

Absorption mostly occurs via passive diffusion and carrier-mediated transport processes. Carrier-mediated transport can be divided into facilitated and active transport [7, 8].

1.2.1.1.1 Passive diffusion

Drug absorption by passive diffusion occurs down its concentration gradient and can be expressed using Fick's First Law applied to membranes, where drug flux across a membrane is directly proportional to the magnitude of the concentration gradient across the membrane. Drug flux also depends on characteristics of the membrane as well as the permeability and solubility (physicochemical properties) of the absorbed compound. For an orally administered drug, drug flux across a membrane refers to the rate that dissolved drug crosses the intestinal wall to reach the portal blood circulation, in other words, the rate of absorption [4, 7, 9]. The rate of absorption via passive diffusion through the biological membranes can be described most simply by Fick's First Law [10]:

$$J = \frac{dQ}{dt} = \frac{(DKA)\Delta C}{h} = Kp\ \Delta C \qquad \text{(Equation 1)}$$

where J or $\frac{dQ}{dt}$ is the rate of passive diffusion; D is the diffusion coefficient of drug in membrane which represents the amount of a drug that diffuses across a membrane of a given unit area per unit time when the concentration gradient is unity; K is lipid–water partition coefficient of drug in the biologic membrane that controls drug permeation; A is the surface area of membrane; and h is the diffusion path length across the membrane; ΔC is the drug concentration gradient across the membrane. D, K, A, and h can be combined and referred to as permeability coefficient:

$$Kp = \frac{DKA}{h}$$

Obviously, this concentration gradient for an orally administered drug will be the concentration of the drug in luminal fluid minus that in cytosol in apical surface; or the concentration of the drug in cytosol minus that in portal blood at basolateral surface. Because the drug distributes rapidly into a large volume after entering the blood, the concentration of drug in the blood initially will be quite low with respect to the concentration at the site of drug absorption. The large concentration gradient is maintained until most of the drug is absorbed, thus driving drug molecules from the gastrointestinal tract (site of absorption) into the plasma hypothetically in a unidirectional manner. However, in reality, the process of absorption is rather complicated and is not unidirectional due to the carrier-mediated processes.

1.2.1.1.2 Carrier-mediated processes

Carrier-mediated transport could occur either by facilitated diffusion or by active transport [7, 8]. Active transport and facilitated diffusion are similar in that both are mediated by membrane-associated transporters (pump); they are saturable; and they are usually selective for certain drugs or nutrients. They differ in the direction of the transport and their requirement for energy [11].

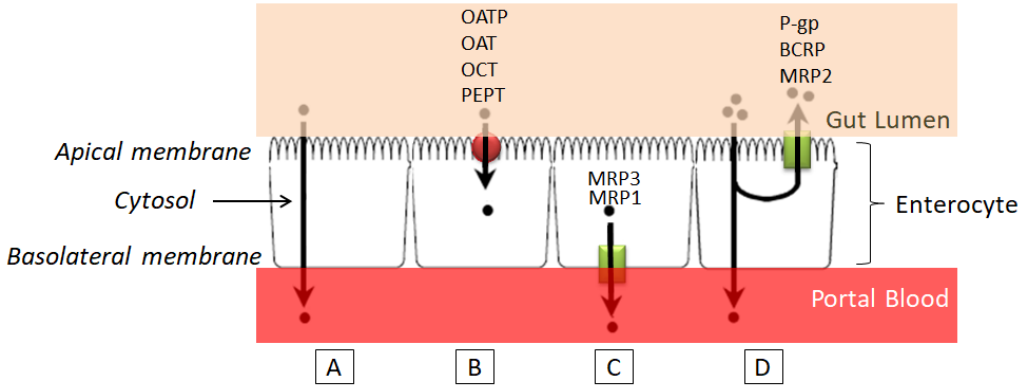
Facilitated diffusion

Facilitated diffusion is performed by diffusion down a concentration gradient and does not require input of energy. In terms of drug absorption, facilitated diffusion seems to play a very minor role and only transports a molecule along its electrochemical gradient.

Active transport

Active transport is performed against the concentration gradient of drug molecules. The transport will be in ‘‘up a concentration gradient” direction which is not favored thermodynamically and, hence, does not occur spontaneously and requires input of energy. The input of energy is commonly supplied by coupled biochemical reactions such as ATP hydrolysis. The active transport pathway is mediated by transporters which are categorized into two major classes – efflux and uptake transporters [11]. The presence of transporters in membranes modulates the traditional theory of unidirectional diffusional absorption towards bidirectional absorption. The role of transporters in governing the absorption is described in more detail in **Section 1.6.1.1**. An overview of the involved mechanisms in absorption is depicted in **Figure 1.**

Figure 1. Major mechanisms of GI absorption. OATP, Organic Anion Transporting Polypeptide; OAT, Organic Anion Transporter; OCT, Organic Cation Transporter; PEPT, Peptide Transporter; P-gp, P-glycoprotein; BCRP, Breast Cancer Resistance Protein; MRP, Multidrug Resistance Protein.



A: Absorption via transcellular Passive diffusion.
B: Active transport which mediates transcellular absorption from gut lumen into the enterocytes at apical surface.
C: Active transport which mediates transcellular absorption from cytosol into the portal blood at basolateral surface.
D: Active transport which exports compounds into the gut lumen subsequent to their absorption into the enterocytes.

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1.2.2 **Percutaneous Absorption**

Drug products applied to the skin surface are categorized into two major classes of topical and transdermal formulations [12-14].

Topical (i.e., dermatological, herein) formulations are not designed to deliver the drug into the systemic circulation. They are rather designed to release drug that penetrates to some extent into the skin layers, where the pharmacological effect is expected. Therefore, the permeation process is expected to stop at the diffusion step through the skin layers, before reaching the cutaneous circulation [15].

The process of drug permeation through different layers of the skin to eventually reach the targeted site within the skin is referred to as input into the skin. Topical formulations are designed to deliver drug into the various layers of skin and exert a local effect in the skin for treating dermal disorders such as acne, dermatitis, erythematous lupus, and psoriasis, and are not designed for delivery of drug into the systemic circulation [12, 16]. Depending on the disease condition the target site for the topical product will vary. For example, in treatment of fungal infections a drug has to exert effect in *stratum corneum*; for management of eczema and psoriasis drug action is needed in the viable epidermis; and amelioration of muscle strains and sprains requires penetration to the deeper tissues [13].

Transdermal formulations, on the other hand, release drug that permeates through the skin and enters the systemic circulation. The process of drug permeation through all layers of the skin to reach the systemic circulation is referred to as percutaneous absorption. Transdermal formulations are designed to ensure that effective plasma concentrations of drug are reached for producing the systemic effect [14, 16, 17].

Topically applied drug products studied in the context of this thesis are topical dermatological formulations with local effect. Hence, the expression “percutaneous absorption” refers to “input into the skin” in the context of this thesis. The mechanisms and pathways of percutaneous absorption is the same for topical and transdermal formulations. The notion of *input* for topical versus *absorption* for transdermal formulations is discriminated only based on the last layer through which the drug permeates. In the following subsections mechanisms and pathways of percutaneous absorption are described.

1.2.2.1 Mechanism of percutaneous absorption

Percutaneous absorption, in the simplest way, can be considered as the permeation of drugs through the skin layers. Percutaneous absorption can alternatively be referred to as percutaneous transport. The general principles of percutaneous absorption are similar to those for oral absorption. From a pharmacokinetic standpoint, permeation of drug, through all percutaneous absorption pathways (**section 1.2.2.3**), occurs via passive diffusion on the basis of Fick's First Law [14, 18].

Some influx and efflux transporters such as Organic Anion Transporting Polypeptide (OATP), Multidrug Resistance protein (MDR), and Permeability glycoprotein (P-gp) have also been found in skin cells (keratinocytes), but their contribution to percutaneous absorption is negligible in comparison to passive diffusion [19].

The kinetics of percutaneous absorption differs from the oral one, mostly due to the different physiology and anatomy of barriers in percutaneous absorption. In oral absorption of drug, enterocytes are the only *line of defense* that a drug has to overcome to be absorbed. In percutaneous absorption, however, drug has to pass different layers of the skin until it reaches the dermis layer. Permeation to dermis brings a drug in contact with lymphatics and cutaneous microcapillaries which eventually open to the systemic circulation. In the following section, different layers of skin which should be overcome in the path of percutaneous absorption are described.

1.2.2.2 Skin structures and the layers in the path of percutaneous absorption

Human skin is a stratified tissue composed of three different layers, which are from the top to the bottom: epidermis, dermis, and hypodermis (or subcutaneous tissues) [20].

Epidermis

Barrier functions of the skin largely confined to the epidermis, and more specifically to the outermost layer of the epidermis, *stratum corneum* (SC). From a skin permeation viewpoint, SC provides the main and the first (mechanical) barrier of the skin. Once drug molecules are able to pass through the SC, the rest of the epidermis provides little resistance. However, the skin's barrier function does not only depend on the SC; but it is also determined by the second (biological) skin barrier formed of enzymes mainly in the viable epidermis [14, 21, 22].

Stratum corneum arises from the sequential differentiation of keratinocytes migrating from the basal epidermal layer (*stratum basale*) to the surface, ending as corneocytes in the SC. *Stratum corneum* is also known as the horny layer of the skin. The total thickness of SC is 10-25 μm [14, 20, 23]. *Stratum corneum* can be described as a brick wall-like structure with corneocytes as the "bricks" in a matrix (the "Mortar") of intercellular lipids. These cells are high density, low hydration hexagonal flattened dead cells without a nucleus and full of keratin. SC consists of about 10-15 tightly stacked layers of corneocytes. Corneocytes are embedded in a lipidic intercellular matrix, mainly composed of ceramides, long-chain free fatty acids and cholesterol [21, 23-25]. Corneocytes are held together by corneosomes that are specialized inter-corneocyte linkages formed by proteins and, together with the lipids, they maintain the integrity of the SC [26-28].

Living epidermis (viable epidermis) comprises of metabolically active cells and is the main location of enzymatic biotransformation of dermally applied drugs. Enzymatic biotransformation of drugs in the skin will be described in more detail in **Section 1.4.1**. Viable epidermis is further divided into 3 layers: *stratum granulosum*, *stratum spinosum*, and *stratum basale* from the surface towards the deeper layers. The total thickness of viable epidermis is about 100 μm [29]. Keratinocyte are held together by desmosomes in the viable epidermis. Both cohesion and the number of desmosomes increase from the surface towards the deeper layers of epidermis which result in the looser packing of corneocytes in the SC and tighter packing of keratinocyte in the deeper layers [30, 31].

Dermis

The dermis provides mechanical strength and flexibility to the skin. Dermis is composed collagen and elastin. Hair follicles and sebaceous glands originate in the dermis and have follicular ducts extending through the epidermis to the skin surface. The dermis also contains large numbers of lymphatics and blood vessels. The capillary plexus between the epidermis and dermis is the major site of cutaneous absorption to the systemic circulation [32].

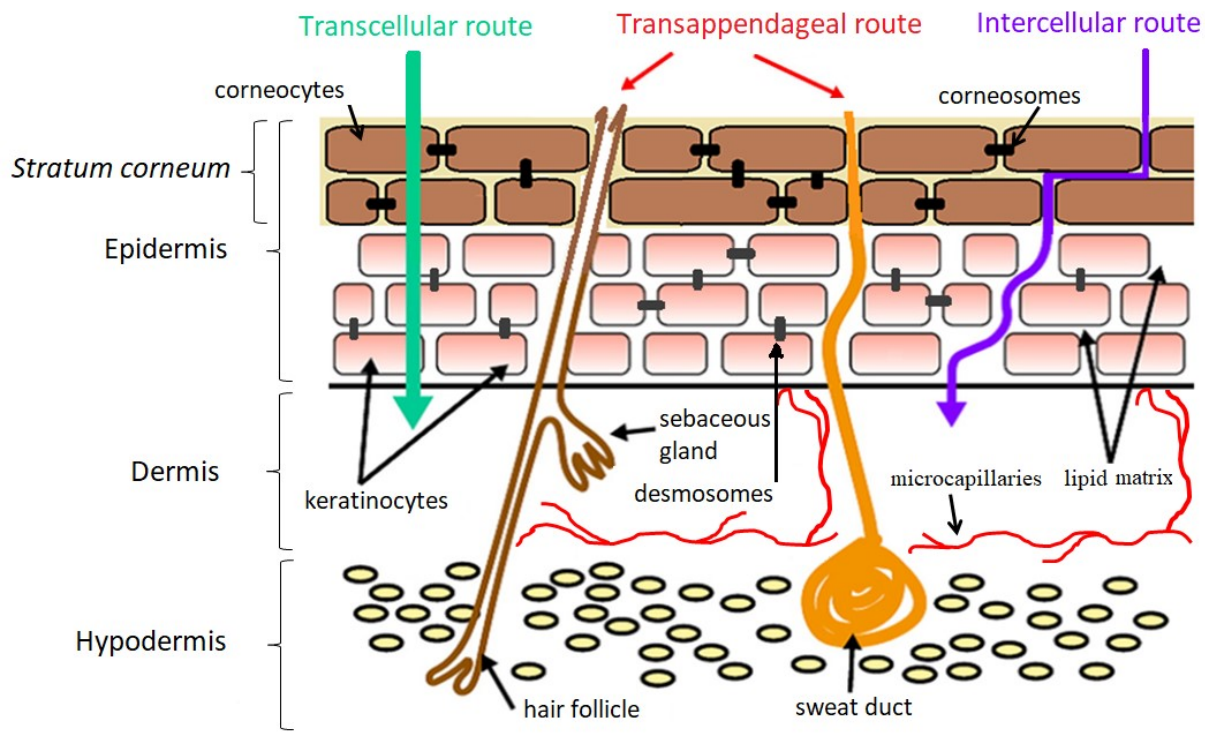
Hypodermis

Hypodermis or subcutaneous tissue refers to the adipose tissue with nerves and blood vessels lying beneath the dermis [33].

1.2.2.3 Percutaneous absorption pathways

The permeation of drugs through the skin occurs via passive diffusion through three pathways: (a) the corneocytes (transcellular route), (b) the lipid matrix between the corneocytes (intercellular route), and (c) the appendages. i.e., hair follicle, sweat and sebaceous glands which form shunts opening to the skin surface (follicular or transappendageal route). These pathways are not mutually exclusive, with most compounds permeating the skin through a combination of pathways based on the physicochemical properties of the permeating molecule. In general transcellular and intercellular routes are the major pathways in percutaneous absorption and the contribution of transappendageal route is considered to be minimal [18, 32]. Structure of the skin and percutaneous absorption pathways are schematized in **Figure 2.**

Figure 2. Structure of the skin and percutaneous absorption pathways



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1.3 Distribution

After a drug is absorbed systemically from the site of administration, they are carried by the blood and will pass from the bloodstream into various tissues and organs. Drugs may

bind to various macromolecular components in the blood, including albumin, α_1-acid glycoprotein, lipoproteins, immunoglobulins, and erythrocytes [35]. Binding to macromolecular components in the blood plays a critical role in drug distribution, since generally only free (unbound) drug is able to cross membranes and reach specific tissues. When drug molecules are delivered to the target sites, they produce targeted pharmacological effect; when they are delivered to other tissues, they may cause side effects or adverse reactions; and when they are distributed to eliminating organs, such as the liver and kidney, they will be eliminated from the body [36]. The topic of drug distribution is beyond the scope of this thesis.

1.4 Metabolism

Metabolism is the chemical conversion of the drug molecule, usually by an enzymatically mediated reaction, into another chemical entity referred to as a metabolite. The metabolite may have the same or different pharmacological effect as the parent drug. More often, the metabolites are either pharmacologically inactive or less active than the parent substance. Drug metabolism is also referred to as biotransformation [37].

Metabolism is divided into phase I and II processes. In phase I, the drug undergoes oxidation, reduction, and hydrolysis that introduce a polar functional group on the drug molecule. Metabolites produced by phase I reactions are rarely of sufficient polarity to undergo the excretion, and many require additional metabolism by phase II enzymes [38]. Catalases, peroxidases, reductases, cholinesterases, dehydrogenases are some examples of Phase I enzymes. However, the CYP superfamily is the major family of enzymes that is responsible for phase I reactions.

In phase II, a polar moiety is usually added into either the parent molecule or its phase I metabolites. The resulting polar metabolites are then excreted from the body through urine or bile, or in some cases sweat or exhalation. Phase II processes involve conjugation of specific endogenous compounds to drugs or their metabolites. For orally administered drugs, the liver and intestine are the principal sites of drug metabolism where both phase I and phase II reactions take place, and therefore, they are also the potential sites for different interactions, such as drug-drug and food-drug interactions [37, 39].

1.4.1 Extrahepatic Cutaneous Metabolism

Metabolism also occurs in extrahepatic organs such as the skin. Nevertheless, the extent of this metabolism is most often negligible. CYP enzymes are among the most studied drug metabolizing enzymes in the skin. Which of the CYP enzyme isoforms are present in human skin has still not been clarified and contradictory data are available in the literature. Among various CYPs, the presence of CYP2E1 enzymes has frequently been reported in keratinocytes, melanocytes, and Langerhans cells in the epidermis, and fibroblasts in the dermis [19, 40, 41]. CYP1A1, CYP1A2, CYP2C9, CYP2D6, and CYP3A4 are other CYP enzymes reported to be found in the skin [42-44].

CYP enzymes in the skin mediate the biotransformation of endogenous and exogenous substrates such as therapeutic drugs, fatty acids, retinoids, steroids including vitamin D, and glucocorticoids [45]. In general, biotransformation of substrates by CYP enzymes in the skin results in protection of the body from external factors or detoxification of xenobiotics. For instance, hydroxylation of vitamin D3 to calcitriol, the most active form of vitamin D3, is essential in offsetting the toxic effects of UV exposure and is mediated by the CYP enzymes in the skin [45-47]. Nevertheless, a number of examples also exist where certain substrates which themselves are not harmful can be biotransformed by cutaneous CYP into ultimate carcinogens. The CYP enzymes in the skin (e.g., CYP1A1) mediate the metabolic activation of polycyclic aromatic hydrocarbons (e.g., benzo(a)pyrene) and form reactive intermediates which can interact with tissue macromolecules, and this interaction is ultimately responsible for inducing skin carcinomas [45, 48-50].

The pharmacological effect of topical and transdermal drugs can theoretically be reduced as the active ingredient passes through the skin layers and may therefore undergo the cutaneous metabolism by CYP and other metabolizing enzymes [19]. For instance, Duell et al. [51] have shown that topical retinoic acid is converted to the less active metabolites 4-OH retinoic acid and 4-oxoretinoic acid, which could limit the pharmacological activity of retinoic acid. Cutaneous metabolism of nitroglycerine by CYP enzymes has also been suggested [52, 53]. However, no data to date has robustly demonstrated whether or not the biotransformation of topical and transdermal products by cutaneous enzymes is of clinical relevance.

In order to overcome cutaneous metabolism, even if its clinical relevance has not yet been documented, prodrugs can be promising. Numerous attempts have been directed to

develop topical prodrugs. Commercial prednicarbate cream is an example topical prodrug which is hydrolyzed to prednisolone 17-ethylcarbonate, and further is metabolized to prednisolone 21-ethylcarbonate and prednisolone in the keratinocytes [54, 55]. Nevertheless, the bioactivation of prednicarbate is not mediated by CYP enzymes. Delivery of naltrexone [56], vitamin E [57], 5-fluorouracil [58], haloperidol [59], nalbuphine [60], and ketorolac [61] were also studied as their prodrugs but none has been commercialized as a drug product.

1.4.2 **Pre-Systemic Metabolism**

The venous drainage system of the stomach and intestines differs from that of most other organs in that the venous drainage of most organs goes directly to the heart, but venous drainage of the GI tract sends blood into the portal circulation, which delivers blood to the liver. Once through the liver, the drug and its metabolites follow the hepatic venous drainage to the heart and into the systemic circulation [39, 62]. The amount of an orally administered drug that reaches the systemic circulation can be reduced by both intestinal and hepatic metabolism. The metabolism of drugs before entering the systemic circulation is referred to as first-pass or pre-systemic metabolism [62]. Drugs that reach the blood are then passed to the liver, where they are subject to further metabolism and biliary excretion. After reaching to the systemic circulation, all subsequent PK paths are the same for oral and any other systemically administered substances. Evidently, orally administered drugs will undergo metabolism once before reaching the systemic circulation (pre-systemic metabolism), and another time after reaching the systemic circulation; while intravenously administered drugs will only undergo the systemic hepatic metabolism and avoid first-pass metabolism [63].

It was widely assumed that the liver is the major site of pre-systemic metabolism because of its size and its high content of drug-metabolizing enzymes. However, discovery of the drug-metabolizing enzyme CYP3A4 in the human intestinal mucosa as well as the liver by Watkins and coworkers [64, 65] have led to a growing interest in the process of gut wall metabolism. Since then many clinical studies have indicated that the small intestine contributes substantially to the overall pre-systemic metabolism [66-69]. Some studies have even suggested that the role of intestinal metabolism is quantitatively greater than that of hepatic metabolism in the overall first-pass effect [67, 68]. This could be a result of better access to the enzymes in the enterocytes; a function of transcellular flux and the large absorptive area.

Almost all of the drug-metabolizing enzymes in the liver, including those in the CYP family, are also found in the small intestine, although their levels are generally much lower in the intestine [63]. Hence, an orally administered drug will be subject to metabolism in small intestine before it enters into the portal vein to the liver. This metabolism is called pre-systemic metabolism at intestinal level. A similar process can occur in hepatocytes, as drug that is absorbed unchanged into the portal vein passes through the liver for the first time and will undergo metabolism in hepatocytes before reaching into the systemic circulation. This metabolism is called pre-systemic metabolism at hepatic level. Due to this first-pass metabolism, a significant fraction of the oral dose can be lost by the actions of both hepatic and intestinal enzymes before the drug ever reaches the systemic circulation.

1.5 Excretion

Excretion is the irreversible removal of drug from the body and mainly occurs via the kidney or biliary tract. Other pathways for drug excretion may include the excretion of drug into sweat, saliva, milk (via lactation), or other body fluids. Excretion should be differentiated from elimination. Drug elimination is divided into two major components: biotransformation (metabolism) and excretion [70, 71]. Drug and its metabolites generated by the metabolism within liver cell, can be excreted either into the bile canaliculus for hepatobiliary excretion or into the bloodstream for subsequent renal excretion. Within the hepatobiliary excretion, a drug and its metabolites are extruded into the bile and then pass into the intestine. A drug that is passed into the intestine may be reabsorbed. The unabsorbed fraction will be eliminated in the feces. Renal excretion is a process by which the intact drug and its metabolites passes through the kidney to the bladder and ultimately into the urine [2, 71].

Drug excretion, beside the distribution and biotransformation, contributes to the decline in plasma drug concentration. Differently said, excretion may affect systemic drug exposure which is expressed by the area under the curve (AUC) of drug plasma concentration-time profile. Nevertheless, systemically available drug (bioavailable drug) is removed from the body through excretion only when a drug has already escaped the barriers of first-pass metabolism. A drug needs to overcome several barriers in order to become available in the systemic circulation but excretion is not among these barriers. Intestinal and hepatic barriers are determinants of drug availability in the systemic circulation (bioavailability). **Section 3** of this thesis is attributed to discuss bioavailability in more detail.

Because the focus of this thesis is relative bioavailability, and because any step following appearance of drug into systemic circulation is not amongst the determining factors of drug bioavailability, excretion processes will not be described any further.

1.6 Factors that Impact Pharmacokinetics of Drugs

Many factors can affect pharmacokinetics of drugs in the absorption and metabolism stages of LADME process. In general, the factors that influence absorption and metabolism can be divided into physicochemical/biopharmaceutical and physiological factors.

Physicochemical and biopharmaceutical factors (e.g. stereoselectivity, molecular size, acidity, lipophilicity, etc.) impact the PK of drug by influencing drug permeability, drug solubility and dissolution characteristics of drug formulation. These properties are inherent in the drug molecules and are not the focus of this thesis [72-74].

Physiological factors influencing the PK of drug are different at the absorption and metabolism sites, depending on the particular gastrointestinal sites involved. Nevertheless, there are also similarities. For instance, metabolizing CYP enzymes and membrane transporters are among the physiological factors that impact the PK. Both are expressed in the tissues at the absorption site (intestine) and elimination (liver) sites. However, CYP enzymes are only involved in the metabolism, while transporters have substantial role in both the absorption and metabolism. The role of CYP enzymes and transporters in the absorption and metabolism stages are described in more detail in the following subsections.

1.6.1 Transporters

Membrane transporters can be major determinants of the pharmacokinetics by acting as the gatekeepers for cells and controlling the uptake and efflux of drugs and other xenobiotics. Transporter-mediated drug disposition is the dynamic interplay between influx and efflux transporters within the epithelial cells.

Functionally, transporters can be classified into transporters mediating the export of drugs or drug metabolites out of cells (efflux transporters) and transporters mediating the uptake of drugs into cells (influx or uptake transporters) [5, 75]. More specifically, efflux transporters export drugs from the intracellular to the extracellular environment, often against high concentration gradients, thereby requiring energy. Most efflux transporters belong to the

ATP-binding cassette (ABC) superfamily and rely on ATP hydrolysis to actively pump their substrates across membranes. Hence, ABC transporters are also called primary active transporters. P-glycoprotein (P-gp), encoded by the Multidrug Resistance-1 (*MDR1*) gene (also termed *ABCB1*[1]), is the best recognized efflux transporters in the ABC superfamily. Breast Cancer Resistance Protein (BCRP; *ABCG2*), Multidrug Resistance Proteins (MRP1-6; *ABCC1-6*), and the Bile Salt Export Pump (BSEP; *ABCB11*) are other members of ABC superfamily. Influx transporters, on the other hand, facilitate the movement of drugs into cells and are members of the solute carrier (SLC) superfamily. Widely recognized SLC transporters include the Organic Anion Transporting Polypeptide (OATP; *SLCO*), the Organic Anion Transporter (OAT; *SLC22A*), the Organic Cation Transporter (OCT; *SLC22A*), and Peptide Transporter (PEPT; *SLC15A*) families [76].

Transporters, in general, are expressed in the basolateral or apical side of epithelial cells lining pharmacological barriers, e.g., in the epithelium contributing to the blood-brain barrier, in excretory sites (the biliary canalicular membrane of hepatocytes, the luminal membrane in proximal tubules of the kidney), and in absorption barriers (such as the brush border membrane of intestinal cells) [77]. Transporters expressed in the enterocytes, hepatocytes, and renal tubular epithelial cells are critical components for regulating the absorption and disposition of orally administered drugs and play a significant role in determining basic PK parameters reflecting the drug exposure [5, 75]. Each transporter has a specific pattern of tissue expression [78, 79]. In other words, expression of transporters in the GI tract displays regional distribution patterns, and their presence at specific physiological barriers complies well with their role in the relevant stage of LADME process where they are involved. For instance, the main function of P-gp transporters is to prevent or restrict the exposure to xenobiotics, either by impeding their absorption or by enhancing their elimination. P-gp transporters should, therefore, be present on the apical or luminal surface of secretory cells in the organs to undertake their function [80]. In the small intestine, P-gp transporters are localized in apical membrane of enterocytes, mediating the efflux of xenobiotics from inside the enterocyte back into the gut lumen, and therefore limiting the absorption [5]. In hepatocytes, they are exclusively found on the biliary canalicular front of hepatocytes and on the apical surface of epithelial cells in small biliary ducts, mediating the

[1] The gene coding for the transporter protein is designated in italics.

export of xenobiotics into bile and therefore enhancing the elimination [80]. It is of value to mention that the excretory versus absorptive role of transporters is ultimately determined by their localization rather than the superfamily under which they are categorized. For instance, MRP1 and MRP3 are members of ABC efflux transporters superfamily. Despite their excretory role, these efflux transporters mediate absorption, due to their localization in basolateral membrane of enterocytes [76, 81, 82]. Thereby, the movement of drugs across the intracellular and extracellular compartments of epithelial cells may be impeded or facilitated depending on the localization of transporters on apical or basolateral membranes.

Although a comprehensive review of the transporters in the kidney is beyond the scope of this thesis, their contribution in mediating the renal excretion deserves a brief mention. Drug transporters in the kidney play a major role in drug secretion from the proximal tubular cells of the kidney into urine. Secretion of drugs is mediated by uptake of drugs (from blood into the renal epithelial cells) via influx transporters (such as OATP4C1, OCT2, OAT1-3) localized in the basolateral membrane and subsequent efflux (from inside these cells into the urine) by efflux transporters (such as MRP2, MRP4, and P-gp) localized in the luminal membrane of proximal tubular cells [76, 83].

In this thesis, the transporters involved in the absorption and metabolism stages of LADME are of particular interest and will be elaborated in more detail below.

1.6.1.1 Transporters Governing Absorption

As specified earlier, transporters are widely expressed in the plasma membranes of many organs. Only those in the small intestine are involved in governing the absorption. P-gp, BCRP, MRP1-6, and BSEP are efflux transporters expressed in the intestine. Among them, P-gp, BCRP, MRP2, and MRP4 are localized at the apical (brush-border) membrane of enterocytes and drive compounds from inside the cell (cytosol) back into the intestinal lumen, thus preventing their absorption into blood. MRP1 and MRP3, on the other hand, are localized on the basolateral membrane of enterocytes where they act as efflux transporters into portal blood. They transport drugs from the cell into the interstitial fluid, rather than moving them out into the intestinal lumen. As a result, MRP1 and MRP3 act as absorptive transporter [76, 81, 82]. OATP, OAT, OCT, and PEPT are influx transporters expressed on the apical membrane of intestinal epithelia mediating the uptake of drugs from intestinal lumen into the cytosol of intestinal epithelial cells [83]. The presence of transporters in the membranes of

enterocytes modulates the traditional theory of unidirectional diffusional pharmacokinetics towards vectorial pharmacokinetics [75].

1.6.1.2 Transporters Governing Metabolism

Because drug-metabolizing enzymes are located intracellularly, the uptake of drugs across the plasma membrane is a prerequisite for their subsequent metabolism in both the enterocytes and hepatocytes. The export of drugs or drug metabolites is also mediated by transporters. Therefore, influx and efflux transporters can affect intracellular metabolism by controlling the access of drug to the metabolizing enzymes in the intracellular environment and its duration [76, 84].

The interplay between transporters and CYP enzymes can increase drug metabolism by extending the intracellular residence time of drug molecules and therefore by elongated exposure to the CYP enzymes, or conversely, it can decrease drug metabolism by reducing the intracellular residence time and therefore by reduced exposure to the CYP enzymes [85]. In order to elaborate the coordinated action of transporters and enzymes, we will use the example of P-gp and CYP3A4 interplay as it is the most commonly known enzyme-transporter interplay, and a substantial overlap in the substrate specificity of P-gp and CYP3A4 exists [86].

In the small intestine, CYP3A4 and P-gp are both localized in enterocytes on the villus tip, with P-gp expressed on the apical brush border membranes of villus of enterocytes and CYP3A4 present in the endoplasmic reticulum just below the brush border membrane. Drugs absorbed into the intestinal epithelium can interact with P-gp and be actively extruded back into the intestinal lumen. If the process of diffusion and active transport occurs repeatedly at absorption site, the circulation of the drug from the lumen to the intracellular compartment will potentially prolong the intracellular residence time of the drug, and therefore, results in increased drug metabolism, in particular by CYP3A4, which constitutes the largest proportion of all CYP enzymes expressed in the small intestine [5, 6, 77, 85]. In summary, CYP3A and P-gp play complementary roles in intestinal drug metabolism, where, through repeated extrusion and re-absorption, P-gp ensures elongated exposure of the drugs to the metabolizing enzyme [77].

In the liver, CYP3A4 and P-gp are co-expressed in hepatocytes, however, their interplay results in an opposite interactive effect on drug metabolism in that organ. In the

liver, P-gp and other efflux transporters (e.g. BCRP, MRP2 and BSEP) are localized in the biliary canalicular membrane of hepatocytes and on the apical surface of epithelial cells in small biliary ducts, mediating the transport of drug molecules from inside the hepatocytes into bile [76, 80]. Therefore, their interplay reduces the exposure of the drugs to the metabolizing enzyme. As a result, the interplay between efflux transporters and CYP enzymes in the liver decreases the metabolism [76]. In summary, the overlap in the substrate specificity of CYP3A4 and P-gp and their joint presence/localization in the absorption and elimination organs lead to a coordinated function of these proteins in governing the PK of drugs [85].

Overlaps in the substrates of other transporters and CYP enzymes have also been observed and it is hypothesized that transporter-enzyme interplays are not limited to that of P-gp and CYP3A4 [84]. For instance, atorvastatin was shown to be a substrate of OATP1B1 transporters as well as CYP3A4 and CYP3A5 and, to a smaller extent, CYP2C8 enzymes [87-91]. Cyclosporine [92, 93] and repaglinide [89, 94] are other example drugs which are the dual inhibitor and substrate of CYP3A and OATP1B1, respectively.

Transporter-enzyme interplay can also exist between transporters and Phase II metabolizing enzymes. The anionic drug conjugates formed by UDP-glucuronosyltransferase, sulfotransferase, and glutathione S-transferase Phase II enzymes can subsequently be substrates to MRP2 and BCRP transporters [95, 96].

1.6.2 **CYP enzymes**

CYP enzymes are the principal enzymes involved in the biotransformation of drugs and other xenobiotics such as food constituents and excipients in drug formulations. CYP enzymes are a superfamily of heme-containing proteins and catalyze the oxidation of drugs, mainly via a monooxygenase reaction [62]. CYP enzymes are prominently concentrated in the liver. In addition to the liver, they are expressed appreciably in the small intestinal mucosa, lung, kidney, brain, olfactory mucosa, and skin. Of these tissues, the small intestine is the extrahepatic site with the most expression of CYP enzymes. Consistent with CYP enzymes protein levels, their levels of activity is also higher in the liver than the small intestine [97, 98].

CYP enzymes are located mainly in the endoplasmatic reticulum of hepatocytes in the liver and enterocytes in the small intestine [6, 99]. CYP-mediated pre-systemic metabolism can eliminate a large proportion of orally administered drugs before they reach the systemic

circulation. Drugs that are highly metabolized not only suffer from low bioavailability, but they are also more likely to be susceptible to Drug-Drug Interactions (DDI), food-drug interaction, and any other interaction with CYP substrates or inducer/inhibitor compounds, and show large inter-individual variability in their PK profiles [100]. In the skin, CYP enzymes are expressed in epidermal keratinocytes and play a major role in biotransformation of the xenobiotics that come in contact with the skin [19, 45].

CYP superfamily is subdivided into families and subfamilies based on the amino acid sequence homology. CYP enzymes in the same family have greater than 40% amino acid identity, whereas those in the same subfamily have greater than 55% identity. Each CYP enzyme is denoted by an Arabic numeral designating the family (e.g., CYP3), a letter indicating the subfamily (e.g., CYP3A), and an Arabic numeral representing the individual enzyme (e.g., CYP3A4) [101]. In humans, approximately 80% of oxidative metabolism and almost 50% of the overall elimination of commonly used drugs can be attributed to one or more of the members of the CYP1, CYP2, and CYP3 families [102, 103]. Among them, CYP3A4, CYP2C9, CYP2C19 and CYP2D6 individual members are described in more detail below as they are responsible for most of the body's hepatic and intestinal metabolism (23), and are involved in the metabolism of approximately 50% of all drugs [104].

CYP3A accounts for more than 80% of the overall amount of CYP enzymes. Like hepatic CYP3A, enteric CYP3A is localized within the epithelial cells (enterocytes) that largely compose the mucosal lining [105]. CYP3A4 enzyme is implicated in the metabolism of 45-50% of drugs [102] and was found to be the most abundant CYP enzyme in both human liver and small intestine, although the liver exhibited a 2- to 5-fold higher level of the enzyme than the intestine [63, 98, 106]. CYP3A4 represents nearly 70% of the total CYPs in human intestine and approximately 30% of the total CYP content in the liver [6, 107].

CYP2C is the second most abundant CYP subfamily. CYP2C9 accounts for approximately 14% of the overall amount of CYP enzymes and for 30% of the hepatic CYP content in the liver [108]. CYP2C9 is the second more abundant intestinal metabolic enzyme at approximately 20% of the CYP3A4 protein content [39, 98]. This enzyme is reported to be involved in the metabolism of 10% of drugs [102].

CYP2C19 is primarily expressed in the human liver and represents only 2% of the intestinal CYP content [98, 109].

CYP2D6 is expressed in the human liver at levels less than that of CYP3A4 and CYP2C9 enzymes and accounts for only 2–5% of total CYP content. The amount of CYP2D6 in the intestine is negligible (0.7% of intestinal CYP content) [98], suggesting that the intestine would have minimal contribution to the overall first-pass metabolism of CYP2D6 drug substrates.

2 Pharmacodynamics

As discussed in **Section 1**, PK describes drug concentration as a function of administered dose and time. However, the time course of drug concentration alone does not suffice to predict the time course or intensity of drug effect. The study of the relationship between drug concentration and effect is the science of pharmacodynamics (PD). More specifically, PD refers to the relationship between drug concentration at the site of action and the pharmacological effects or adverse effects [110].

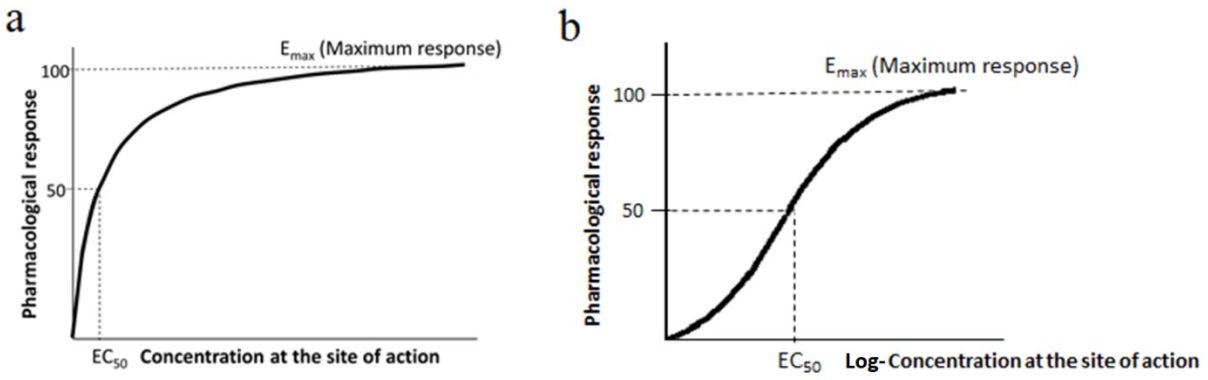
In order for drugs to elicit their pharmacological effects, they have to reach the site of action. The simple fundamental principle of clinical pharmacology is that PD is intrinsically linked to PK. It means that a relationship always exists between a desirable (efficacy) or undesirable (safety/toxicity) effects of a drug and the concentration of the drug at its site of action. For some drugs, this relationship may be more complicated, but the lack of an apparent relationship does not undermine this fundamental principle [111].

2.1 Pharmacokinetic-Pharmacodynamics Relationships

Different types of PK-PD relationships exist. In the simplest case, drug effects are directly related to plasma concentrations, but this does not necessarily mean that the relationship between drug concentration and effect is linear. In the majority of cases, drug effects do not parallel the time course of concentrations and there is often a delay in exerting the PD effect in relation to the changes in plasma concentration. This delay may be due to the time required for the drug to distribute from plasma to the site of action referred to as biophase. Biophase is the target tissue/site where the drug elicits its PD effect. However, the exact location of biophase is often unknown and theoretical. [112].

The relationship between PD effect (pharmacological response) and drug exposure is usually demonstrated in terms of an E_{max} model. In this type of PK-PD relationship, as the dose increases, the drug concentration at the site of action (exposure) increases, and the pharmacological response increases up to a maximum effect (E_{max}) at the plateau. A plot of the pharmacological response versus concentration on a linear scale *generally* results in a hyperbolic curve (**Figure 3, a**) and the plot of the same data using a logarithmic scale on the x-axis results in a sigmoid curve (**Figure 3, b**) [113, 114].

Figure 3. Plot of pharmacological response versus drug exposure(a) on linear scale, (b) on semi-logarithmic scale. Fifty percent of the maximum effect (E_{max}) is achieved at the EC_{50} concentration.



The base (simple) E_{max} model is defined by **Equation 2**, where E_{max} is the maximum possible pharmacological effect that can be produced by a drug, EC_{50} is the drug concentration which produces 50% of E_{max} achieved at the effect site, and C represents the drug concentration at the effect site.

$$E = \frac{Emax \,.\, C}{EC_{50} + C} \qquad \text{(Equation 2)}$$

It should be noted that the PK-PD relationship can also be established by plotting the pharmacological response versus dose or pharmacological response versus dose duration. In both cases the relationship can be demonstrated in terms of an E_{max} model. When plotting pharmacological response versus dose, ED_{50}; and when plotting pharmacological response versus dose duration, DD_{50} should be used instead of EC_{50}. Accordingly, ED_{50} would represent the dose which produces 50% of E_{max}, and DD_{50} would represent the dose duration at which the PD response is half-maximal. The PK-PD relationship for topical corticosteroid formulations is an example for the E_{max} model where the pharmacological response (skin blanching) is plotted versus different dose durations of the same dose. The characterization of response versus dose duration relationship for topical corticosteroid formulations is one of the focuses of this thesis (**Chapter 3**).

For many drugs with the pharmacodynamics that follow the E_{max} model, the plot of the PD response on a normal scale versus the PK measures (concentration, dose, or dose duration) on a logarithmic scale shows a linear relationship at the concentration/dose/dose duration range required to produce 20% and 80% of the maximum response. When comparing

products for equivalence, the range between 20% and 80% of the maximum response is known as the sensitive or discriminative portion of the E_{max} curve, and ED_{50} as the most sensitive point. The majority of comparisons between the Test and Ref formulations for bioequivalence (BE) purposes are done at ED_{50} point as the optimum discrimination of formulation differences can be detected [115, 116]. Above the 20%-80% range, the change in PD response may be very little despite the large change in dose or in other relevant PK measures. For instance, when administered doses are too high *(*e.g., ≥ 4 times ED_{50}*)*, the PD response would be at the plateau of the dose–response curve and increasing the dose will not lead to a further increase in PD response. If the administered dose is too low (e.g., ≤ ¼ of ED_{50}), the PD response may remain at the base of the E_{max} curve, and as a consequence therapeutic efficacy may not be obtained. Therefore, the products cannot be compared [111].

The base E_{max} model with the hyperbolic shape of the curve may not always characterize the PK-PD relationship adequately. In this case, sigmoidal E_{max} model can be used for dose-response characterization. In sigmoidal E_{max} model, a sigmoidicity constant, also known as hill coefficient, is introduced to the base E_{max} model (**Equation 3**). Hill coefficient (γ) influences the slope (steepness) of the E_{max} curve. When the values of γ is less than or equal to unity (1), the E_{max} curve has broader slope, and as γ increases the steepness of the relationship increases.

$$E = \frac{Emax \,.\, C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}} \qquad \text{(Equation 3)}$$

The PK-PD relationships other than E_{max} model are beyond the scope of this thesis. The reader is referred to available sources in the literature [111, 113, 114, 117] for further information on other types of PK-PD relationships.

3 Bioavailability and Bioequivalence

The three fundamental questions that need to be answered during the drug development process are: Does a pharmaceutical product have acceptable quality? Is it safe? And, is it effective? Answers to these questions are developed through a series of non-clinical and clinical tests during the drug development process, with study reports and summaries submitted to a regulatory agency. Based on the subsequent efficacy and safety assessment by regulatory agency and mutual agreements between an applicant and agency, a product with defined quality, as expressed in product labeling and manufacturing controls and specifications, becomes available at the market. Both bioavailability (BA) and BE focus on the processes by which the drug substance is released from a dosage form followed by absorption and distribution to the site of action. At these sites, the active ingredient and/or its active metabolites produce the safety and efficacy outcomes reflected in product labeling [118, 119].

Based on different study goals and designs, BA studies are intended either (1) to provide PK information (PK BA studies) or (2) to focus on product quality (product quality BA studies) [118, 120]. An explicit regulatory objective for a BA study is to assess the performance of the drug products used in the pivotal clinical trials that provide evidence of safety and efficacy, while that of a BE study is to compare the drug product performance between two formulations. Drug product performance for a solid oral dosage form (e.g., tablets, capsules) is the entire process of disintegration of the drug product, the subsequent release of the Active Pharmaceutical Ingredient (API), and its dissolution in an aqueous environment [3].

Bioequivalence and product quality BA studies are interrelated to each other in that both focus on drug product performance. Therefore, similar approaches such as developing a systemic exposure profile by monitoring drug concentrations in plasma or serum over time are generally employed to measure BA and demonstrate BE in support of drug applications, including Investigational New Drug Applications (INDs), New Drug Applications (NDAs), and Abbreviated New Drug Applications (ANDAs) [118, 121].

The only difference between BA and BE lies in the study goals, hence the study designs and statistical analysis of study outcome. Bioavailability studies can be employed to assess the PK and performance of a drug product with a new molecular entity (NME) during

the IND and NDA periods to provide evidence of safety and efficacy. In contrast, BE studies are primarily utilized to compare formulations. The ultimate purpose of BE studies is to provide evidence to support that the safety and efficacy are the same between two formulations. In other words, BE is a formal test that compares BA of the same drug substance from various drug products, using specified criteria and acceptance limits [119, 121].

Bioequivalence studies can be conducted in different phases of the drug development process, such as IND, NDA, ANDA, and post-approval periods. In the context of this thesis, BE studies refer to the relative BA studies for generic drug applications. Only the BE studies for ANDAs and a specific type of PK BA studies (food effect BA study) are the focus of this thesis. In the following sections, BA and BE concepts will be elaborated on. The factors that impact BA and BE outcomes will also be discussed. Due to the inter-related nature of BA and BE, many factors that influence BA can also affect BE outcomes. Whether these factors influence either or both BA and BE will be specified when these factors are discussed.

3.1 Bioavailability

Bioavailability via various routes of administration is the fraction of the unchanged drug or the active moiety that is absorbed intact from a pharmaceutical dosage form and becomes available in the site of action. The site of action could either be the systemic circulation from where systemically active drug products exert the pharmacological effects, or be the relevant target site for locally acting drug products [62, 122].

Under the US Code of Federal Regulations (CFR) Title 21 Part 314.3, BA is defined as "*the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, BA may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action*" [123].

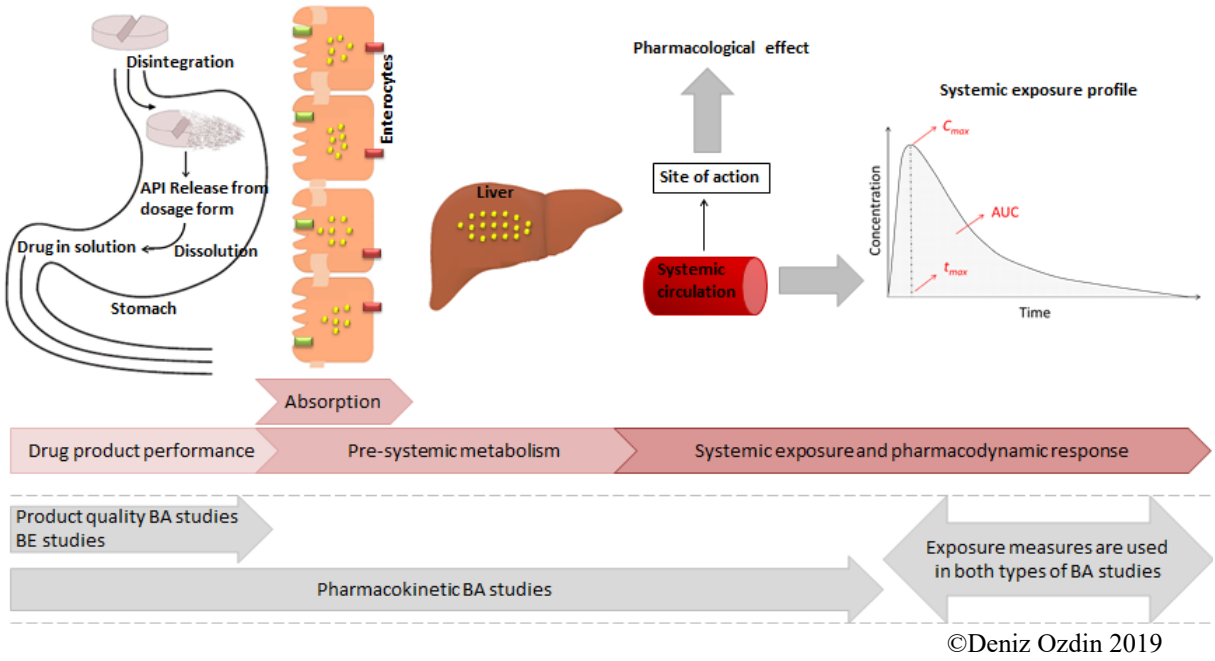
Bioavailability studies are generally the clinical PK studies within which a systemic exposure profile is obtained by measuring the concentration of drug and/or the active metabolite(s) concentrations in the systemic circulation (blood, plasma, and/or serum) over time [124]. This approach rests on an understanding that measuring the active ingredient

and/or its active metabolite(s) at the site of action is generally not possible and, furthermore, that some relationship exists between the efficacy and safety (pharmacodynamic) and concentration of active moiety and/or its metabolite(s) in the systemic circulation (pharmacokinetics). Given that there is a relationship between the drug exposure and produced pharmacological effect (both desirable and undesirable), BA studies provide evidence of safety and efficacy.

In addition to systemic exposure, BA studies may provide additional information about ADME of the drug substance and its metabolites, dose proportionality, linearity in PK, effect of various intrinsic and extrinsic factors on the PK of the drug (e.g., the influence of pre-systemic enzymes and transporters on PK), effect of nutrients on BA (i.e., food effect BA studies), and etc. These types of BA studies are called PK BA studies. Among PK BA studies, food effect BA studies are the focus of this thesis (Article#1) and will be described in more detail in **Section 3.3.1.2**.

From a drug product performance perspective, BA studies are clinical studies used to define the effect of changes in the physicochemical properties of the drug substance, the drug product (dosage form), and its manufacture process, *in vivo*. These types of BA studies are called product quality BA studies and provide evidence of the safety and efficacy of drug product. It should be noted that BE studies for ANDAs are in fact the product quality relative BA studies [118]. **0** illustrates different types of BA studies in the clinical phase of drug development process.

Figure 4. Applications of product quality and pharmacokinetic bioavailability studies in the clinical phase of drug development process. Transporters at the apical and basolateral membrane of enterocytes are represented by green and red rectangles, respectively. CYP enzymes are represented by yellow circles. API, Active Pharmaceutical Ingredient; BA, Bioavailability; BE, Bioequivalence; C_{max}, Maximum plasma concentration; t_{max}, Time to reach C_{max}; AUC, Area under the concentration-time curve.



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3.1.1 Components of Bioavailability

As mentioned in Section **1.4**, the liver and intestine are the principal organs in drug metabolism. Due to the actions of both hepatic and intestinal enzymes and transporters during the first-pass metabolism, a significant fraction of the orally administered dose can be lost before the drug ever reaches the systemic circulation. The fraction of drug extracted by these organs as drug passes through is called extraction ratio (E). The extraction ratio is used to describe the total ability of the liver and intestine to metabolize a drug in the absence of blood flow limitations, reflecting the inherent activities of the CYP and all other enzymes [71]. The extraction ratio has no units and the value of E may range from 0 (no drug removed by the organ) to 1 (100% of the drug is removed by the organ). An E of 0.35 indicates that 35% of the incoming drug concentration is removed by the organ as the drug passes through. Oral bioavailability (F_{oral}) is the product of the fraction of the drug dose absorbed into and through

the gastrointestinal membranes (F_{abs}), the fraction of the absorbed dose that passes through the gut into the hepatic portal blood unmetabolized (F_G, intestinal availability), and the fraction of the drug that passes through the liver into the systemic circulation unmetabolized (F_H, hepatic availability), as described below [67]: $F_{oral} = F_{abs} \times F_G \times F_H$.

F_{abs} may be defined as one minus the fraction of drug that is either locally degraded or not absorbed (E_A) further to the drug release from dosage form: $F_{abs} = 1 - E_A$. If a highly permeable API is orally administered as a perfectly formulated drug product, i.e., the dosage form dissolves completely and the API released is 100% absorbed, F_{abs} will be equal to 1. Gut (F_G) and hepatic (F_H) availability may be defined as one minus the extraction ratio (E) at each site:

$$F_G = 1 - E_G$$

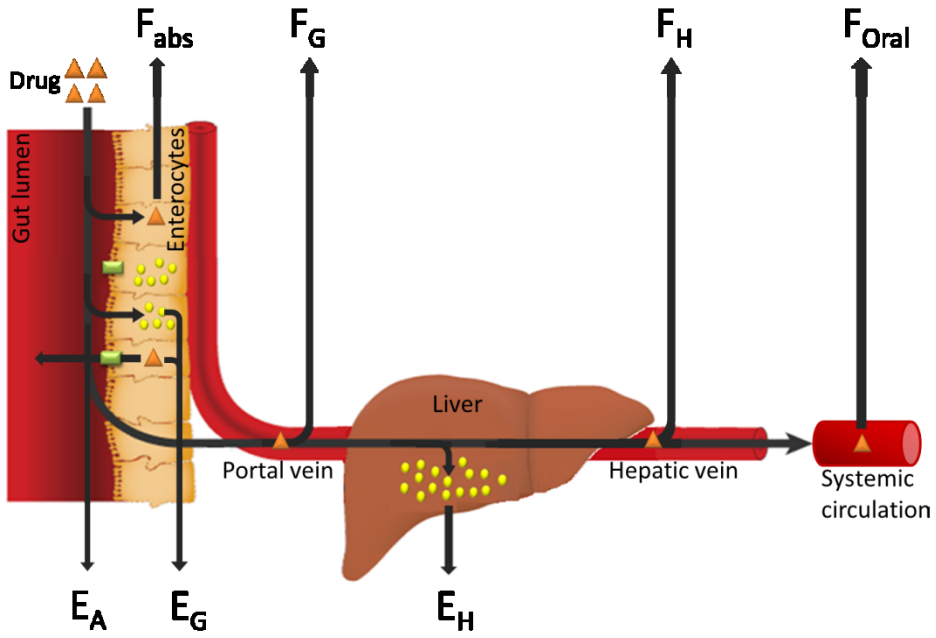
$$F_H = 1 - E_H$$

Therefore, oral bioavailability (F_{oral}) can alternatively be described as below:

$$F_{oral} = (1 - E_A) \times (1 - E_G) \times (1 - E_H)$$

Obviously, an intravenously administered drug reaches the systemic circulation without undergoing the pre-systemic metabolism and is 100% bioavailable. For an intravenous dose of a drug, bioavailability is defined as unity, and for a drug administered by other routes of administration, bioavailability is often less than unity. A drug that is highly metabolized by the liver and/or by the intestinal mucosal cells, has a high extraction ratio and demonstrates poor systemic availability (bioavailability) when given orally [122]. The loss of drug during the pre-systemic metabolism and the components of oral bioavailability are illustrated in **Figure 5**.

Figure 5. Relative contribution of formulation factors, hepatic and intestinal pre-systemic metabolism on oral bioavailability. Efflux pumps at the brush borders of enterocytes are represented by green rectangles. CYP enzymes are represented by yellow circles.



3.1.2 **Absolute and Relative Bioavailability**

As mentioned above, a drug product's bioavailability provides an estimate of the relative fraction of the administered dose that is absorbed into the systemic circulation. Determining the fraction (F) of the dose absorbed into the systemic circulation involves comparing the drug product's systemic exposure with that of a suitable reference product. For systemically available drug products, systemic exposure is represented by determining the area under the drug plasma concentration-time curve (AUC). When the systemic exposure of a drug product is compared to that of intravenous formulation absolute BA is obtained; when it is compared to any other extravascularly administered drug product relative BA is obtained. Absolute and relative BAs are described in more details below. Different types of relative BA are studied in the context of this thesis.

3.1.2.1 Absolute Bioavailability

Absolute BA compares the BA of the drug substance in the systemic circulation following extravascular administration with the BA of the same drug following IV administration. The route of extravascular administration can be oral, transdermal, subcutaneous, topical, rectal, sublingual, inhaled, intramuscular, etc. As an example, when the bioavailability of a capsule is reported to be 20%, it implies that only 20% of the administered dose of the API from capsule becomes available in the systemic circulation.

The absolute bioavailability is the dose-corrected AUC of the extravascularly administered drug product divided by the AUC of the drug product given intravenously. Thus, for an oral formulation, the absolute bioavailability is calculated as follows:

$$F_{absolute} = \frac{AUC_{oral} \,.\, Dose_{IV}}{AUC_{IV} \,.\, Dose_{oral}} \qquad \text{(Equation 4)}$$

where $F_{absolute}$ is the fraction of the dose available in the systemic circulation, expressed as a percentage by multiplying $F_{absolute}$ x 100. AUC_{oral} and AUC_{IV} are the AUCs following oral and IV administrations, respectively. $Dose_{oral}$ and $Dose_{IV}$ are the doses administered orally and intravenously, respectively.

Conducting a BA study with an IV reference enables the assessment of the impact of route of administration on BA [124].

3.1.2.2 Relative Bioavailability

Relative BA compares the BA of the drug substance from a designated formulation (generally referred to as Test formulation) with the BA of the same drug from a reference (Ref) formulation. Reference formulation could be different dosage forms of the same drug substance administered via the same route. Reference formulation can also be the same dosage form of the drug substance, but formulated as a different drug product. For instance, for an oral tablet, a Ref could be an oral solution, suspension, or capsule. Similarly, for a to-be-marketed generic oral tablet a Ref could be an existing tablet which is already on the market. Relative bioavailability is calculated as follows:

$$F_{rel} = \frac{AUC_{Test} \,.\, Dose_{Ref}}{AUC_{Ref} \,.\, Dose_{Test}} \qquad \text{(Equation 5)}$$

where F_{rel} is the fraction of the dose which becomes available in the systemic circulation from Test formulation relative to that from Ref, expressed as a percentage. For

instance, F_{rel} = 114% implies that the drug was 14 times more available in systemic circulation from Test than from Ref formulation. AUC_{Test} and AUC_{Ref} are the AUCs obtained from Test and Ref formulations, respectively. $Dose_{Test}$ and $Dose_{Ref}$ are the doses of drug substance in Test and Ref formulations, respectively. In general, a new formulation, or a formulation produced by a new method of manufacture, or a new strength is considered as the Test and the prior formulation, or the formulation produced by prior method of manufacture, or prior strength as the reference formulation. Depending on the type of BA study, Test and Ref can also refer to the Test and Ref treatments, respectively, rather than Test and Ref formulations. This will be better comprehended in the following subsections where the applications of relative BA studies are elaborated.

3.1.3 Relative Bioavailability Studies

Relative BA studies are commonly conducted for regulatory submissions. Relative BA studies have a wide area of application and can be performed in different phases of drug development process. Relative BA studies could be conducted for different objectives. For instance, they could be conducted for NDA purposes. Under CFR title 21, 320.25(d)(2) and (3), an oral BA of a new drug should be assessed by comparing its BA to an oral solution or suspension containing the same quantity of the active ingredient to assess the impact of formulation on BA [125]. Drug-Drug Interaction (DDI) and food effect BA studies (**Section 3.3.1.2)** are other types of relative BA studies used in NDAs [126, 127].

Relative BA studies can also be conducted for ANDA purposes where sponsors seek for the approval of generic drug(s) [121, 127, 128]. The relative BA studies performed for ANDA are commonly known as BE studies (comparative BA studies) and are the main focus of this thesis. Bioequivalence studies will be discussed in **Section 3.2**.

3.1.4 Measures of Bioavailability

Bioavailability measures are frequently expressed in terms of systemic exposure measures, and provide information about the PK of a drug. The area under the plasma or serum concentration-time curve (AUC) is considered the most reliable measure of a drug's bioavailability, as it is directly proportional to the total amount of unchanged drug that reaches the systemic circulation. The units for AUC are concentration x time (e.g., $\mu g.h.ml^{-1}$) [120, 129].

The statutory definition of BA is expressed in terms of rate and extent of absorption [121, 130, 131], suggesting the use of PK measures which are reflective of these parameters. Differently said, regulatory assessment of BA frequently relies on measures of rate and extent of absorption, especially when BA studies are performed for BE purposes (i.e. comparative BA studies).

Finding clinically relevant metrics for the rate and extent of absorption has historically been a challenge for BA and BE assessment. The extent of absorption is well estimated by the AUC, but measures for rate of absorption in BA studies have been more problematic [132-136]. The rate of absorption is difficult to measure and has little clinical relevance. Peak plasma or serum concentration (C_{max}) and time to peak concentration (t_{max}) have commonly been used as the metrics for the rate of absorption for decades. However these parameters are indirect measures of absorption rate, as 'rate' is defined by a rate constant (k_a) with units of $time^{-1}$ (e.g., min^{-1}) or $mass.time^{-1}$ (e.g., $mg.min^{-1}$) (depending on the PK of drug) while C_{max} is expressed in concentration units (e.g., $mg.ml^{-1}$, $ng.ml^{-1}$) and t_{max} in units of time (e.g., h, min) [136, 137].

In 2000, recognizing the fact that systemic exposure is the key for drug safety and efficacy, and that the rate and extent of absorption lack clinical relevance, FDA recommended a change in focus from the *measures of absorption* to *measures of systemic exposure* in BA and BE studies.

According to the FDA's Guidance to Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations [121], the PK measures for BA and BE assessment are defined relative to the total, peak, and early portions of the blood/plasma/serum profile. These measures are the same as those formerly referred to as the metrics for the rate and extent of absorption. More specifically, peak exposure is expressed by C_{max}; and early and total exposures by AUC.

The measures of exposure are directly obtained from the plasma or serum concentration-time profile without interpolation or model-fitting. The C_{max} is assessed by measuring the maximum plasma or serum drug concentration (C_{max}) from the plasma or serum profiles and the AUC is calculated using either linear trapezoidal method or mixed linear-log trapezoidal method. The linear trapezoidal method uses linear interpolation between concentration points to calculate the AUC for the entire time interval. This method can

overestimate the AUC during the elimination phase. Mixed linear-log trapezoidal method, also called "linear-up log-down" method, on the other hand, uses linear interpolation when concentrations are increasing (in the portion of the concentration-time profile before t_{max}), and logarithmic interpolation when concentrations are decreasing (in the portion of the concentration-time profile after t_{max}). Unlike the linear trapezoidal method, mixed linear-log trapezoidal can accurately estimate mono-exponential decline of drug concentrations during the elimination phase.

The trapezoidal methods used for the AUC calculations may differ for generic submissions to different regulatory agencies. While the Office of Generic Drugs (OGD) of the FDA requires the linear trapezoidal method, HC accepts the mixed linear-log trapezoidal method as an alternative. The formula in **Equation 6** is used to calculate AUC:

$$\text{AUC} = \sum_{i=0}^{n-1} \frac{C_i + C_{i+1}}{2} \text{ x } (t_{i+1} - t_i) \qquad \text{(Equation 6)}$$

where C and t represent the measured concentration and relevant time point, respectively.

The early exposure is measured by a partial AUC from time zero to the population median of t_{max} of the Ref ($pAUC_{Ref,tmax}$). Partial AUC can also be truncated at any earlier time after drug administration, depending on the PK/PD relationship of the drug product under examination [121].

Peak exposure metric (C_{max}) and early exposure (pAUC) are frequently considered as the measure of both the rate and the extent of exposure in regulatory settings [137, 138]. Total exposure is expressed either by AUC_{0-t} or by $AUC_{0-\infty}$.

The AUC_{0-t} is the AUC from time zero after a single dose drug administration to time t, where t is the last time point with detectable concentration. The $AUC_{0-\infty}$ is the AUC from time zero after a single dose drug administration extrapolated to time infinity and is calculated by **Equation 7**:

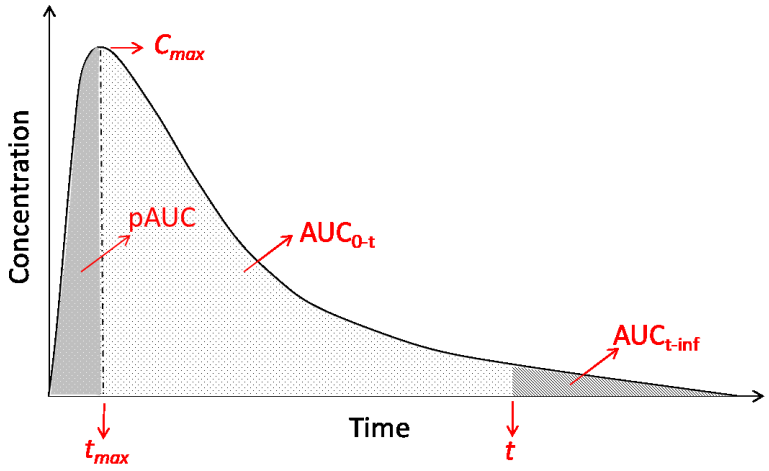
$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_t}{\lambda_z} \qquad \text{(Equation 7)}$$

where C_t is the last quantifiable drug concentration and λ_z is the terminal or elimination rate constant. The last quantifiable concentration either is determined directly from the observed plasma or serum drug concentration-time profile (as per the US FDA)

[121], or is estimated from fitting the observed concentration-time profile (as per Health Canada) [130].

The PK metrics for total exposure (AUC_{0-t} or $AUC_{0-\infty}$) are used as measures of the extent of exposure. **0** illustrates the PK metrics which are used to represent the rate and extent of absorption or exposure. These metrics could be compared between formulations to assess BE.

Figure 6. Hypothetical plasma concentration–time curve after a single oral dose administration and derived PK metrics. The shaded portion represents the area under the curve (AUC). C_{max}, Maximum observed concentration; t_{max}, Time to reach C_{max}; t, Last time point with a measurable concentration; pAUC, Partial AUC; AUC_{0-t}, AUC from time 0 to time "t"; AUC_{t-inf}, AUC from the time "t" to infinity.



For most approved drugs, a relationship may be found between the plasma concentrations and the PD effect. Therefore, systemic exposure has a direct impact on efficacy and safety outcomes. C_{max} provides indications on whether the drug is sufficiently systemically absorbed to provide a pharmacodynamic response, depending on established therapeutic range. In addition, it can provide warning of possibly toxic levels of drug. Therefore, C_{max} can be considered as a surrogate measure of both safety and efficacy of the drug product. Partial AUC is also considered as a surrogate measure of both safety and efficacy. For instance, early exposure ($pAUC_{Ref,tmax}$) for immediate-release (IR) dosage forms is considered as a surrogate of efficacy when a rapid onset of action is required [121]. Partial AUC for modified-release (MR) dosage forms is considered as a surrogate of safety when a

slow input rate of the drug is required for safety concerns (e.g. to ensure there is no dose dumping) [139]. Given that the systemic exposure of a drug is often well correlated with its efficacy, the measure of total exposure (AUC_{0-t} or $AUC_{0-\infty}$) is used as a surrogate measure of efficacy [120, 129]. Common BA measures are summarized in **Table 1**.

Table 1 Common bioavailability measures and their implementations in safety and efficacy

PK metrics	Former attribution	Current attribution	Surrogate of…
C_{max}	Rate and extent of absorption	Peak exposure	Safety and efficacy
pAUC	-	Early exposure	Safety and efficacy
AUC_{0-t}	Extent of absorption	Total exposure (Extent of exposure)	Efficacy
$AUC_{0-\infty}$	Extent of absorption	Total exposure (Extent of exposure)	Efficacy

3.2 Bioequivalence

In its simplest form, bioequivalence can be defined as the regulatory science of establishing that two drug products are equivalent in terms of clinical safety and efficacy when administered at the same molar dose under similar conditions, and therefore, they can be substitutable in a given patient, regardless of whether the two products are exactly in the same or comparable dosage forms [138].

The US FDA's regulations define BE as "*the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study, in either a single dose or multiple doses. Where there is an intentional difference in rate, pharmaceutical equivalents or alternatives may be considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of action. This applies only if the difference in the rate is intentional and reflected in the proposed labeling, is not essential to the attainment of effective drug concentrations on chronic use, and is considered of no clinical relevance*" [123].

Health Canada (HC) defines BE as "*a high degree of similarity in the bioavailabilities of two pharmaceutical products (of the same galenic form) from the same molar dose that is unlikely to produce clinically relevant differences in therapeutic effects, or adverse effects, or both*" [130].

As per European Medicines Agency (EMA), two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effect, with respect to both efficacy and safety, will be essentially the same [140].

Regardless of the subtle differences in statutory definitions of BE, the study of BE serves one ultimate goal, which is the assurance of the same (equivalent) clinical safety and efficacy between two drug products. As per the US FDA, when two pharmaceutically equivalent products are shown to be bioequivalent, the two products are expected to be therapeutically equivalent in that they produce the same safety and efficacy profiles when administered under the conditions listed in the product labeling [141].

Drug products are considered pharmaceutical equivalents if they are in identical dosage forms and route(s) of administration, they contain identical amounts of the identical active drug ingredient (i.e., the same salt or ester of the same therapeutic moiety), and meet the same compendial or other applicable standards (i.e., strength, quality, purity, identity, content uniformity, disintegration times, and/or dissolution rates); but do not necessarily contain the same inactive ingredients (excipients) [123].

The most crucial concept in the understanding of BE is that the sole objective is to measure and compare formulation performance between two or more pharmaceutically equivalent drug products. Formulation performance is one step before the absorption and involves disintegration of the drug product, subsequent release of the drug substance, and dissolution of the drug substance in an aqueous environment. Once the drug substance is dissolved, it will be available to be absorbed across intestinal cell membranes into the systemic circulation. All other steps following *in vivo* drug substance absorption are subject- or patient-determined processes and are not directly related to formulation factors [141, 142]. In the current practice of BE, only the steps prior to absorption are the focus of the study, and any other step after absorption is considered as not of importance.

3.2.1 Bioequivalence in Drug Development Process

Bioequivalence studies may be required in different stages of the drug development and approval process such as the periods before the Investigational New Drug Applications (INDs), New Drug Applications (NDAs), Abbreviated New Drug Applications (ANDAs), and even in post-approval period [124, 128, 143] (**Figure 7**). For a better perspective of where BE studies fit in the drug development and approval process, different stages of the process are briefly presented in the following subsection.

3.2.1.1 Drug Development and Approval Process

The ultimate goal of the drug development process is to effectively assure the safety and efficacy of drug products that receive regulatory approval for marketing. A large number of preclinical and clinical studies are conducted to ensure the safety and efficacy of marketed drug products. In the context of this thesis, we classify the drug development process based upon the approval periods.

IND period

The period before IND refers to the drug development stage where a New Chemical Entity (NCE) is discovered and large numbers of *in vitro* and animal studies are conducted to investigate the biological activity and safety of the new compound to make sure that it will be potentially effective and reasonably safe to administer to humans. The IND application includes all toxicity and safety data from preclinical animal studies [114, 144].

NDA period

After discovery and preclinical testing, the NCE enters the clinical trials phase. The clinical trials involve the administration of a drug product to human subjects and consist of Phase I, Phase II and Phase III studies.

The first series of experiments in humans are conducted during Phase I. Clinical studies in Phase I are generally conducted in a small number of healthy volunteers (20-80), with the exception of some drugs (such as chemotherapeutic agents) which are administered to patients. Phase I studies aim to characterize the PK characteristic of the NCE and assess its safety and tolerability in human. Phase I studies may also include DDI studies, studies to assess the effect of food on PK (food effect BA studies), special population studies such as patients with renal or hepatic impairments [145].

Phase II studies shift the focus from safety to efficacy. Clinical studies in Phase II are conducted in patients suffering from the target illness with the goal to investigate whether the drug exhibits anticipated therapeutic benefit in the intended population and to establish the PK-PD relationship for the drug. In comparison to Phase I, a larger number of subjects (100–300 patients) are enrolled in Phase II.

Phase III studies are the longest and most comprehensive clinical studies for the evaluation of new drugs, conducted in significantly larger numbers of patients (1000–3000) with the target illness. Phase III studies aim to confirm the efficacy and safety of the new drug that was demonstrated in Phase II. The drug product that is tested at this stage is generally the one that the company is planning to market in terms of composition, formulation, and strength. Phase III studies are considered pivotal trials. Typically two adequate and well-controlled pivotal studies are required, by regulatory authorities, where the new drug may be compared to an already marketed drug or a placebo [146].

Once the Phase III trials have been completed, all preclinical and clinical data for the new drug products with an NCE that previously has not been approved by the regulatory agency (e.g., the US FDA) are filed as an NDA. This type of application is also known as a "stand-alone" NDA. The analogous application to NDA as per HC is New Drug Submission (NDS). The products approved under this regulatory pathway are commonly called brand name products (or innovator drugs) and they are usually used as reference drug products upon which applicants rely in seeking approval for other types of applications such as ANDA for generic products.

ANDA period

The drug development and approval process for an innovator drug product is costly and long. The entire process from discovery stage to the marketing of an innovator drug usually requires 10-15 years. The costly and exhaustive nature of preclinical and clinical studies for innovator drugs raised a need for a simpler and shorter process for the approval of duplicate new products which are identical in API and dosage form with the drug products previously approved as safe and effective [146, 147]. The identical copies of innovator drug products are referred to as generic drugs. In 1984, The Drug Price Competition and Patent Term Restoration Act, commonly known as the Hatch-Waxman Amendments, amended the US Federal Food, Drug, and Cosmetic Act and established the present-day ANDA approval

process for generic drugs. With the passage of the Hatch-Waxman Amendments, the majority of the costly animal and human studies required for innovator drugs do not need to be repeated for generic copies based on the premise that the safety and efficacy of the innovator counterpart have already been established through animal and clinical Phase I-III studies. For generic drugs, in most situations one or two BE studies suffice to replace the animal and clinical Phase I-III studies [148, 149].

Note: The analogous application to ANDA as per HC is Abbreviated New Drug Submission (ANDS).

Post-approval period

Certain changes in components and composition, chemical synthesis, analytical methods, manufacturing site, manufacturing process, equipment, batch size, or labeling can be made post-approval, either after the approval of an innovator drug or a generic drug. Any of these changes to the marketed drug product are often termed Scale-Up and Post-Approval Change (SUPAC). Depending on the magnitude of the change(s) in components, composition and/or method of manufacture, and the impact the change may have on the drug product performance and its BA, additional *in vitro* or *in vivo* studies may be required by the regulatory agency to show that the change will not affect the identity, strength, purity, quality, BA, safety and efficacy of the approved drug product and to prove the BE between the pre- and post-change innovator product (NDA) or post-change generic product and reference listed drug (ANDA) [143, 150]. Different levels of SUPAC are beyond the scope of this thesis. For details of different levels of SUPAC as per the US FDA and HC, the reader is referred to guidance documents [143, 150-152].

Bioequivalence studies in IND and NDA periods

During new drug (innovator drugs) development, BE studies verify the clinical equivalence between different formulations and sometimes between different strengths. For instance, BE studies may be required to compare (a) early and late clinical trial formulations; (b) formulations used in clinical trials and stability studies, if different; (c) clinical trial formulations and to-be-marketed drug products, if different; and (d) product strength equivalence, as appropriate. The initial safety and clinical efficacy studies during new drug development usually use a simple formulation (e.g., a hard gelatin capsule containing only the API diluted with lactose) which is different than the to-be-marketed drug product. Therefore,

pharmaceutical manufacturers must demonstrate an equivalent drug product performance between these two formulations. Equivalent drug product performance is generally demonstrated by an *in vivo* BE study in Healthy Volunteers (HV), and under certain conditions by *in vitro* comparative drug release or dissolution profiles [121, 124].

Bioequivalence studies in ANDA period

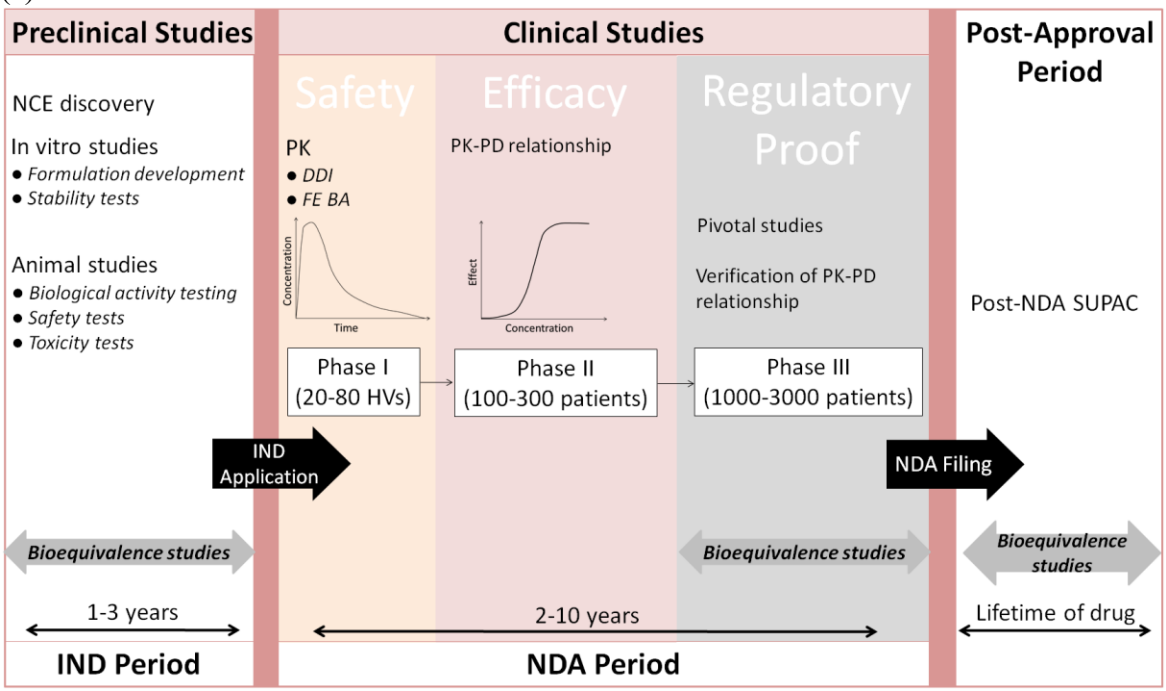
The major implementation of BE studies is for ANDAs, where the performance of a to-be-marketed generic drug product is compared to an already marketed drug product (reference). Reference, also referred to as Reference Listed Drug (RLD) product, is usually an innovator drug product but sometimes another generic product if the innovator product is withdrawn. For a generic drug, BE studies replace the animal, clinical safety and efficacy, and BA studies that were previously conducted for the reference counterpart. Bioequivalence studies for generic drugs confirm the clinical equivalence in terms of safety and efficacy between the generic and reference products. The BE between the comparator generic and Ref products is often established by *in vivo* PK BE studies in HVs under both fasted and fed states. Other types of BE studies as alternative for documentation of BE between generic and RLD products are described in more detail in **Section 3.3**.

Bioequivalence studies in post-approval period

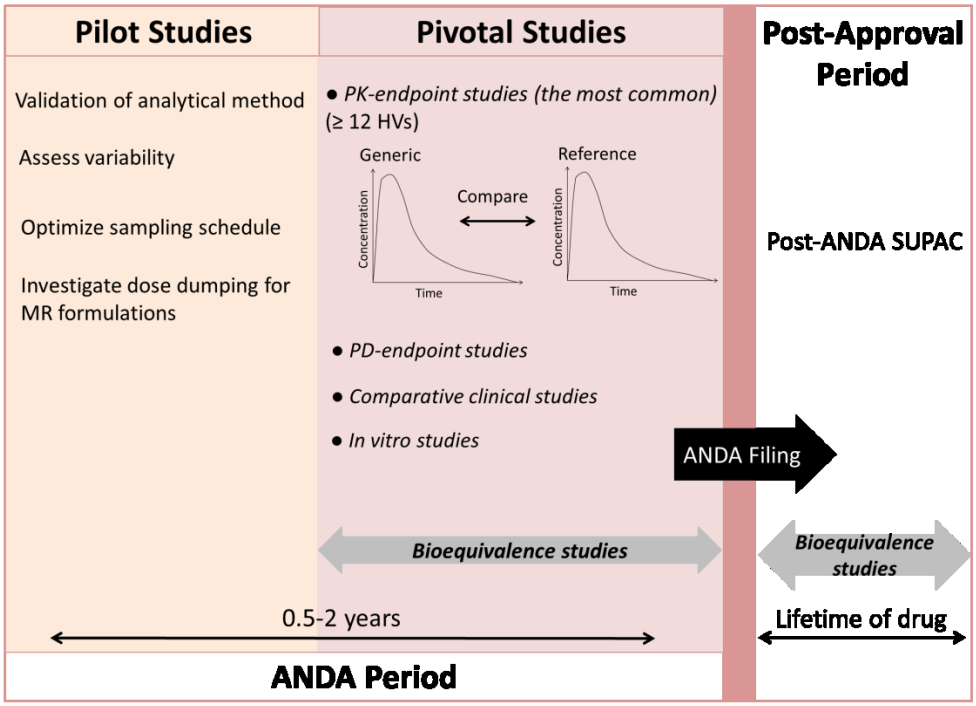
Depending on the type of formulation (i.e., IR or MR) and SUPAC level, different types of evidence may be required to ensure that formulation performance did not change and is the same for the drug product manufactured before and after the SUPAC [143, 150]. For instance, if the total additive effect of excipient changes is more than an absolute total of 5% (w/w) of the dosage form weight for an IR solid oral formulation (SUPAC Level II), *in vitro* dissolution tests demonstrating similar dissolution profiles of the pre-change and post-change formulations would be required by the regulatory agency to decide that the changes are approvable [150]. Nevertheless, if the total additive effect of release controlling excipients is greater than 10% (w/w) of total release controlling excipient content in a MR solid oral dosage form (SUPAC Level III), a single-dose BE study in HV would be required, unless a predictive *In Vitro–In Vivo* Correlation (IVIVC) is available [143].

Figure 7. Schematic of the drug development and approval process (a) for innovator drug, (b) for generic drug. The figure exemplifies the process pertinent to the US FDA. BA, Bioavailability; DDI, Drug-Drug Interaction; FE, Food Effect; HV, Healthy volunteer; NCE, New Chemical Entity; PD, Pharmacodynamic; PK, Pharmacokinetic, SUPAC, Scale-Up and Post-Approval Change; MR, Modified-release.

(a)



(b)



3.3 Different Approaches to Document Bioavailability and Bioequivalence

Bioavailability can be measured or bioequivalence can be demonstrated by several *in vivo* and *in vitro* methods. The most accurate, sensitive, and reproducible approach must be selected depending upon the objectives of the study, the ability to analyze the drug (and metabolites) in biological fluids, the PK and PD of the drug substance, the route of drug administration, and pharmaceutical dosage form. The following *in vivo* and *in vitro* approaches, in descending order of preference, are used to measure BA and assess BE: (a) *in vivo* measurement of active moiety or moieties in biological fluid (PK-endpoint studies); (b) *in vivo* PD effect studies (PD-endpoint studies); (c) well-controlled clinical trials; and (d) *in vitro* comparison studies [153].

The comparison of PK profiles *in vivo* (PK-endpoint study) is the most widely used method to demonstrate BE where the BA of a test drug product (generic) is compared with its corresponding reference. In most situations, one or two PK-endpoint studies in HVs will suffice for the demonstration of BE. However, sometimes a PK-endpoint study is only one component of the proof for BE, and alternative approaches, in addition to PK-endpoint study, may be required for establishing BE. When PK-endpoint studies are not applicable, PD, clinical, and/or *in vitro* studies may be used for BE assessment.

Pharmacokinetic-endpoint studies are mostly used for BE demonstration of systemically acting drug products and are conducted in healthy volunteers while PD and clinical studies are generally employed for locally acting drug products that are not systemically absorbed, such as those administered topically or those that act locally within the GI tract and are conducted in patient population [121, 153-155]. In the following subsections, these approaches are described in more detail with more emphasis on PK- and PD-endpoint studies.

3.3.1 PK-Endpoint Studies

The statutory definition of BA and BE [123, 130, 140], expressed in terms of rate and extent of absorption of the active ingredient or moiety, emphasizes the use of PK measures in an accessible biological matrix such as whole blood, plasma, serum or urine (when the drug

cannot be quantified in serum/plasma/blood and when it is excreted mainly in urine) for the assessment of BA and BE.

Implementation of PK approach for BA and BE assessment rests on an understanding that measuring the active moiety or active ingredient at the site of action (biophase) is generally not possible and on the assumptions that (a) the therapeutic effect of a drug product is a function of systemic exposure and (b) concentrations of a drug in the systemic circulation (whole blood, plasma, or serum) are in equilibrium with concentrations in biophase (**Section 2.1**).

The PK approach is particularly applicable to all systemically active drugs dosage forms which are intended to deliver the active moiety to the bloodstream for systemic effect such as oral drug products, or to non-orally administered drug products in which reliance on systemic exposure measures is suitable for documenting BA and BE (e.g., transdermal delivery systems and certain rectal and nasal drug products), or to locally effective dosage forms from which the drug will reach the systemic circulation at the same time or before reaching site of activity [153, 156].

3.3.1.1 Pharmacokinetic Measures

Bioavailability and BE assessment based on the PK approach relies on the PK measures that can be obtained either from non-compartmental (NCPT) or compartmental (CPT) analysis of the PK profile of drug substance. The PK profile is usually generated by plotting the concentrations of active ingredient or active moiety, and when appropriate its active metabolite(s), in an accessible biological fluid (e.g., whole blood, serum, plasma, urine) as a function of time in HVs. Depending on NCPT or CPT analysis of PK profile, different PK measures can be derived and used for BA and BE assessment.

The $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and C_{max} are the principal and minimum PK metrics upon which the BE of Test (e.g., generic) versus Ref is established by conducting the statistical procedure of Two One-Sided Tests (**Section 3.5**). The information on t_{max} is also provided in generic submissions, but it is not the official metric for BE assessment and statistical procedures are not used to compare the t_{max} of Test and Ref. Nevertheless, the regulatory agencies routinely examine t_{max} in BE studies as supportive data to verify that the Test and Ref have the same rate of exposure [130, 157, 158]. It is worth to note that the BE demonstration of generics to their reference products majorly relies on the C_{max} and $AUC_{0\text{-}t}$. If the equivalence of a generic

to its Ref product is only demonstrated in terms of C_{max} and AUC_{0-t}, but not $AUC_{0-\infty}$, the generic and its Ref product may be considered bioequivalent by regulatory agencies. This is due to the differences that may exist in the calculation of extrapolated portion of $AUC_{0-\infty}$ as described below:

1. The $AUC_{0-\infty}$ is calculated by summing up AUC_{0-t} and C_t/λ_z where C_t is the last quantifiable concentration, and λ_z is the terminal rate constant. The AUC_{0-t} must account for a minimum of 88% of $AUC_{0-\infty}$ as per the US FDA [121], while it must account for a minimum of 80% $AUC_{0-\infty}$ as per HC and EMA [130, 140, 159].

2. The extrapolated portion of $AUC_{0-\infty}$ (C_t/λ_z) may be calculated differently for submissions to different regulatory agencies. In the extrapolated portion of $AUC_{0-\infty}$, λ_z is calculated by ln-transformation of the terminal portion of the concentration-time profile and estimation of the slope of this transformed data using linear regression [128, 130]. Nevertheless, the approaches required by different regulatory agencies for the assessments of the last quantifiable concentration, C_t, are not the same. While US FDA requires C_t to be directly obtained from the observed concentration-time profile [121], HC defines C_t as the estimated concentration at the Lowest Quantifiable Concentration Time (LQCT), which requires fitting the observed concentration-time profile [130].

Depending on the PK characteristics of the API (e.g. half-life), desired pharmacological effect (e.g. rapid onset of an analgesic effect), release profile of the dosage form (immediate versus modified release), dosing regimen (single versus multiple dosing), pharmaceutical dosage form, and route of administration, different or additional PK metrics such as partial AUC (pAUC), AUC_{0-tau}, and K_a may be required for BE assessment. For instance, when a better control of drug absorption into the systemic circulation is required to achieve a rapid onset of action such as analgesic effect for an orally administered IR drug product, the FDA and EMA Guidances [121, 159] recommend the use of $pAUC_{Ref,tmax}$ or the pAUC truncated at any early time after drug administration depending on the PK/PD relationship of the drug product as an early exposure measure [137]. Partial AUC can also be used for any segment of the concentration-time profile with appropriate truncation time point for a better characterization of PK profile. This is exemplified by multiphasic MR drug products that combine both immediate- and extended-release components in one formulation to achieve a quick onset of action as well as a sustained response afterward [160-162]. In the

following subsections, the two approaches for PK analysis of data and the relevant PK measures are described.

3.3.1.1.1 Non-compartmental analysis

At the present time, regulatory assessment of BA and BE frequently relies on the PK metrics which are derived from NCPT analysis. Non-compartmental analysis has proven to be reliable and robust for BE assessment of most products. In this method, the PK profile is generated by plotting the observed concentrations over the course of time. From concentration-time profiles, conventional measures of systemic exposure including peak exposure (C_{max}), early exposure ($pAUC_{Ref,tmax}$), and total exposure ($AUC_{0\text{-}t}$ or $AUC_{0\text{-}\infty}$) and are directly derived or calculated (**Section 3.1.4**). In addition to C_{max} and AUC, the time required to reach C_{max} after drug administration, t_{max}, can directly be derived from concentration-time profile (**Figure 6**) [3].

Non-compartmental analysis is considered to be the gold standard approach for deriving PK metrics for BE assessment. The simplicity and robustness of NCPT analysis are the main reasons for which NCPT analysis has become a standard method for characterizing the PK of drugs. The calculation of PK metrics with NCPT analysis is straightforward and simple [163]. In addition, NCPT approach is free from the subjectivity associated with CPT analyses that could lead to a different results or different interpretation of the same data, due to the factors such as using different models, different fitting methods or algorithms, and different assumptions [164-168]. The results of the NCPT PK analysis are the same regardless of the software that is used [138].

While there are many advantages to using NCPT approach, it has certain limitations. In order to perform NCPT analysis in a robust manner, it is necessary to meet the following conditions (i) a sufficient number of samples (e.g. plasma concentrations) at appropriate time points must be collected (i.e., ≥12 per subject after a single dose administration) [121, 130, 169], (ii) the duration of sampling should cover plasma concentration-time profile adequately (i.e., $\frac{AUC_{0-t}}{AUC_{0-\infty}} \geq 80\%$) [170] (iii) the drug should display linear PK; in other words, exposure increases in proportion with increasing dose, and PK metrics are stable over the course of time, (iv) the drug is eliminated from the same compartment as sampling is done (e.g., the plasma), and (v) the PK is assessed either after a single-dose or at steady-state [169, 171, 172].

3.3.1.1.2 Compartmental analysis

Compartmental analysis, also known as model-based approach, is an alternative approach for characterization of PK profile of the drugs that violate the assumptions required for a robust NCPT analysis (**Section 3.3.1.1.1).** The PK of drugs with complex or non-linear PK, or the drugs which are not eliminated from the sampling compartment can be robustly characterized with CPT analysis. Robustness of CPT analysis to the violation of assumptions required for NCPT approach is the major advantage of this approach for its application in BE assessment. Other advantages of compartmental analysis will be further discussed in **Section 5**.

As explained earlier, C_{max} derived by NCPT analysis is not a pure measure of rate. It rather represents a combination of both rate and extent of exposure. In fact, the absence of a specific metric for rate has been considered as one of the weaknesses of the NCPT analysis. Compartmental analysis, on the other hand, enables us to estimate absorption rate constant (K_a) – with units of $time^{-1}$ or $mass.time^{-1}$ – directly by fitting the PK data [173-176].

Unlike C_{max}, K_a is not a function of various processes such as absorption, distribution, and elimination, and is purely representative of the rate of absorption. The caveat with K_a is that it may be too discriminative for BE assessment. A difference of 20%-25% in K_a usually causes negligible changes in the plasma or serum concentration-time profile, C_{max}, and AUC, suggesting that large differences purely in K_a may not always be clinically relevant. Furthermore, inherent variability associated with K_a is another factor that can make concluding equivalence even more difficult, even if the two products are in fact equivalent. Therefore, although similar criteria for declaring BE are presently used for the PK metrics from NCPT and CPT approaches, further research is warranted to investigate whether different criteria may be more appropriate (for example, a wider confidence interval for K_a comparisons between Test and Ref) [136, 138].

The use of K_a for BE assessment is advantageous, especially for locally acting drug products such as topical dermatological formulations, which do not produce measurable concentrations in an accessible biological fluid. For these products, there is no established link between the concentration of the drug in the systemic circulation and the therapeutic effect even when they produce systemic measurable concentrations. For this reason, equivalence studies with clinical endpoints are the only available approach for BE documentation for most

of locally acting drug products [12, 177-179]. The use of CPT analysis could circumvent the need for large and indiscriminative equivalence studies with clinical endpoints. By Fitting the PK data of Test and Ref products, obtained from the measurements at the site of action (e.g., skin for topical dermatological formulations), to a suitable CPT model, the rate (K_a) and extent of absorption (or input) at the local site of action can be estimated for each products. The BA factor F from CPT analysis is analogous to AUC from NCPT approach and represents the fraction of applied dose which reaches the systemic circulation or site of action. The estimated K_a and F parameters can then be compared for BE assessment.

In the implementation of CPT analysis in BE assessment, the data of Test and Ref are fitted simultaneously. In this way, PK parameters which are independent of formulation factors (those describing the PK of the API such as volumes of distribution, clearances, etc.) would be identical for both formulations, while formulation-related PK parameters such as absorption rate constants ($K_{a(Test)}$ and $K_{a(Ref)}$) or relative BA (F_{rel}) would be different. The formulation-related PK parameters can then be compared for BE assessment in a manner analogous to the comparison of AUC and C_{max} derived from NCPT analysis [138, 173, 180]. It should be mentioned that CPT analysis not only enables the characterization of K_a, but also estimates C_{max}, pAUC, and $AUC_{0-\infty}$ parameters which are conventionally derived by NCPT analysis. **Table 2** summarizes potential PK metrics derived from NCPT and CPT analysis and their application in BE assessment.

Table 2 PK metrics derived from non-compartmental and compartmental analyses and their application in BE assessment

Absorption/Exposure	NCPT	CPT	Application in BE assessment
Rate	-	K_a	Systemically acting oral drug products (IR and MR), locally acting drug products
Extent	AUC_{0-t} $AUC_{0-\infty}$ (Trapezoidal method)	AUC_{0-t} $AUC_{0-\infty}$ (Predicted)	Systemically acting oral drug products (IR and MR)
	-	F	Systemically acting oral drug products (IR and MR), locally acting drug products
Mixed Rate and Extent	C_{max} (Observed)	C_{max} (Predicted)	Systemically acting oral drug products (IR and MR)
	$pAUC_{Ref,tmax}$ (Observed)	$pAUC_{Ref,tmax}$ (Predicted)	Systemically acting oral drug products (IR and MR)

3.3.1.2 PK-Endpoint Studies under Fed Conditions

Altered physiological conditions can influence drug BA from formulation and BE outcomes. The presence of food in the GI tract can and does alter physiological conditions by different mechanisms such as, but not limited to, causing delay in gastric emptying, stimulation of bile flow, causing an increase in splanchnic blood flow, changing the GI pH, altering the solubility and/or permeability of a drug substance, physical or chemical interact with a dosage form or a drug substance, and inducing or inhibition of transporters and metabolizing enzymes [127, 181]. As a result, formulations may perform differently under fasted and fed conditions, and BA of a drug may not be the same under fasted and fed conditions. Similarly, two formulations assessed to be bioequivalent under fasted conditions may not necessarily be bioequivalent under Fed conditions. For this reason, the US FDA and other regulatory agencies require the BA studies (for NDA/NDS) and BE studies (for ANDA/ANDS) be performed under both fasted and fed conditions [127, 159, 182].

In evaluating the exposure changes due to food intake, in both food effect (FE) BA studies for NDA/NDS and fed BE studies for ANDA/ANDS, regulatory agencies seek information on the ‘‘potentially extreme condition’’, i.e., the largest food effect likely resulting from co-administration of drugs with meals. For this reason, regulatory agencies

(e.g., the US FDA and HC) recommend that the meal to be used under fed conditions should be of high calorie (800–1000 Kcal) and high fat (50%) content as it is widely accepted that the high-fat, high-calorie meal will likely provide maximal GI perturbation and the greatest effects on formulation performance *in vivo* [127, 130]. As such, the presence of high-fat, high-calorie meal in GI environment renders the physiological conditions more discriminative of formulation differences for BE purposes. The meal composition for FE BA and Fed BE studies is the same. For the details of the nutrient, caloric contents and volume of the standard meal for FE BA and fed BE studies, the reader is referred to the regulatory guidance documents [127, 130]. In the following subsections, two types of regulatory studies under fed conditions are described.

3.3.1.2.1 Food-Effect Bioavailability Studies

Administration of a drug product with food may change the BA by affecting either the drug substance or the formulation factors such as excipients. In practice, it is difficult to determine the exact mechanism by which food changes the BA of a drug product and to predict the direction and the magnitude of FE on formulation BA. Therefore, a FE BA study is recommended for all new chemical entities (new drug substances), irrespective of their BCS class and release mechanisms (IR and MR) of their formulations during the IND period for INDs and NDAs. The purpose of FE BA study is to assess the effects of food on the rate and extent of exposure or absorption of a drug when the drug product is administered shortly after a meal (fed conditions) as compared to administration under fasted conditions (an overnight fast of at least 10 hours).

The guidance documents recommend a randomized, balanced, single-dose, two-treatment (fed vs. fasted), two-period, two-sequence crossover design for studying the effects of food on the bioavailability of either an IR or a MR drug product. The formulation to be tested should be administered on an empty stomach (fasted condition) in one period and following a test meal (fed condition) in the other period. An adequate washout period should separate the two treatments [127, 130].

The study should be conducted in HVs, unless safety concerns preclude the enrollment of HV. A minimum of 12 subjects should complete the study to achieve adequate power for a statistical assessment of FE on BA and conclude the absence of FE.

In general, the highest strength of the drug product intended to be marketed should be dosed in the FE BA study, unless safety concerns warrant the use of a lower strength.

Data analysis and FE evaluation

The complete shape of the plasma concentration-time profile for the active ingredient (and when applicable, active moieties or active metabolites) should be characterized in both fasted and fed treatment periods. The exposure measures, particularly C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, should be obtained from concentration-time profiles in both treatment periods. Consequently, the exposure measures under fed conditions (Test treatment) are compared versus those under fasted conditions (Ref treatment).

An equivalence approach based on the Two One-Sided Tests (TOST) procedure (**Section 3.5**) is recommended for FE evaluation. Exposure measures should be ln-transformed prior to analysis, and the 90% confidence interval (CI) for the ratio of population geometric means of test versus reference treatment should be calculated for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$.

The absence of FE on BA is concluded only if the 90% CI for the ratio of population geometric means of fed versus fasted treatment, based on ln-transformed data, is contained in the equivalence limits of 80.00-125.00% for both $AUC_{0-\infty}$ (or AUC_{0-t}) and C_{max}. When the 90% CI fails to meet the limits of 80.00-125.00% for either of PK parameters, the absence of FE cannot be concluded. However, it does not necessarily mean that the effect of food on drug BA is of clinical relevance. The clinical relevance of FE could be interpreted only based on clinical studies and/or PK-PD relationships of the drug under study.

3.3.1.2.2 Fed Bioequivalence Studies

Inactive ingredients (excipients) in a generic dosage form can differ from the inactive ingredients in the reference counterpart [159, 183, 184]. May studies have documented the interaction between food ingredients and formulation excipients and demonstrated that the influence of food-formulation interaction on drug BA differs between formulations with different formulation factors (release mechanisms, excipients, manufacturing method, etc.) [185-188]. Therefore, the relative direction and magnitude of FE on drug BA from generic and Ref products are not predictable. For this reason, regulatory agencies ask generic drug applicants to conduct studies comparing the BA of the generic and corresponding reference

drug products under fed conditions [189]. Such studies are called fed BE studies. The purpose of fed BE study for ANDA/ANDS is to establish the equivalence of Test (i.e., generic) to reference product under fed conditions, which means that the drug BA from both the test and reference products should be affected similarly by food.

For a generic product to be bioequivalent to its reference, they need to be bioequivalent under both fasted and fed conditions. For all IR and MR oral dosage forms, the US FDA asks that applicants conduct two *in vivo* BE studies, one under fasted conditions and one under fed conditions. However, if the drug is classified as Biopharmaceutical Classification System (BCS) Class I (highly solubility, high permeability), or if drug product labeling strongly recommends administering only on an empty stomach for either efficacy or safety reasons, then only an *in vivo* study under fasted conditions is necessary to meet the requirements to demonstrate BE [127].

As per HC, unlike the US FDA, only for MR oral dosage forms BE should be demonstrated under both fasted and fed conditions, and for IR oral dosage forms fasted BE studies suffice to demonstrate the BE between the generic and reference products [182].

The guidance documents recommend a similar, two-treatment, two-period, two-sequence crossover design with an adequate washout period between the two treatments as recommend for FE BA studies except that the treatments should consist of both Test (generic) and reference formulations administered under fed conditions [127, 130].

Similar to FE BA studies, fed BE studies should be conducted in HVs, unless safety concerns preclude the enrollment of HV. A minimum of 12 subjects should complete the study to achieve adequate power for a statistical assessment of BE data to claim BE between Test (i.e., generic) and reference products under fed conditions.

In general, the highest strength of the drug product intended to be marketed should be dosed in the fed BE study, unless safety concerns warrant the use of a lower strength.

Data analysis and bioequivalence evaluation

The complete shape of the plasma concentration-time profile for the active ingredient (and when applicable, active moieties or active metabolites) should be characterized for both Test (i.e., generic) and reference products. The exposure measures, particularly C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$, should be obtained from concentration-time profiles for each product.

Consequently, the exposure measures of generic product (Test treatment) are compared versus those of reference product (Ref treatment).

An equivalence approach based on the Two One-Sided Tests (TOST) procedure (**Section 3.5**) is recommended for bioequivalence assessment. Exposure measures should be ln-transformed prior to analysis, and the 90% confidence interval (CI) for the ratio of population geometric means of test versus reference treatment should be calculated for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

Bioequivalence of a test (generic) to the reference product under fed conditions is concluded if the 90% CI for the ratio of population geometric means of the test versus reference product, based on ln-transformed data, is contained in the bioequivalence limits of 80.00-125.00% for both $AUC_{0-\infty}$ (or AUC_{0-t}) and C_{max}. Otherwise, the BE cannot be demonstrated under fed conditions. Although no criterion applies to t_{max}, the t_{max} values for the test and reference products are expected to be comparable based on clinical relevance. If BE is concluded under fed conditions, the statements with regard to food in the package insert of the generic product should be the same as the reference product.

3.3.2 PD-Endpoint Studies

In situations where a drug cannot reliably be measured in blood or other accessible biological fluids, BE may be established with a PD-endpoint study, provided that suitably validated PD or clinical endpoints are available, which can be quantitatively measured and evaluated with sufficient accuracy, sensitivity, and reproducibility [158]. This is the case for most locally acting drug products, some systemically acting drug products for which drug levels are too low to be measured in biological fluid, or when there is a safety concern for using the PK approach to assess BE. For locally acting drug products, another reason for not using the PK approach to demonstrating BE lies in the fact that drug concentrations in the systemic circulation may not reflect the BA of the drug at the site of action [120, 154, 190].

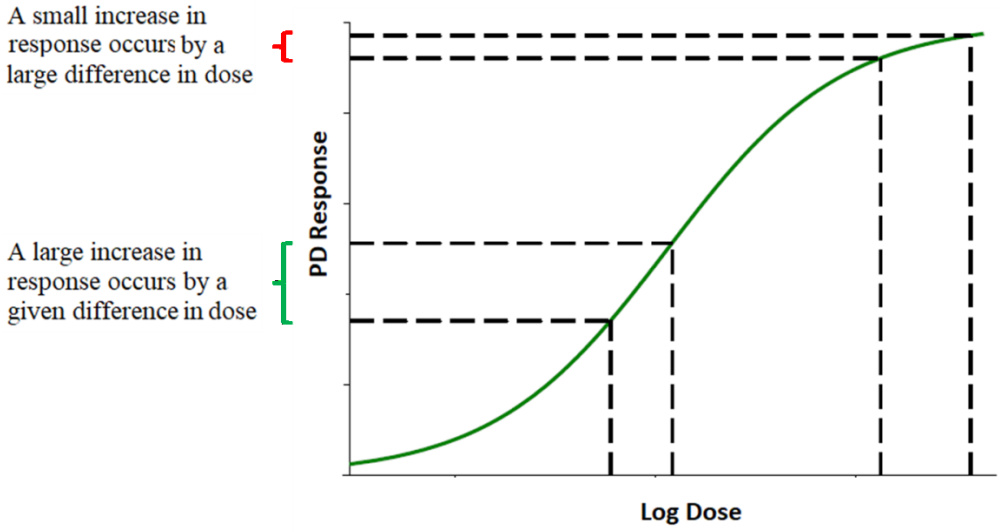
PD-endpoint studies are not as accurate, sensitive, and reproducible as the PK-approach based on plasma concentration measurements in detecting the formulation performance differences between the Test (generic) and Ref products. The reason is that after the absorption of the API from the site of delivery, it reaches to the site of activity and, through binding to a receptor or other mechanisms, elicits a quantifiable PD response [112, 113]. The variability of the measured endpoint increases with each additional step in the

process of delivery to the site of action. As a result, the variability of PD or clinical measures is quite high compared to that of blood concentration measures [191].

An essential component of the PD-endpoint study is the characterization of a dose-response (or exposure-response) relationship. The dose-response relationship is characterized by plotting the PD effect versus dose, often log-transformed dose, which yields a sigmoidal curve and is commonly described by the E_{max} model (**Section 2.1**) [141].

To establish BE it is crucial to conduct the BE study in the discriminative region of the dose-response curve. The dose at which the BE is studied should be in the range that the PD response is sensitive to small changes in dose. A dose that is too high will produce a minimal change in response at the plateau phase of the dose-response curve, such that even large differences in dose will show little or no change in PD effect (**Figure 8**). Therefore, a BE study conducted near the plateau of response will be insensitive to differences in drug delivery between the Test and Ref products. Lower doses are usually recommended for a PD-endpoint BE study. The discriminative dose for PD-endpoint BE studies is determined in a pilot study prior to the pivotal BE study [113, 154, 155].

Figure 8. Pharmacodynamic response versus dose on logarithmic scale



Topical dermatological corticosteroid products and locally acting nasal aerosols and oral inhalers (e.g., albuterol metered-dose inhalers) are the examples for which PD endpoints are used to evaluate BE [154, 155]. The BE for topical dermatological corticosteroid products is described in more detail in **Section 4.1.1**.

3.3.3 **Comparative Clinical Trials**

BE studies with clinical endpoints would be considered only when both PK and PD approaches are impossible for BE determination. This type of BE study is conducted in patients and is based on the evaluation of a therapeutic, i.e., clinical response. The clinical response is often located near or at the plateau of the dose–response curve, thus insensitive to distinguish the therapeutic difference between the Test (generic) and Ref products [119]. As a result, conduct of these studies requires a large number of patients to detect formulation differences. To gain adequate statistical power for establishing BE clinical studies may require as many as 1000 patients, which increases the study costs. High variability and the subjective nature of clinical evaluations are other reasons that the clinical response is often not sensitive enough to differences in drug formulation performance [191]. For these reasons, the clinical approach is the least accurate, sensitive, and reproducible of the *in vivo* approaches to determining BE and is recommended only for those products with local site of action for which it is not possible to develop reliable PD assays. For systemic effective drug products, comparative clinical trials may be acceptable only when analytical methods cannot be developed for the measurement of drug concentrations. Bioequivalence studies with clinical endpoints are also used for some oral drug products that are not systemically absorbed, such as sucralfate tablets [121]. Topical antifungal drug products (e.g., ketoconazole), topical acne preparations, ketoconazole shampoo, miconazole nitrate vaginal cream, and sucralfate tablets are the examples of drug products for BE assessment with clinical endpoints. For more examples, the reader is referred to several FDA Guidance documents [119, 141, 192, 193].

Bioequivalence studies with clinical endpoints generally employ a randomized, double-blinded, placebo-controlled, parallel design. Studies compare the efficacy of the Test (generic) product, Ref product, and placebo to determine if the two products containing active ingredients are bioequivalent. The placebo is included to ensure that the two active treatments (Test and Ref) are actually being studied at a dose that affects the therapeutic response. Failure to assure that the treatments are clinically active in the trial would show that the trial has no sensitivity to differences in formulations. Bioequivalence is established if the Test product is equivalent to the Ref product and superior to the placebo treatment [190, 191].

3.3.4 *In Vitro* Comparison Studies

In vitro studies are seldom used alone for BE determination except with some special cases. A comparative *in vitro* dissolution (or release) test is the most commonly used *in vitro* method for BE assessment. *In vitro* dissolution data along with the *in vivo* study data is routinely submitted by drug sponsors for BE documentation of orally administered drug products [158]. *In vitro* dissolution data are also utilized to support biowaiver for *in vivo* BE studies for lower strengths of a drug product, provided that an acceptable *in vivo* study has been conducted for the higher strength and compositions of these strengths are proportionally similar [143, 158]. Dissolution profiles of Test and Ref products may be considered similar if the similarity factor (*f2*) is greater than 50%. The similarity factor, expressed the percentage (%), is a measurement of the similarity in dissolution between the Test and Ref products (e.g., pre-change and post-change products) [18, 143].

In vitro dissolution data can also be used to establish a correlation with *in vivo* data (e.g., plasma concentrations) [194, 195]. This correlation is called *In Vivo-In Vitro* Correlation (IVIVC). A bioequivalence study can be waived based on the IVIVC if the predicted mean AUC and C_{max} of the Test and Ref do not differ from each other by more than 20%. The presence of an adequate IVIVC can also serve as a surrogate for BE studies to assess the impact of some pre- and post-approval changes [194].

In vitro approaches other than comparative dissolution study may also be used for establishing BE. The use of *in vitro* biomarkers and *in vitro* binding assays has been proposed to establish BE for orally administered drug products with no systemic absorption such as cholestyramine resin and calcium acetate tablets [196, 197]. *In vitro* physicochemical characterization such as *in vitro* rheological tests, pH, viscosity, specific gravity or density, surface tension, buffer capacity (if the product contains a buffer), droplet size or volume (if administered as drops), and droplet size distribution (if an administered as a topical spray) are other types of *in vitro* approaches that may be required by regulatory agencies for the BE assessment of topical drug products [198, 199]. For more examples, the reader is referred to several product specific FDA Guidance documents [192].

3.3.5 Alternative Approaches

The Hatch-Waxman Act was modernized in 2003 to better accommodate the evaluation of BE for a drug that is not intended to be absorbed into the bloodstream [200]. Further to a 2003 addition to the US Federal Food Drug and Cosmetic Act at Section 505(j)(8)(A)(ii), the US FDA authorized to ‘‘establish alternative, scientifically valid methods to show BE if the alternative methods are expected to detect a significant difference between the drug and the listed drug in safety and therapeutic effect’’ [201].

The tape stripping method and population compartmental modeling are the examples to these alternative approaches. Model-based analysis of tape stripping data is the focus of Article #3 within the context of this thesis (**Chapter 4**). Tape stripping and population compartmental modeling will be further described in **Sections 4.2.1** and **5** respectively.

3.4 Design and Conduct of Bioequivalence Studies

3.4.1 Pilot and Pivotal Studies

Bioequivalence studies may be conducted in two parts, pilot and pivotal studies.

A pilot study is usually a smaller study in the number of subjects and is conducted prior to the pivotal studies where the BE between the Test (e.g., generic) and Ref products is investigated. The purpose of pilot study is to establish an appropriate study design for the pivotal BE study. For instance, pilot studies can be used to estimate the variance, and therefore, to determine the appropriate number of subjects needed to provide adequate statistical power for concluding BE in the pivotal studies. A pilot study can also be used to validate analytical methodology and optimize sampling scheme for the pivotal PK-endpoint studies [128, 158].

The conduct of a pilot study is especially useful prior to the PD-endpoint BE studies. For a PD-endpoint BE study, a pilot study is recommended to investigate the dose-response relationship and to determine the appropriate dose (or dose-duration) that is on the linear portion of the PD response-dose curve, so that it can later be used in the pivotal study as the dose or as the time point at which the Test and Ref products are compared. Gastrointestinal tract locally acting acarbose tablet, lanthanum tablet, and orlistat capsule; topically applied fluticasone propionate cream; and orally inhaled albuterol sulfate metered-dose aerosol are the

examples of the products which require a pilot study using the Ref product to determine the most sensitive dose for their pivotal PD-endpoint BE study [155, 202].

The pivotal study is the BE study where the Test and Ref products are compared in order to investigate the BE between them.

3.4.2 Bioequivalence Study General Recommendations

(a) Two-treatment, two-sequence, two-period, single-dose, crossover study designs, (b) single-dose, parallel study designs, or (c) replicated crossover study designs are the most frequently recommended study designs for BE studies [130, 158, 203]. Several factors may be considered when choosing appropriate designs for a BE study. For instance, the preferred study for most orally administered dosage forms is a two-sequence, two-period (two-way) crossover, single-dose study performed in HVs. However, this design could be impractical for drugs with long half-lives, i.e., longer than 30 h. For BE studies conducted in patients or for drugs with a long half-life where crossover studies are difficult or impossible to perform, parallel design is often used.

For BE purposes, single-dose studies are required by regulatory agencies as they are more sensitive in detecting differences in formulation performance than multiple-dose studies [120, 204, 205].

Most BE studies are conducted on the highest strength of the drug in question unless it is necessary to use a lower strength for safety reasons. For drugs for which rate and/or extent of exposure increases less than proportional with an increase in dose, the BE study will be most discriminative with the lowest strength.

3.4.3 Number of subjects

The number of subjects enrolled in BE studies is determined based upon many factors, such as study design, anticipated intra-subject variance (or variability) for crossover design and inter-subject variance in parallel design, the expected mean difference between the Test and Ref drug products, desired power of the study, study endpoints, etc. In crossover design relatively smaller number of subjects and in parallel design higher number of subjects are required to obtain sufficient power for accurate evaluation of BE. The number of subjects should be calculated based on maintaining the overall Type I error rate at 5%, and usually to

have 80% or 90% power to conclude BE between Test (generic) and Ref products. A minimum of 12 subjects are required in pivotal BE studies by regulatory agencies [130, 203], but usually a larger number is required. Most BE studies submitted in support of ANDAs/ANDSs enroll 24-36 subjects.

3.4.4 Experimental Study Design

In this section we describe three of the most common BE study designs in more detail.

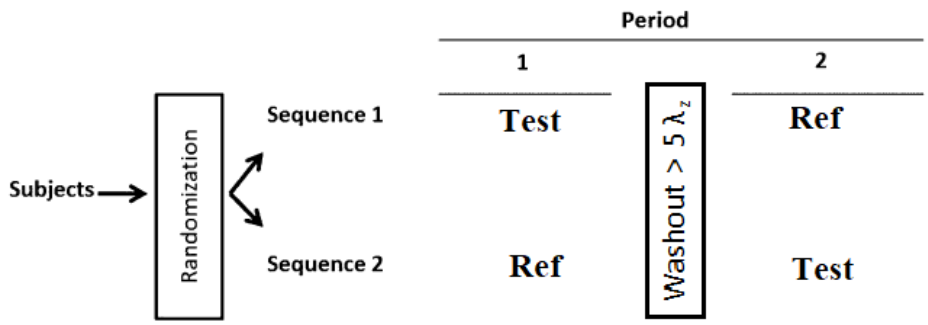
3.4.4.1 Crossover Design

The two-formulation, two-sequence, two-period (two-way) crossover study design is generally conducted with HVs for most of the systemic effective oral dosage forms. In this design, each subject is randomized either to sequence one, where the subject receives the Test drug (or Test treatment) in the first period then Ref drug (or Ref treatment) in the second period, or to sequence two, where the subject receives the Reference drug (or Ref treatment) in the first period and then the Test drug (or Test treatment) in the second period as shown in **Figure 9**. In this way, any bias in the data due to a residual effect from the previous treatment (carryover effects) will be minimized.

In two-way crossover design, each subject will receive each treatment (e.g., Test and Ref drug) only once, with an adequate time period between treatments. The time period which separates the two treatments is called washout period. The length of the washout period should be approximately more than 5 elimination half-lives (λ_z) of the drug so that the drug received in the first period will be completely eliminated from the body and drug level at the beginning of the second period is almost zero or negligible as in the first period [142, 158].

Because each subject received both treatments, the design allows a within subject comparison between the test and the reference treatments. Therefore, it enables us to evaluate BE with greater precision than would a study of two parallel groups. In this design, each subject serves as his/her own control and inter-subject variability is reduced. Therefore, relatively lower number of subjects are required for BE assessment. In summary, crossover design allows us either to evaluate BE with greater precision than a parallel group study with the same number of subjects, or to evaluate BE with equal precision to parallel design but with fewer subjects.

Figure 9. Two-formulation, two-period, two-sequence crossover design.Test, Test treatment; Ref, Reference treatment, λ_z, elimination half-life.



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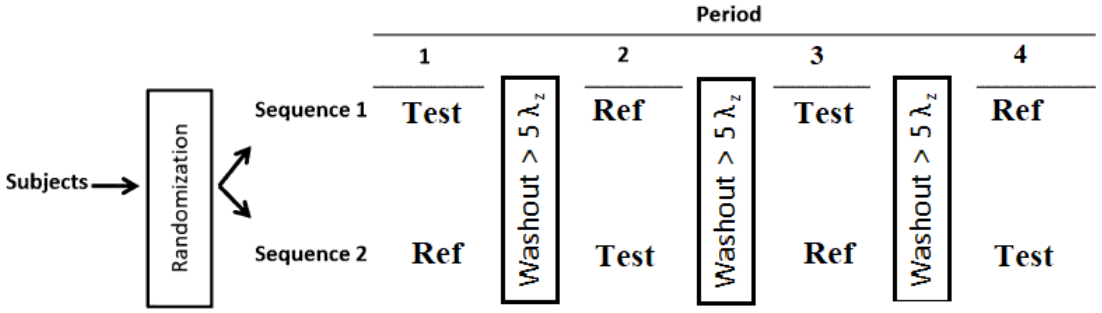
3.4.4.2 Replicated Crossover Design

In a replicated crossover design, at least one treatment is repeated and there are usually more periods than there are treatments. By giving the same drug product twice to the same subject in replicated crossover design, it enables us to estimate within-subject (intra-subject) variability, while it is not possible with a two-way crossover design. The estimation of intra-subject variability is of importance when Scaled Average BE (SABE) approach is employed for BE assessment, which is beyond the scope of this thesis. Highly variable drugs (with intra-subject variability ≥30%) and narrow therapeutic index drugs are the common examples where the replicate design is employed for the study of BE. Similar to any other crossover design, the periods in replicated designs are separated with an adequate washout period.

A two-formulation, two-sequence, four-period crossover design, also known as fully replicated crossover design (TRTR/RTRT), as shown in **Figure 10**, is one of the most well-known replicated crossover designs. In this design, each subject is randomized to either sequence one, where the subject receives the Test in the first period, the Ref in the second period, then the Test in the third period, and finally the Ref in the fourth period or sequence two where the subject receives the Ref in the first period, the Test in the second period, then the Ref in the third period, and finally the Test in the fourth period. This design is usually used for narrow therapeutic index drugs [207-209].

Figure 10. Two-formulation, two-sequence, four-period crossover design (Fully replicated crossover design, TRTR/RTRT). Test, Test treatment; Ref, Reference treatment, λ_z, elimination half-life.



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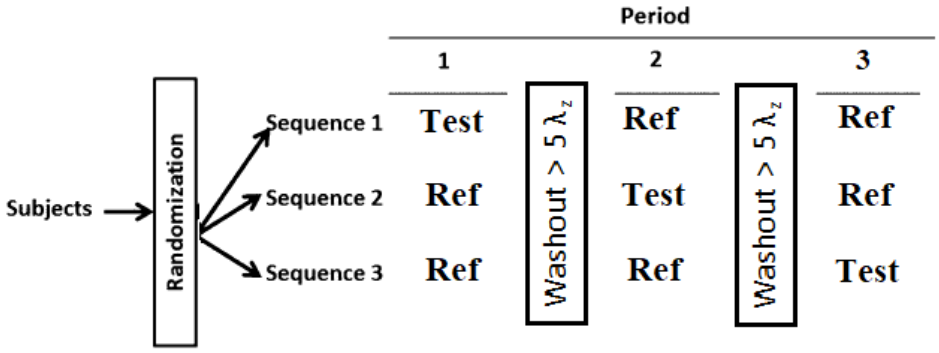
The second example of the replicated crossover design is a two-formulation, three-sequence, three-period, crossover design, also known as partially replicated crossover design (TRR/RTR/RRT), which is usually employed for highly variable drugs [207, 210, 211]. partially replicated crossover design is shown in **Figure 11**.

Figure 11. Two-formulation, three-sequence, three-period crossover design (partially replicated crossover design, TRR/RTR/RRT). Test, Test treatment; Ref, Reference treatment, λ_z, elimination half-life.



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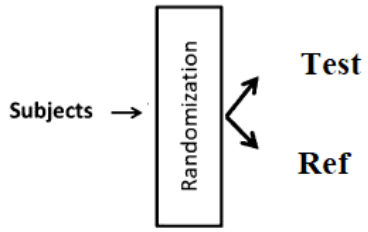
3.4.4.3 Parallel Design

In a parallel design, each treatment is administered to a separate group of subjects with similar demographics and no washout period is needed between treatments. In the parallel design, each subject is randomized to only one treatment group, and usually each treatment group has the same number of subjects. The simplest form of such a design is the two-group parallel design as shown in **Figure 12**.

When treatment groups of parallel design have the same number of subjects, the design of the study is known as balanced parallel, and when the treatment groups have a different number of subjects, the design is referred to as unbalanced parallel design.

A parallel design is not commonly used in BE studies since it cannot distinguish between the inter-subject variability and the intra-subject variability because each subject receives only one treatment. Therefore, more subjects are necessary to achieve the same statistical power compared to other designs such as the crossover [203, 206].

Figure 12. Two-group parallel design. Test, Test treatment; Ref, Reference treatment.



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3.5 Statistical Considerations of Bioequivalence

3.5.1 Clinical Pharmacology and Background

As mentioned previously, BE is concluded when the active ingredient or active moiety has an equivalent rate and extent of absorption or exposure at the site of action from two pharmaceutical alternative or pharmaceutical equivalent drug products. In practice, it is difficult to obtain the exact same rate and extent of absorption or exposure of API from two drug products. Even if two drug products are of the same dosage form and contain the same active ingredient in the same quantity, or even if the same product administered to the same subject on two separate occasions, completely identical and superimposed profiles, and hence completely the same rate and extent of exposure, are rarely obtained. For this reason, demonstration of BE relies on similarity rather than identicality of PK metrics (C_{max} and AUC) that are representative of rate and extent of exposure, and some degree of difference is considered acceptable without compromising clinical safety and efficacy.

Based on historical PD data and FDA medical experts, a significant difference in a clinical effect is not observed when a difference in plasma drug concentrations following the administration of Test and Ref products is less than 20%. Approved Drug Products with

Therapeutic Equivalence Evaluations (The FDA Orange Book) indicates that a difference of greater than 20% for each of the AUC and C_{max} is determined to be significant [212].

The cut-off of 20% is not an arbitrary number, but indeed has a great clinical relevance. In clinical practice, a DDI has been considered to be clinically relevant and necessitating dosage adjustments only if it results in 20% alteration in the AUC of the affected drug, with the exception of narrow therapeutic index drugs for which small differences in doses will lead to significant efficacy and safety changes [138, 207, 208]. As such, the BE limits for a Test product have generally been considered within 20% of the Reference Listed Drug (RLD) product [213, 214]. In the early 1970s, as per the US FDA, the approval of generic products was based on mean data in that the mean AUC and C_{max} values for the generic product had to be within ±20% of those of the corresponding Ref product [215]. In addition, plasma concentration-time profiles had to be reasonably superimposable. Beginning in the late 1970s, the FDA started using the power approach. This approach required both AUC and C_{max} to be within ±20 % of the innovator product at an estimated power of 80% (with an 80% probability). The power approach was limited in that it only considers differences in the calculated averages of AUC and C_{max}. With this approach, two approved products can have equal AUC and C_{max} mean values but differ in variability. In an attempt to consider variability, the 75/75 (or 75/75-125) rule was added to the criteria. According to the 75/75 rule, BE would be met if (a) the Test/Ref ratios of AUC and C_{max} were within 0.75-1.25 for at least 75% of the subjects, and (b) there was no more than 20% difference in mean AUC and C_{max} [141, 215, 216].

In 1986, the US FDA discontinued the use of the 75/75 rule and power approach due to their limitations and introduced the Two One-Sided Tests (TOST) statistical procedure, which establishes the present-day statistical approach for BE assessment. The TOST procedure is mostly used for a comparison of BA measures in PK-endpoint studies, however, it can also be useful in assessing BE based on PD or clinical endpoints. Two alternative approaches, termed Population Bioequivalence (PBE) and Individual Bioequivalence (IBE), are other approaches of BE assessment, both of which incorporate scaling for reference variability and they may be useful, in some instances, for analyzing *in vitro* and *in vivo* BE data [203]. Population and Individual Bioequivalence approaches are beyond the scope of this thesis and will not be further described.

In the following sections, the TOST procedure and BE evaluation based on this approach are described.

3.5.2 Two One-Sided Tests (TOST) Procedure

The two One-Sided Tests (TOST) statistical procedure was first put forward in 1987 by Schuirmann [217]. The TOST procedure is commonly referred to as the 90% CI approach or Average BE (ABE) approach. The TOST procedure consists of decomposing the interval hypotheses H_0 and H_1 into two sets of one-sided hypotheses as follow:

Null hypothesis	$H_{01}: \mu_T - \mu_R \leq -\delta$	and	$H_{02}: \mu_T - \mu_R \geq +\delta$
Alternative hypothesis	$H_{11}: \mu_T - \mu_R > -\delta$	and	$H_{12}: \mu_T - \mu_R < +\delta$

Where μ_T and μ_R are mean BA measures for Test and Ref, respectively; $-\delta$ and $+\delta$ are the lower and upper limits of equivalence interval. Obviously, the interval remaining between $-\delta$ and $+\delta$ inclusive is called equivalence interval.

In this statistical hypothesis, the null hypothesis (H_0) states that Test (μ_T) and Ref (μ_R) are not (bio)equivalent, and the alternative hypothesis (H_1) states that they are (bio)equivalent. Therefore, the equivalence of Test and Ref can be concluded if and only if the null hypothesis is rejected at a chosen level of significance ($\alpha = 0.05$).

The level of significance is related to the probability of incorrectly rejecting H_0 when it should have been accepted. This level of error is called Type I error. Differently said, Type I error is committed when one rejects H_0 when, in fact, H_0 is true. Type II error is committed when H_0 is accepted as being true when, in fact, it is false and should have been rejected [218-220].

The probability of committing a Type I error is defined as the significance level of the statistical test and is denoted as α (alpha). The probability of a Type II error is denoted as β (beta). To reduce Type I and Type II errors, the sample size needs to be increased. The power of a statistical test is the probability that the statistical test results in the rejection of the null hypothesis if H_0 is really false. The larger the power, the more sensitive is the test. Power is defined as $1-\beta$, which means the smaller the β error, the larger is the power. The variability in the studied samples, sample size, and desired level of significance will affect the power of the statistical test. Usually, the greater the variability in the samples, the larger will be the sample size needed to obtain sufficient power [219, 220].

Given the null and alternative hypotheses in the TOST procedure, in the context of BE, the probability of rejecting H_0 when H_0 is true (Type I error) corresponds to the probability of concluding BE while Test and Ref products, in fact, are not bioequivalent, and it is called consumer's risk. The probability of accepting H_0 when H_0 is false (Type II error) corresponds to the probability of concluding that Test and Ref products are not bioequivalence while they are, in fact, bioequivalent, and it is called manufacturer's risk. Power in the TOST procedure is the probability of concluding BE when the two products are truly bioequivalent. It should be mentioned that if one rejects the H_0 (concludes BE), there is no way that one could have made a Type II error (failing to conclude BE); this means the study was of sufficient power to conclude BE. However, if one accepts the H_0 (fails to prove BE), it does not necessarily mean that Test (generic) and Ref products are not bioequivalent, and it could be due to the insufficient power of the study. In this case, the study is known to be under-powered [221]. By increasing the sample size of the study, sufficient power may be obtained and the study may pass BE. As mentioned earlier (**Section 3.4.3**), the sample size for a BE study is usually determined based on achieving a minimum of 80% power at the significant level of 5% (α=0.05). As also mentioned earlier, a minimum of 12 number of subjects is required for a pivotal BE study for generic submission. [130, 140, 203, 222-224]. A summary of null and alternative hypotheses and Type I and Type II errors in the context of BE are presented in **Table 3**.

Table 3 Interval H_0 and H_1 hypotheses in the TOST procedure and Type I and Type II errors. H_0, null hypothesis; H_1, alternative hypothesis; BE, Bioequivalence

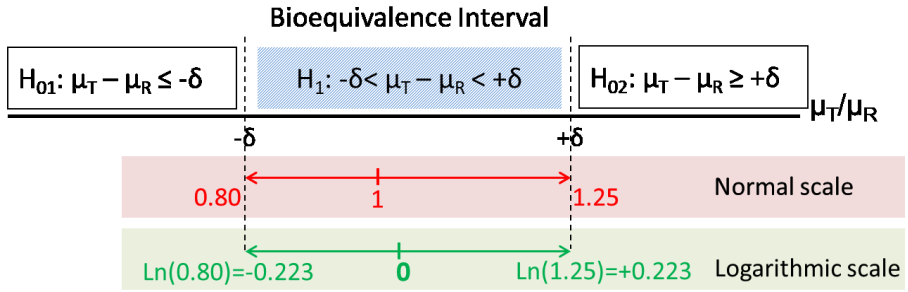
	Fail to Reject H_0	Reject H_0
H_0 is true (Test ≠ Ref)	Correct decision (Failing to prove BE could be due to the insufficient power)	Type I error (consumer's risk): Concluding BE when Test and Ref are not bioequivalent.
H_1 is true (Test = Ref)	Type II error (manufacturer's risk): Concluding that Test and Ref are not bioequivalent when they are bioequivalent.	Correct decision (Any probability of insufficient power is ruled out)

The TOST procedure, or the ABE approach, involves the calculation of a 90% CI for the ratio of the averages (population geometric means), based on ln-transformed data, of the Test and Ref products. To establish BE, the calculated 90% CI should fall within the equivalence limit (equivalence interval). In the current practice of BE, the lower limit (-δ) and

upper limits (+δ) of equivalence range are set as 0.80 and 1.25, respectively. The rational for choosing these values is based on the TOST procedure. The TOST procedure tests two questions. The first question asks if the Test product is significantly less bioavailable than the Ref product. The second question asks if the Ref product is significantly less bioavailable than the Test product. A significant difference is defined as 20% at α=0.05. In the first case, the Test/Ref ratio (or the BE limit) is 0.80; and in the second case, the Ref/Test ratio (or BE limit) is 0.8. Since by convention, BE ratios are expressed as Test/Ref, the second BE limit will be the reciprocal of 0.80, that is, 1.25. This illustrates the rationale for the equivalence range of 0.80-1.25 (or 80.00-125.00%) which is set by regulatory agencies for BE demonstration [141].

The 80.00-125.00% range is the equivalence range on the original scale (normal scale). As mentioned above, it corresponds to a range of ±20% relative difference between the Test and Ref products, where the Ref product must be in the denominator of the ratio. This criterion is not symmetric around unity (Test/Ref=1) on the original scale for the ratio of the average BA, but it is symmetric on the logarithmic scale (log or ln scale) around zero as Ln(0.80)= -0.223 and Ln(1.25)= +0.223 are at equal distance from unity (Ln(1)= 0). The principles of the TOST procedure are illustrated in **Figure 13**.

Figure 13. Decomposition of the Two One-Sided Tests procedure and the conventional equivalence interval



3.5.2.1 Construction of Confidence Interval and Data Analysis

The ABE approach focuses on the comparison of the population averages of the ln-transformed BA measures (AUC and C_{max}) after the administration of the Test and Ref products. To this purpose, the Geometric Mean Ratios (GMR) for the BA measures of Test versus reference should be calculated, and the 90% CI for calculated GMRs should be

constructed. If the 90% CI is entirely contained in the regulatory-specified equivalence interval of 80.00-125.00%, then the BE can be concluded. The calculation of 90% CI for the GMR of Test/Ref ratio is conducted in logarithmic scale in the following steps:

1. The values of PK parameters in each treatment (Test and Ref) are ln-transformed
2. The difference between the Ln(Test) and Ln(Ref) is calculated for each subject
3. These values are then averaged (arithmetic mean) across all subjects
4. The 90% CI for the difference in the means of the ln-transformed PK parameters [Average(Ln(Test)-Ln(Ref)] is calculated using equations and methods appropriate to the experimental design. For instance, **Equation 8** is used for two-way crossover and balanced parallel designs, and **Equation 9** is used for unbalanced parallel design, where the treatment groups (here, the Test and Ref treatments) have unequal number of subjects [225, 226].

$$\text{CI} = \text{Exp}[(ln\frac{\mu_{Test}}{\mu_{Ref}}) \pm \text{z}\ \frac{SD}{\sqrt{n}})] \qquad \text{(Equation 8)}$$

where μ_{Test} and μ_{Ref} are population geometric means of BA measures of Test and Ref, respectively; z is the critical value in the normal distribution (z=1.645) and it is replaced with $t_{1-\alpha}$ for small samples (n≤30) which are not normally distributed, instead they have a t-distribution; SD is the standard deviation and stands for intra-subject variability for a crossover design, and intra-subject variability for a parallel design [226, 227].

$$\text{CI} = \text{Exp}[(\text{Ln}\frac{\mu_{Test}}{\mu_{Ref}}) \pm t_{1-\alpha,\, n_{Test}+n_{Ref}-2} \cdot SD_{pooled} \cdot \sqrt{\frac{n_{Test}+n_{Ref}}{n_{Test} \cdot n_{Ref}}}] \qquad \text{(Equation 9)}$$

where μ_{Test} and μ_{Ref} are the population geometric means of BA measures of Test and Ref, respectively; $t_{1-\alpha,\, n_{Test}+n_{Ref}-2}$ denotes the critical value of the t-distribution with $n_{Test} + n_{Ref} - 2$ degrees of freedom at the $1-\alpha$ probability level; SD_{pooled} is the pooled standard deviation which is calculated as the square root of the pooled error variance ($\sigma^2{}_{pooled}$) estimate (**Equation 10**) [225]:

$$SD_{pooled} = \sqrt{\sigma^2{}_{pooled}} = \sqrt{\frac{[(n_{Test}-1)\sigma^2{}_{Test}]+[(n_{Ref}-1)\sigma^2{}_{Ref}]}{(n_{Test}+n_{Ref}-2)}} \qquad \text{(Equation 10)}$$

where $\sigma^2{}_{\text{Test}}$ and $\sigma^2{}_{\text{Ref}}$ are the variance estimates associated with Test and Ref, respectively.

The results of the statistical analysis are then presented in terms of the GMRs of AUC and C_{max} and the 90% CI for these GMRs on normal scale. The GMR, termed Point Estimate (PE), is determined by applying the anti-log (i.e., exponential) function to the [Average(Ln(Test)-Ln(Ref)]. Similarly, anti-log (i.e., exponential) function is applied to the Lower Limit and Upper Limits of the 90% CI (LLCI and ULCI).

3.5.3 Bioequivalence Interpretation

3.5.3.1 Bioequivalence

From the regulatory perspective, BE is generally concluded if the GMR (Test/Ref) for the BA measure and the 90% CI around the GMR are completely contained within the equivalence interval of 80.00-125.00% [158, 203].

3.5.3.2 Failure to Conclude Bioequivalence (Non-bioequivalence)

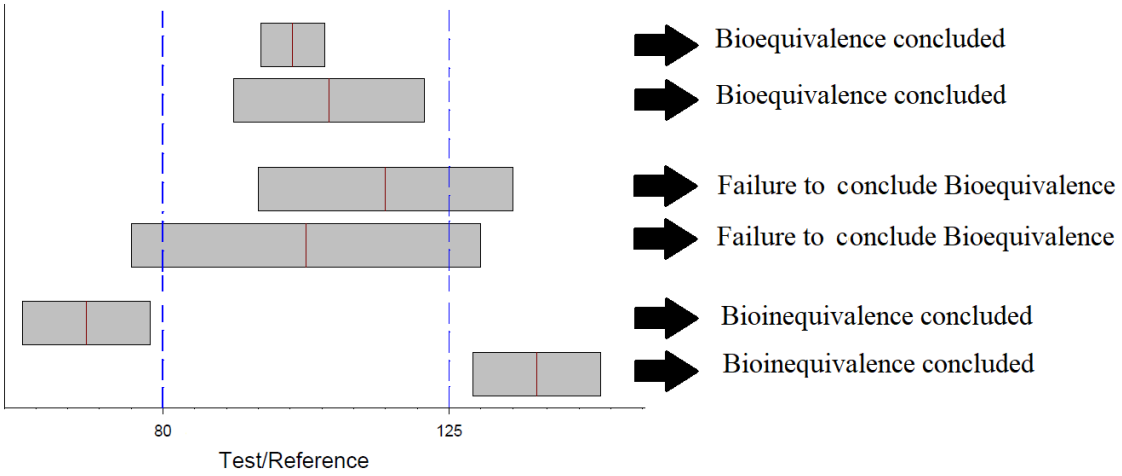
If either side or both sides of the 90% CI fall outside the 80.00-125.00% equivalence interval, the Test product fails to demonstrate BE to its Ref. The failure to demonstrate bioequivalence may lie in different reasons. First, it could be due to the fact that the Test product is poorly formulated and is truly different in performance from its Ref. Second, it could be due to the study design, and the Test product could have been shown to be bioequivalent to its Ref if the study was better designed. For instance, an inadequate number of subjects may have been enrolled, and therefore, the study did not have sufficient power to conclude BE.

The width of the 90% CI is proportional to the estimated drug variability (intra-subject variability for a crossover design and inter-subject variability for a parallel design) and inversely proportional to the number of subjects in the study. If an inadequate number of subjects is enrolled, or if the BE data is associated with high variability, the 90% CI will be wider. The wider the 90% CI, the more chance is for the 90% CI to exceed the 80.00-125.00% equivalence interval [129, 141].

3.5.3.3 Bioinequivalence

Bioinequivalence (BIE) is concluded if the GMR (Test/Ref) for the BA measure and the 90% CI around the GMR completely fall outside the 80.00-125.00% equivalence interval [129]. Different examples of BE interpretation are illustrated in **Figure 14**.

Figure 14. Possible bioequivalence study results. Gray bar represents the width of the 90% confidence interval. The red vertical line in the middle of the bar represents the point estimate.



3.6 Factors that Impact Bioavailability

In section **3.1.1,** BA of a drug from an oral formulation was described to be a product of series of rate processes before absorption (disintegration and dissolution), absorption, and pre-systemic metabolism in the intestine and in the liver. Any factor that can affect these processes, can impact drug BA. In broad terms, pharmaceutical factors and physiology of the GI system can impact drug BA.

Pharmaceutical factors refer to physiochemical properties of the drug substance such as permeability, solubility, pK_a, stability in pH, partition coefficient, particle size; and formulation characteristics such as pharmaceutical dosage form, release profile, matrix, and excipients.

Physiology of GI, on the other hand, refers to individual-related factors such as GI environment (e.g., acidity of the stomach, contents of the GI tract, hormones, intestinal transit time, etc.), hepatic and intestinal transporters and metabolizing enzymes, pathophysiological conditions, and genetic polymorphism [7, 228]. Many factors can alter the physiology of the GI tract, which in turn can influence the absorption and metabolism processes, and eventually the BA of drug. These factors can affect the physiology of GI tract via numerous mechanisms.

As described in **Section 1.6,** the extent of intestinal and hepatic metabolism of a drug is determined by the content of CYP enzymes within the liver and intestinal epithelial cells

and the intracellular residence time of the drug substance subject to biotransformation. Depending on the expression site of transporters, they can govern the residence time of drug molecules within the cells, and enhance or impede the metabolism by CYP enzymes either by mediating the intake of drug substances into the cells or by expelling drugs out of the cells at both intestinal and hepatic levels [85]. In addition to governing the duration of exposure of drug molecules to CYP enzymes, transporters at the intestinal level regulate the absorption. Due to the significant role of intestinal and hepatic transporters and CYP enzymes in modulating drug absorption, metabolism, and excretion, any factor affecting the functionality of these proteins can also affect drug BA. Among these factors, food constituents, pharmaceutical excipients, and physiological factors such as genetic polymorphism can alter the expression and functionality of CYP enzymes and transporters. The influence of these factors on CYP enzymes and transporters will be elaborated on in **Section 3.7**.

In the following subsections, the factors that impact BA are briefly summarized in three main categories based upon the phases of the LADME process.

3.6.1 Factors that Impact Pre-Absorption Processes

Drug substance is not usually given as a pure chemical compound but is formulated into a finished dosage form (i.e., drug product). The drug product includes the active drug substance combined with additional ingredients (excipients). Physicochemical properties of drug substances and excipients can impact BA by influencing the disintegration of drug product, subsequent drug release, and dissolution of drug substance before absorption [229-231].

3.6.2 Factors that Impact Absorption

The rate and extent of absorption is a function of pharmaceutical and physiological factors. Alteration of any of these factors can affect absorption. For example, pharmaceutical factors and excipients by influencing the rate of drug disintegration and dissolution, altering membrane permeability, changing membrane integrity, modifying GI transit time, affecting transporters at absorption site [229, 231-233]; dietary constituents of food by influencing drug dissolution, disintegration, binding to drug, altering GI motility, gastric emptying rate and gastric pH, inhibition and/or induction of CYP enzymes and gut membrane transporters can influence the rate and extent of drug absorption [3, 181, 234].

3.6.3 Factors that Impact Metabolism

Similar to drug absorption, metabolism can be affected by pharmaceutical and physiological factors. Pharmaceutical factors such as physicochemical properties of drug (e.g., stereoselectivity, molecular size, acidity, lipophilicity, etc.), and physiological factors (genetic polymorphism, age, gender, hormone balance, disease, etc.) can impact metabolism. Food intake can alter the intestinal and hepatic pre-systemic metabolism by inducing/inhibiting enzymes and transporters, influencing the hepatic blood flow, and many other mechanisms [73, 74].

3.7 Mechanisms that Impact Bioavailability

In the previous section, the factors that impact BA and the employed mechanisms were briefly indicated. A comprehensive review of all mechanisms involved in the alteration of BA is beyond the scope of this thesis. Given the relevance of CYP enzymes and transporters in the first project of this thesis, only the mechanisms that relate to transporters and CYP enzymes will be elaborated on.

The functionality and the level of expression of CYP enzymes and transporters can be affected by different extrinsic (e.g., xenobiotics) and intrinsic (e.g., physiology) factors. Xenobiotics such as drug substances, excipients in pharmaceutical dosage forms, and food constituents by their inhibitory or inductive effect can alter the function or even the level of expression of these proteins. Induction is an increase in the number and activity of the CYP enzymes and transporters and usually results from their increased biosynthesis in the liver and intestine, while inhibition is a decrease in the activity of the CYP enzymes and transporters and usually results from their inactivation further to substrate binding rather than a change in enzymes and transporters biosynthesis [235, 236].

Drugs and other xenobiotics that increase enzyme and transporter activity are known as inducers, and those which decrease enzyme and transporter activity are known as inhibitors. Induction of CYP enzymes results in an enhanced metabolism of their substrates, and therefore, a decrease in the level of the parent molecule and an increase in the level of metabolites. The induction of transporters results in an increase in the movement of their substrates across the cellular compartments. For instance, induction of efflux transporters on apical membrane of intestinal epithelia will increase the efflux of their substrate from inside

the enterocyte back into the gut lumen, and therefore attenuates the absorption, and eventually decreases the BA. The opposite occurs with inhibition [237]. Briefly speaking, induction or inhibition of CYP enzymes and transporters in enterocytes will eventually influence the absorption and metabolism while these interactions will influence only the metabolism of the substrates in hepatocytes.

In the following subsections we are presenting the examples demonstrating the interaction of different intrinsic and extrinsic factors with CYP enzymes and transporters, and the consequences on the BA of drugs.

3.7.1 Interaction of CYP Enzymes and Transporters with Food Constituents

Food intake may influence drug BA owing to different mechanisms that can initiate physiological changes in the GI tract. To date, many studies have reported the inhibitory and/or inductive effect of food constituents on transporters and CYP enzymes. In this section, some of these studies are presented.

Saquinavir, a substrate of CYP3A4 and P-gp, is an HIV-protease inhibitor with a low 5% oral bioavailability due to its low intrinsic solubility and high first-pass metabolism. Saquinavir is a weak base drug that dissolves in the acid pH of the stomach but would enter the upper small intestine at concentrations three folds higher than its intrinsic solubility. Saquinavir administration with a high-fat meal increases oral bioavailability five- to ten folds [238]. This tremendous increase in BA was explained to be likely due to a combination of solubilization of the drug in the intestine by lipid meal components and the resultant saturation of its transporters and CYP enzymes during its pre-systemic metabolism, particularly in the intestine [181].

Kakuda and Falcon [239] later tested the BA of saquinavir in a concomitant administration with a histamine H2-receptor antagonist ranitidine alone, ranitidine and food, and when taken concomitantly with food in order to investigate the mechanism underlying the effect of food on saquinavir absorption. More specifically, they investigated whether the increased saquinavir BA under fed condition is related to gastric pH changes/elevations or other mechanisms. No correlation was found between BA measures and gastric pH. Gastric pH elevation due to the administration of ranitidine were significantly greater than the gastric

pH elevation due to the ingestion of food. Despite the significantly higher pH elevation with ranitidine, the lowest C_{max} and AUC occurred when saquinavir was administered with ranitidine alone. Saquinavir's BA with coadministration of ranitidine alone was 15.9% [90% CI: 10–25%] of its BA in concomitant intake with food. Therefore, it was unlikely that the increase in saquinavir BA with food was caused by the increase in gastric pH. Author postulated that the decreased clearance of saquinavir due to the inhibition of CYP enzymes or intestinal efflux transporters can be one of the potential mechanisms for elevated saquinavir BA in concomitant intake with food.

Indinavir, a drug from the same pharmacological family as saquinavir, with an additional weak base moiety for increased solubility, showed 60% decrease in BA when administered with a high-caloric meal. All meal types produced a significant negative meal effect on indinavir oral BA without causing significant gastric pH elevation, except protein meals. Protein meal produced the most significant negative effect on indinavir BA and was found to provide the greatest potential for poor dissolution and/or precipitation of indinavir in the stomach as a function of elevated pH. Significant indinavir plasma concentration reductions observed with the administration of the other meals in the absence of elevated gastric pH indicated that other factors than pH should be playing a role in the meal effects [240]. Later, rat intestinal perfusion and permeability studies showed that indinavir is metabolized by CYP3A4 in both the upper intestine and the liver and it is also a substrate of intestinal P-gp [241].

The contradictory effect of food on saquinavir and indinavir BA at this point is not explainable, but the greater magnitude of food effect on saquinavir can be explained by the interaction between food and transporters. Similar to many other Biopharmaceutical Drug Disposition Classification System (BDDCS) Class II compounds (with low solubility and extensive metabolism), saquinavir is a dual substrate of CYP3A and P-gp efflux transporters [242]. The low solubility of saquinavir will limit the concentrations coming into the enterocytes. Thereby there will be little opportunity to saturate intestinal P-gp transporters and CYP3A enzymes. As a result, changes in expression, and inhibition or induction of efflux transporters will cause changes in its intestinal metabolism and will affect the extent of its oral BA. The majority of poorly soluble and extensively metabolized BDDCS Class II compounds have been found to be vulnerable to food effects, and in general high fat meal increases BA for Class II compounds, suggesting that high-fat meal results in an inhibitory effect on

intestinal transporter function [181, 242], which can be an explanation for five- to ten folds increase in saquinavir oral BA. Indinavir, on the other hand, although is similarly classified as a BDDCS Class II drug [242], has higher solubility than saquinavir. Higher solubility of indinavir, along with its high permeability will allow indinavir to pass through the plasma membranes more readily, leading to its higher concentrations in the GI tract/gut and a higher driving force for absorption as compared to saquinavir. The higher intestinal indinavir concentrations can saturate P-gp transporters, making intestinal transporters relatively less important in indinavir disposition. As a result, the influence of any interaction between food constituents and transporters would be less reflected on indinavir oral BA.

Several studies have reported that bile salts and certain types of monoglycerides, breakdown products from a high-fat meal inhibit P-gp transporters [242-245]. In a study conducted by Custodio et al. [242], the inhibitory effect of monoglycerides on the P-gp efflux of vinblastine was demonstrated in MDCK and MDR1-MDCK cell lines.

To date, a number of clinically important interactions between herb and drugs, many of which are substrates for CYP enzymes and/or P-gp transporters, have been reported. The common drugs that interact with herbal medicines include warfarin, midazolam, digoxin, amitriptyline, indinavir, cyclosporine, tacrolimus, and irinotecan. Common herbal medicines that interact with drugs include St John’s wort (Hypericum perforatum), ginkgo (Ginkgo biloba), ginger (Zingiber officinale), ginseng (Panax ginseng), and garlic (Allium sativum). For example, St John's wort significantly reduced the BA of cyclosporine, midazolam, tacrolimus, amitriptyline, digoxin, indinavir, warfarin, phenprocoumon and theophylline. The flavonoids and other ingredients present in fruits, vegetables and herbs have been found to modulate the activity of P-gp [246] and BCRP transporters [247]. For instance, estrone, 17-β-estradiol, and dietary flavonoids such as chrysin and biochanin are known as BCRP inhibitors [247].

One of the most recognized food-drug interactions is the ingestion of grapefruit juice with drugs. A significant amount of research has been done on the effect of grapefruit on drug disposition. Studies have shown that grapefruit juice mainly enhanced the BA of P-gp and CYP3A substrates by inhibition of CYP enzymes and P-gp mediated intestinal efflux while it reduced the BA of OATP substrates, by its inhibitory effect on OATP uptake transporters. Several studies reported that concomitant ingestion of grapefruit juice increased the oral BA of cyclosporine (a substrate of CYP3A and P-gp) [84, 248-250] and talinolol (a substrate of P-

gp transporters) [251], suggesting the inhibitory effect of grapefruit juice on CYP3A enzymes and especially on intestinal P-gp transporters.

A case study reported a possible interaction between pomelo and tacrolimus, a substrate of CYP3A and P-gp. Monitoring the blood concentrations of tacrolimus in a patient revealed higher concentrations than usual after the intake of a fresh pomelo [252].

Furanocoumarins in grapefruit and related citrus fruits are known to inhibit CYP3A enzymes. Several studies investigated the possible relationships between different furanocoumarins and extents of inhibition of microsomal CYP3A activity, *in vitro*. The results suggested that all the major furanocoumarins such as 6',7'-Dihydroxybergamottin and Bergamottin in grapefruit, Seville orange, and Pomelo juice contributed to the CYP3A inhibition [250, 253, 254]. In a clinical study with felodipine, a substrate of CYP3A4, an increase of 76% and 93% in plasma concentrations of felodipine was observed after Seville orange juice and grapefruit juice ingestion. The increased exposure was explained by inactivation of intestinal CYP3A4 by Bergamottin and 6',7'-dihydroxybergamottin [166]. Flavonoids (e.g. naringin) are another class of compounds in grapefruit that can inhibit CYP3A and P-gp. However, they are generally considered to play a minor role in the overall grapefruit juice effect *in vivo* [255].

The interactions between food constituents and transporters have also been demonstrated for transporters other than P-gp. Many studies have documented diminished oral BA of OATP substrates when taken with fruit juices due to the inhibition of these uptake transporters. Decreased BA of OATP1A2 substrates such as celiprolol, acebutolol, talinolol, L-thyroxine with grapefruit juice [256, 257], and that of atenolol, ciprofloxacin, and celiprolol with orange juice [258, 259] have been reported. The oral BA of OATP2B1 substrate, aliskiren, was reduced to clinically relevant extent in concomitant ingestion with grapefruit [260]. Reduced BA of fexofenadine, OATP1A2 substrate, in concomitant ingestion with grapefruit and orange juices was observed in different studies [261-263]. Dresser et al. [263] showed that grapefruit, orange, and apple juice decreases fexofenadine BA by 30% to 40% in human subjects. Based on *in vitro* drug transport study results grapefruit, orange, and apple juices inhibit the uptake of fexofenadine in a concentration-dependent manner by inhibiting OATP transporters [263]. Catechins in green tea (Camellia sinensis) were also reported to have an inhibitory effect on the function of OATP1A2 and OATP2B1 [264].

In summary, numerous studies have demonstrated the inhibitory or inductive effect of food constituents on CYP enzymes and transporters. The interaction between food constituents and CYP enzymes and/or transporters is of great importance in producing the food effect and altered BA of drugs [185, 234, 265-270].

3.7.2 Interaction of CYP Enzymes and Transporters with Excipients

Pharmaceutical excipients or non-medicinal ingredients are generally considered as inert substances in the dosage forms in that they are assumed to not influence the PK of API. Pharmaceutical excipients are rather known for their important role in enhancing the disintegration and/or solubilization of API, and governing the release of the API. However, accumulating evidence from the last decade studies has shown that routinely used pharmaceutical excipients are not pharmacologically inert and can alter drug disposition and BA by influencing CYP enzymes and efflux and influx transporters [271-273]. To date, more than 20 excipients have been shown to inhibit CYP3A4 function, either through suppression of gene expression at transcription and/or protein levels or via direct interference with enzyme activity. The induction of enzyme activity was uncommon, with only one case reporting the minor induction of CYP3A4 transcription [233]. The majority of the studies directly investigating the influence of excipients on CYP enzymes and transporters are limited to *in vitro* assays and *in vivo* animal studies. Only limited numbers of clinical studies are available in the literature that suggest the significance of excipients in governing the BA of API through interaction with CYP enzymes and transporters. In this section, we will present some of the *in vitro*, *in vivo* animal, and clinical studies, which highlight the influence of excipients on CYP enzymes and transporters.

The inhibitory effects of commonly used surfactants on CYP3A4 and other CYP enzymes were reported in several studies. Commonly used nonionic surfactants, Cremophor® EL and Tween® 80 (Polysorbate 80), significantly reduced the intrinsic clearance of midazolam, a CYP3A substrate, in rat hepatocytes and microsomes [274] and inhibited CYP3A4-mediated metabolism of testosterone in human liver microsomes *in vitro* [275]. Tween 20 and Tween 80 were shown to inhibit human cDNA expressed CYP3A4 at concentrations of 0.005% and above [273]. Similarly, PEG-40 stearate was shown to inhibit CYP3A4 activity in human liver microsomes [276]. In another study, PEG 400 has been shown to inhibit intestinal P-gp and CYP3A at concentrations of 1% and above in the excised

rat jejunum [277]. In a study performed by Ren et al. [278], the effects of Tween® 20, Cremophor® EL, PEG-40 stearate, and Pluronic® F68 on CYP3A were examined, using midazolam as a probe. All tested surfactants inhibited CYP3A-mediated metabolism of midazolam in isolated rat liver and intestinal epithelial cell microsomes *in vitro*. Martin et al. [279] also showed that Tween® 80 and Pluronic® F68 inhibited CYP3A4 activity *in vitro*.

Apart from interactions between excipients and CYP enzymes, many others have been documented between excipients and transporters. Common pharmaceutical excipients have been shown to inhibit or at least attenuate P-gp efflux function in the GI tract. Inhibition of transporters by excipients can result in a more than fivefold decrease in efflux, which may lead to a significant increase in the exposure of respective drug substrates [233]. In a study conducted by Zhu et al. [276] the inhibitory effect of polyoxyethylene (40) stearate on P-gp transporters was reported. Cosolvents (e.g., PEG 400) [280], the Cremophor® class of pharmaceutical excipients (e.g., Cremophor EL) [281, 282], and nonionic surfactants such as Tween® 20, and Tween® 80 have been identified as P-gp inhibitors [281, 283] and were found to enhance the transfer of P-gp substrates such as digoxin across the intestinal mucosa in different *in vitro* cell models by ≈2 folds. Although the inhibitory effect of Cremophor® EL on P-gp transporters and CYP3A enzymes was demonstrated in several *in vitro* studies, its influence on BA of drug substances in clinical studies was found to be unpredictable. In a clinical study conducted by Tomaru et al. [284], Cremophor® EL significantly decreased the BA of saquinavir, a substrate of CYP3A and P-gp while increased the C_{max} and AUC of another P-gp substrate, fexofenadine, by 1.3 and 1.6 folds, respectively.

Several *in vitro* and *in vivo* animal studies demonstrated the inhibitory effect of poloxamer type surfactants on P-gp transporters. The inhibitory effect of Pluronic® F68 on P-gp transporters was shown within *in vitro* and *in vivo* studies. In an *in vitro* study, Pluronic® F68 was shown to decrease the efflux ratio of P-gp substrate celiprolol by two-fold by inhibiting P-gp transporters in Caco-2 cells [285]. In an *in vivo* study in rats, rifampicin was orally administered with and without Pluronic® F68. Increased intestinal absorption of rifampicin was found and it was attributed to the inhibition of intestinal P-gp transporters by Pluronic® F68 [286]. Other poloxamer type surfactants such as Pluronic® P123 and Pluronic® F127 also inhibited the intestinal P-gp activity and decreased the efflux ratio by 6.8-fold and 1.6-fold in the human Caco-2 cell model, respectively. Pluronic® P123 and Pluronic® F127

enhanced the absorption of a P-gp substrate by 6.6-fold and 2.7-fold in rat everted gut sacs, an *in vitro* drug absorption model [287].

The inhibitory effect of excipients on transporters other than P-gp has also been reported. Cremophor® EL, Tween® 20, Span 20, and Pluronic® P85 were found to inhibit BCRP transporters [288-290]. Tween® 20 and Pluronic® P85 were also shown to increase the oral BA of topotecan by 2.0- and 1.8-fold, respectively, by inhibiting intestinal BCRP function, *in vivo* in mouse [289]. The inhibitory effects of PEG 400, Solutol HS 15 and Cremophor® EL on OATP transporters have also been documented [291].

Pharmaceutical excipients by influencing CYP enzymes and transporters as well as other mechanisms, can extensively alter physiological conditions and influence the BA of drugs. The unexpected effects of excipients on drug BA and BE outcomes will be further discussed in **Section 3.8**.

3.7.3 **Polymorphism**

Polymorphism is the genetic variations in the genes encoding proteins such as transporters and CYP enzymes. These variations in DNA sequence are often single nucleotide polymorphisms (SNPs) and can occur in either the coding region of a gene, causing an amino acid change, or in the regulatory region of a gene, thereby causing an alteration in the absolute amount of protein produced. As a result, polymorphism can influence the expression, localization or functionality of CYP enzymes and transporters [292, 293].

The normal catalytic speed of CYP enzyme activity is called extensive. There are two major metabolizer phenotypes due to polymorphism: poor (slow) and ultra-extensive (rapid) [294]. Reduced activity of a polymorphic CYP enzyme would lead to increased exposure and potential toxicity for the relevant substrate of that enzyme. Conversely, a drug may not reach a therapeutic concentration because the patient is an ultra-extensive metabolizer [295].

Accumulating data has shown that the frequency of functional SNPs varies widely between ethnic groups, and genetic polymorphism accounts for a large part of inter-ethnic differences in drug BA. The CYP2C19, CYP2C9 and CYP2D6 genes are highly polymorphic. The CYP2D6 gene is associated with ultra-extensive and poor metabolism, while the CYP2C19 and CYP2C9 genes are only associated with poor metabolism [296, 297].

In European Caucasians and their descendants, the functional group of CYP2D6 alleles is predominant with a frequency of 71%, while in Asians and their close descendants functional alleles represent only ~ 50% of the frequency of CYP2D6 alleles [298-300]. The prevalence of CYP2D6 poor metabolizers (PMs) is 7–10% in Caucasians, 3-8% in African-Americans/blacks, and less than 2% in Asians [296, 300].

The two common defective alleles that result in a non-functional CYP2C19 enzyme are CYP2C19*2 and *3 alleles. These two alleles account for almost all PMs in Asian and Black African populations. The main defective allele, CYP2C19*2, accounts for 75%-85% of the CYP2C19 alleles responsible for PMs in Orientals and Caucasians [301]. The second variant allele, CYP2C19*3, is extremely rare in Caucasian populations, while it accounts for almost all the remaining defective alleles in Orientals [302]. The frequency of CYP2C19*2 allele in the Chinese population (30%) has been reported to be twice the frequency in blacks (17%) or Caucasians (15%), and CYP2C19*3 allele was shown to occur in approximately 5% of Chinese but less than 1% of blacks or Caucasians [303]. Many other studies similarly reported that the prevalence of PM genotype of CYP2C19 is much lower in White U.S. or European populations, and African-Americans/blacks (3%-5%) than in Japanese, Asian, or Oriental populations (18%-23%) [296, 301, 304, 305]. The fourfold larger AUC of omeprazole, a CYP2C19 substrate, in Asian patients compared to Caucasian ones, indicated in the label [306], is in support of this information.

For CYP2C9, the variant alleles, CYP2C9*2 and CYP2C9*3, result in decreased enzymatic activity. The frequency of these alleles in Asians is higher than in Caucasians, and the frequency in African Americans is significantly lower than in Caucasians, leading to specific race or genetic information on warfarin label as per the US FDA [307, 308].

Ethnic differences in allele frequencies and functional activities of genetic variants are not limited to CYP enzymes, but also exist for transporters. The c.388A>G variant of OATP1B1 was found to show the highest expression of OATP1B1, approximately 1.5-1.3 folds greater than the wild-type allele. Clinical studies showed that the higher hepatic OATP1B1 expression of the c.388A>G variant could result in increased clearance (and therefore decreased exposure) of OATP1B1 substrates, pravastatin [309, 310], and repaglinide [311]. A high level of hepatic expression of this variant was found in Caucasian livers [312, 313]. An SNP of another OATP transporter, OATP2B1, was found in 31% of the Japanese population. This variant is associated with a greater than 50% reduction in transport capacity

[314]. In OATP transporters family, genetic variations for OATP1A2 have also been reported which markedly reduced the uptake capacity for the OATP1A2 substrates, estrone-3-sulfate and the d-opioid receptor agonists, *in vitro* [315].

The frequency of variant alleles of BCRP transporters with reduced efflux transport activity was reported to be 2% and 4% in White and Black populations, respectively, while it was reported to be 45% in South East Asians (non-Chinese, non-Japanese) [316-318]. The higher frequency of the BCRP polymorphism in the Asian population contributed to the increased systemic exposure of its substrates, statins, in Asians compared with Caucasians, as a result of the reduced BCRP-mediated enteric luminal efflux and/or biliary efflux of statins [319].

Inter-ethnic differences in drug exposure due to the genetic polymorphism of drug-metabolizing enzymes and transporters have led to different dosing recommendations for some drugs. Warfarin [308], rosuvastatin [320], tacrolimus [321], and carbamazepine with box warning [322] are some of the examples of currently marketed drugs with labelings that include dose recommendations for populations with specific race or genetic characteristics. As per the label of Warfarin, a substrate of CYP2C9, Asian patients may require lower initiation and maintenance doses, partly due to the higher prevalence of polymorphic CYP2C9 enzyme with reduced enzymatic activity in the Asian population [308].

In the tacrolimus label, no significant PK difference among the African American, Latino American, and Caucasian ethnic groups following IV tacrolimus infusion is documented. However, after single oral dose administration of tacrolimus, the C_{max} in African Americans (23.6±12.1 ng/mL) was significantly lower than in Caucasians (40.2±12.6 ng/mL) and Latino Americans (36.2±15.8 ng/mL), leading to specific dose recommendations in the tacrolimus label. As per tacrolimus label, a higher dose in Black patients is required to attain comparable trough concentrations to Caucasian patients [321]. Lower oral BA of tacrolimus in African Americans than in non-African Americans and Caucasians was also concluded elsewhere. 1.2–1.8 times greater oral BA in White and Latin American subjects was reported compared to African American subjects [323, 324]. Lang et al. [325] suggested that African American people are characterized with more active CYP3A enzymes than White people. Hence, the lower BA of tacrolimus in African Americans was concluded to be a function of differences in the level of activity of intestinal P-gp and CYP3A enzymes that may exist between different ethnic populations [324].

Similarly, the ethnic differences in the PK of drugs such as cyclosporine [326, 327], triazolam [325], nifedipine [328-331], and midazolam [332], have also been attributed to different levels of activity of CYP3A enzymes and P-gp transporters.

An FDA review of drug approvals between 2008 and 2013 found that approximately one-fifth of new drugs demonstrated differences in exposure and/or response across racial/ethnic groups [333]. As for rosuvastatin, lower initial starting doses in Asians have been recommended, but no clinically relevant differences in PK among Caucasians, Hispanics, Blacks, or Afro-Caribbeans have been observed [320]. Results of PK studies have demonstrated an approximate 2-fold elevation in median exposure in Asian subjects (having either Filipino, Chinese, Japanese, Korean, Vietnamese or Asian-Indian origin) living in Japan when compared with a Caucasian control group, which renders a dose of 40 mg contraindicated in Asians. Similar results have been observed between Asian racial subgroups and Caucasian subjects living in the same environment; plasma exposure to rosuvastatin was markedly (64% and 84%) higher in Asian racial subgroup (Chinese, Filipino, Asian-Indian, Korean, Vietnamese, Japanese) compared with Caucasian subjects, both residing in California, USA [334]. The same difference has been observed between Asian racial subgroups (Chinese, Malay, and Asian-Indian) and White subjects living in Singapore [335]. As specified in the monograph of rosuvastatin, it is a substrate of OATP1B1 and BCRP transporters. Therefore it is very probable that ethnic differences in its PK be due to the inter-ethnic polymorphism in its transporting pathways. In a clinical PK study of rosuvastatin [334], its exposure was found to be higher in subjects carrying the SLCO1B1 521C and ABCG2 421A alleles compared with the exposure in non-carriers of these alleles, suggesting that polymorphisms in the SLCO1B1 and ABCG2 genes contribute to the inter-ethnic variability in rosuvastatin exposure.

Another example of inter-ethnic differences in the BA of drugs is the case of simeprevir, a substrate of CYP3A4, P-gp and OATP1B1/3. In Phase 3 trials documented in the FDA's NDA Clinical Pharmacology and Biopharmaceutics Review, a 3.4-fold increased exposure of simeprevir in Asians compared to Caucasians is documented which was attributed to the saturation of its clearance pathway (i.e. CYP3A metabolism and OATP1B1/3 hepatic uptake) that occurs at lower doses in Asians (≤100 mg QD) compared to Caucasians, likely due to the smaller liver size and lower abundance of functional CYP3A, P-gp and OATP in populations with Asian ancestry [336].

In summary, the literature data shows that the inter-ethnic polymorphisms in the level of expression and activity of intestinal and hepatic enzymes and transporters can lead to dissimilar drug BA across different ethnic or regional populations.

3.8 Impact of Pharmaceutical Excipients on Bioequivalence

Despite the conventional understanding, the literature data shows that pharmaceutical excipients can influence drug BA and consequently BE outcomes significantly.

The influence of excipients on BE outcomes depends on many factors such as the type and concentrations of the excipients relevant to the pharmaceutical formulation, different combinations of excipients in formulation, and PK and physicochemical characteristics of incorporated API as they can vary in susceptibility to excipients [231, 337-339]. For instance, drugs with high permeability are known to be less susceptible to excipient influence on their BA than drugs with low permeability, and therefore the influence of excipients on BE between their formulations is expected to be of no significance [121]. Another instance is that when the amount of excipient does not exceed the maximum amount used in a US FDA-approved product as per US FDA's Inactive Ingredient Search for Approved Products [340], the impact of excipients on drug BA and BE outcomes is not expected. Nevertheless, the published data shows that the influence of excipients on BE outcomes is unpredictable.

In a clinical study conducted by Chen et al. [271], the influence of two different sweeteners (sorbitol versus sucrose) on BE outcomes was studied for two drugs, ranitidine, a BCS Class III drug with low intestinal permeability, and metoprolol, a BCS Class I drug with high intestinal permeability. In this study, Ref and Test aqueous solutions of each API were prepared with the same amounts of sucrose and sorbitol, respectively. The results of the study showed that sorbitol decreased the rate and extent of exposure, but to different extents for ranitidine and metoprolol. The C_{max} and AUC of ranitidine from sorbitol Test solution were found to be ≈ 50% of those from sucrose Ref solution; however, C_{max} and AUC of metoprolol in the presence of sorbitol decreased only by 23% and 7%, respectively. Regardless, sorbitol Test aqueous solutions of both drugs failed to show BE to their Ref solutions with sucrose. This is an example study demonstrating not only the differential effect of excipients on drug BA depending on drug’s physicochemical characteristics, but also the impact of excipients on BE outcomes.

Another clinical study with remarkable importance in undermining the traditional assumptions is the BE study of a highly permeable, highly soluble drug, risperidone's oral solutions [231]. In this study a manufacturer developed two oral test solutions of risperidone containing 50 and 7 mg/ml of sorbitol in addition to the same qualitative and quantitative excipients included in the Ref product (Risperdal® 1 mg/ml oral solution). The influence of small amounts of sorbitol on the BA of a highly permeable, highly soluble drug, risperidone, from oral solutions was not expected. Nevertheless, both Test solutions failed to show BE to their Ref despite low intra-subject variability and sufficient power of the study. The results of this study showed that; (1) commonly used excipients can impact not only the BA and BE outcomes for drugs with low permeability, but also those of drugs with high permeability, high solubility which is in contradiction to what is stated in the regulatory guidance documents [121, 341], (2) excipients at much lower doses than maximum dose reported in literature and FDA-approved drugs can impact BA and BE, and (3) the impact of excipients on BA and BE should be expected even when they are used in solutions, where the release of the drug substance from the drug product is self-evident.

Another example illustrating the unexpected impact of excipients on BE outcome is the BE study of risperidone tablets incorporating Sodium Lauryl Sulfate (SLS) [231]. Generic risperidone tablets incorporating a small amount of SLS (3.64 mg) failed to demonstrate BE to the Ref product, although the Ref products also had SLS in an amount within the normal range for that type of dosage form according to the US FDA database [340]. This is happening when FDA Guidance states that using excipients that are currently in FDA-approved IR solid oral dosage forms will not affect the rate or extent of absorption of a highly soluble highly permeable drug substance that is formulated in a rapidly dissolving IR product which is the basis for current biowaiver practice for IR solid oral dosage forms.

Similar outcomes were observed in the BE study of generic alendronate tablet versus its Ref product, Fosamax® 10 mg tablet [231]. Generic alendronate tablets incorporated mannitol and SLS in the amounts within the normal range for tablets as per the US FDA database [340]. Nevertheless, generic tablets failed to demonstrate BE to the Ref. The presence of SLS in generic tablet caused a 5- to 6-fold increase in BA compared to Ref tablets.

In summary, the impact of excipient on drug BA and BE outcome cannot be predictable without conducting a clinical PK study.

The above examples are only some among many, demonstrating the impact of excipients on drug BA and BE outcomes. Many other examples are published in the literature [231, 271, 337, 342].

4 Bioequivalence for Topical Drug Products

Topical dermatological drug products are semisolid preparations such as creams, ointments, lotions and gels which are designed to deliver drug into the various layers of skin, for treating dermal disorders. These products are applied in very small doses (typically 2–5 mg of product/cm^2) and exert a local effect in the skin following application on the skin surface [12, 13].

The objective in application of topical formulations is to maximize drug concentration at the site of action within the skin. Topical dermatological drug products are generally not intended for systemic absorption and their application does not produce measurable concentrations of drug in an accessible biological fluid such as in serum, plasma, or urine [343]. Even when the drug is systemically absorbed and the systemic concentrations are measurable, there is no established link between the concentration of the drug in the systemic circulation and the clinical efficacy for topical dermatological products [344]. Therefore, commonly used *in vivo* PK-endpoint studies which are an established approach for BE assessment of systemic effective solid oral dosage forms are generally not applicable to topical dermatological drug products, and other approaches for BE assessment must usually be employed.

PK-endpoint studies for topical BE assessment can only be employed if there is a safety concern based on unintended systemic exposure such as lidocaine (ointment and patch), a local anesthetic drug, and diclofenac gel, an anti-inflammatory/analgesic drug as the only examples to this case; or if the drug product is intended for systemic absorption such as transdermal delivery system of estradiol [13, 198, 343]. The US FDA currently requires PK-endpoint studies for lidocaine ointment and patch and diclofenac gel [343, 345, 346], and for transdermal delivery systems of estradiol patches or extended release films [347].

Documentation of BE for topical dermatological drug products has been a challenge for manufacturers and regulators. Many attempts have been made to propose alternative methods for BE assessment of these products. The following sections present the accepted methods for regulatory purposes and the methods which are still under investigation.

4.1 Accepted Methods

4.1.1 PD-Endpoint Studies

The BE assessment with PD-endpoint studies is based on the measurement of a pharmacological response of a drug as a function time. Currently the Stoughton–McKenzie test, also known as vasoconstrictor assay or skin blanching assay, is the only PD approach accepted by the US FDA [155, 343]. However, this approach is limited only to topical dermatological corticosteroid drug products. This PD approach is based on the property of corticosteroids to produce a visible blanching response as a result of vasoconstriction of the skin microvasculature [348-350]. The extent of skin blanching presumably relates to the amount of the drug that enters the skin and thus becomes a basis for the comparison of drug delivery from two pharmaceutically equivalent corticosteroid products [350, 351]. For documentation of BE for topical dermatological corticosteroid products, the US FDA recommends conducting two *in vivo* studies in HVs: (1) a pilot study to identify the appropriate dose duration (DD_{50}) for use in the subsequent pivotal BE study, and (2) a pivotal BE study to compare test and reference products [155].

4.1.1.1 Pilot Study

The initial pilot study is conducted in order to establish the exposure-response relationship from which the appropriate dose durations for use in the subsequent pivotal BE study could be determined. Duration of exposure of the skin to the topical corticosteroids is used to control the dose [343]. Therefore, the exposure-response relationship for these products is characterized in terms of dose duration-response.

The pilot study is conducted using a range of dose durations of the same strength of the Ref product and measuring the PD response at each dose duration. To obtain the PD response for each dose duration, an Area Under the Effect Curve (AUEC) is calculated over a time course after drug removal, with time 0 hour representing the time at which the residual drug product was removed until 24 hours later ($AUEC_{(0\text{-}24)}$) using trapezoidal method [348, 349]. The calculated PD responses ($AUEC_{(0\text{-}24)}$) are then plotted as a function of dose duration to obtain the response vs. dose-duration relationship in terms of an *Emax* model (**Equation 11**). From the relationship, the dose duration that results in half-maximal response (DD_{50}) is

estimated. Once the DD_{50} is obtained, shorter dose duration calibrator ($D1$= 0.5* DD_{50}), and longer dose duration calibrator ($D2$= 2* DD_{50}) are determined.

$$\text{AUEC} = 0 - \frac{AUEC_{max} \cdot \ Dose\ Duration}{ED_{50} + Dose\ Duration} \qquad \text{(Equation 11)}$$

Characterization of the skin blanching-dose duration relationship is the focus of Article#2 of this thesis (**Chapter 3**).

4.1.1.2 Pivotal Study

In the pivotal BE study, replicates of Test and Ref products are applied to both arms for a dose duration approximately equal to DD_{50} determined from the pilot study. Untreated control sites are also assigned on each arm. The BE is assessed by comparing the local PD response of the test (generic) versus the Ref product at DD_{50} in evaluable subjects, that is, the subjects with an ability to discriminate between PD responses at $D1$ and $D2$ which is when $D2/D1$ ratio of PD responses meets specified minimum value of 1.25 ($\frac{\text{AUEC at D2}}{\text{AUEC at D1}} \geq 1.25$) [155, 343].

The AUEC values obtained from skin blanching study are generally a mixture of positive and negative values. For this reason, the standard TOST procedure [217] could not be implemented for BE assessment as the data would not permit log transformation. Accordingly, “Locke's method" is recommended by the FDA 1995 Guidance which could provide an exact 90% CI from untransformed data. To conclude BE, the ratio of the average AUEC response due to the test product (average of four replicates) to the average AUEC response due to the reference product (average of four replicates) is calculated and consequently the 90% CI for this ratio is constructed using Locke's method [343, 352, 353]. The generic and reference drug products should meet the acceptable BE criteria in which the 90 % CI is between 80.00-125.00% for the observed skin blanching response.

The PD-endpoint study for BE assessment of topical corticosteroids is relatively inexpensive, fairly reproducible, and requires a considerably lower number of subjects to obtain a sufficient level of sensitivity than BE studies with clinical endpoints performed in patients.

4.1.2 **Comparative Clinical Trials**

Comparative clinical trials are currently used to establish BE for most dermatological drug products except in the case of topical corticosteroids. These studies are usually conducted over several weeks and the endpoints are mostly assessed visually based on scoring scales or dichotomous endpoints where it is based on success (completely resolved) or failure (not resolved) scales. Therefore, they are in general less sensitive than other approaches. Furthermore, these studies require a large patient population (averaging from 200 to 300 patients), they are generally expensive, and labor-intensive. Given the limitations of clinical trials to establish BE, new approaches are needed [198, 354].

4.1.3 ***In Vitro*** **Studies**

Bioequivalence studies with *in vitro* endpoints rely on *in vitro* characterization of the proposed generic and reference products. This is directly based on the formulation performance of the drug products. As of 2012, the US FDA has begun accepting of *in vitro* endpoint studies for simple topical drug formulations on the basis of 21 CFR 320.24 (b) [355]. There are multiple tests that can be employed with this approach, namely *in vitro* rheological tests and *In Vitro* Release Testing (IVRT) [13, 198, 356].

In vitro rheological tests are used to assess the physicochemical properties of the formulation such as pH, viscosity, specific gravity or density, particle size distribution in suspensions or emulsions, extent of cross-linking in gels, arrangement of matter, state of aggregation, surface tension, buffer capacity (if the product contains a buffer), and droplet size distribution (if an administered as a topical spray). Physiochemical properties of formulations are commonly referred to as microstructure of formulation [13, 357].

Another *in vitro* approach is *In Vitro* Release Testing (IVRT). This approach uses diffusion cells such as the Franz diffusion cell system with a synthetic membrane that separates the product from a receptor [13]. The IVRT approach can be used to estimate the rate of drug release from formulation. A difference in the drug release is expected to reflect the differences in physicochemical characteristics of the test and reference formulations.

The relevance, reliability and reproducibility of the *in vitro* approach have not yet been demonstrated to the level necessary for the regulatory purposes, due to many concerns such

as, but not limited to, the quality control of the experiments, method standardization (issues such as leakage of diffusion cells), the type and quality of the membrane, and the absence of an effective clearance mechanism from the membrane [12]. In addition, the literature data demonstrates contradictory results on the sensitivity of *in vitro* methods in detecting formulation differences [358, 359]. Therefore, *in vitro* methods are implemented only under certain conditions for the purpose of topical BE assessment. In general, the minimum requirement for this approach to be considered is that the generic must have the same ingredients (Q1) in the same amount (Q2) as the reference product and should not differ significantly in the microstructure of formulation (Q3) [13, 199]. If the generic product is Q1/Q2 equivalent with the RLD, *in vitro* characterization to show Q3 equivalence may be sufficient to demonstrate BE and *in vivo* testing may be waived depending on the *in vitro* results [13, 360]. When the test product is not Q1/Q2 equivalent, both *in vitro* and *in vivo* tests are usually required to demonstrate BE [13].

4.2 Promising Approaches

Given that the BE assessment of topical dermatological drug products is mostly limited to the comparative clinical trials and given the caveats associated with this method, significant efforts have been directed to alternative methods with surrogate markers, better sensitivity, and lower cost. A number of methods, such as the tape stripping technique for measuring drug quantity in the skin termed dermatopharmacokinetics (DPK), confocal Raman spectroscopy, and microdialysis have been developed and are still under investigation. Among them tape stripping is considered to be the most accurate, sensitive, reproducible, and non-invasive method from which the rate and extent of exposure at the site of action, the skin itself, can be obtained [190, 357, 361, 362]. In Article #3 of this thesis, a novel approach for the BE assessment of topical acyclovir cream formulation is proposed based on the model-based analysis of tape stripping data (**Chapter 4**). For a better understanding of Article #3, tape stripping is described in more detail in the following subsection.

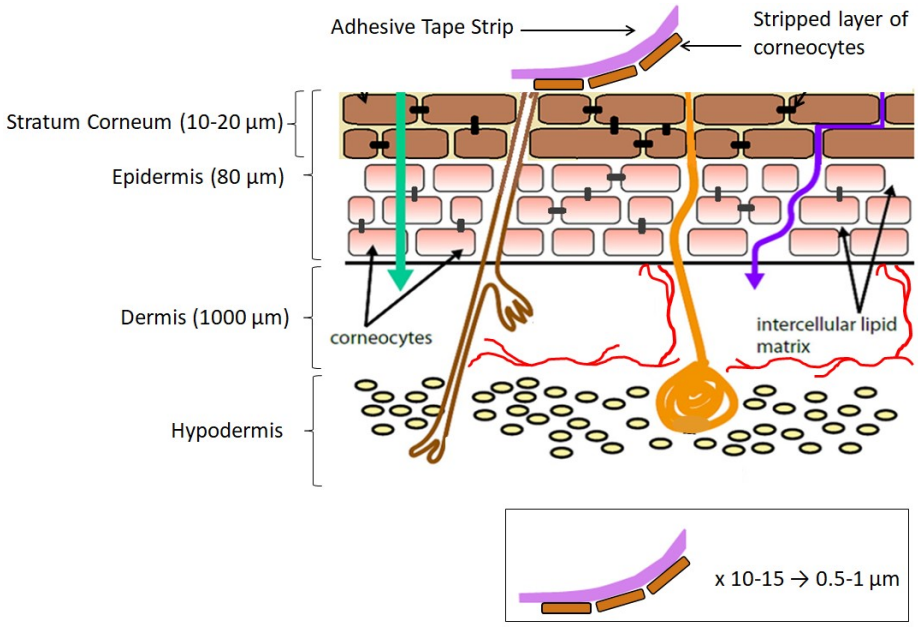
4.2.1 DPK Approach

The DPK approach for topical dermatological drug products could be considered comparable to the PK-endpoint studies for systemically available drugs [357]. Since the target organ of topical dermatological drug products is the skin, it is logical to determine drug PK in

the skin. From PK profile in the skin, the rate and extent of exposure at the local site of action, the skin itself, can be obtained, as the rate and extent of exposure in the systemic circulation can be obtained from plasma or serum PK profile for systemic effective oral dosage forms [12, 13].

The DPK approach includes measurement of drug quantities in the skin over a period of time. This may be possible by measuring drug quantity in the outermost layer of the skin (SC), termed tape stripping. Tape stripping is a process of sequentially removing microscopic layers (typically 0.5–1 µm) of the SC by successive application and removal of adhesive tapes (**Figure 15**), which are subsequently analyzed for the drug quantities. In this process, an adhesive tape strip onto the skin surface is placed, followed by gentle pressure to ensure good contact, and subsequently is removed by a sharp upward movement. The procedure is conducted in human subjects with healthy skin [12, 156, 362].

Figure 15. Removal of a layer of stratum corneum with an adhesive tape strip



Tape stripping is usually performed by applying a minimum of 12 to a maximum of 30 strips to the same site of the skin to collect the majority of the drug in the SC [156, 363, 364] and should be stopped either when the volunteer expressed discomfort, or when the value of Trans Epidermal Water Loss (TEWL) is 6-8 times greater than a baseline measurement made before tape stripping was begun. By this time, at least 75–80% of the SC is considered to have been removed [364, 365]. Complete removal of the SC may require over 70 tape strips [366,

367] and occurs when a constant value of TEWL is obtained. The implementation of TEWL in the DPK approach is discussed in more detail in **Section 4.2.1.1**.

When implementing tape stripping for BE assessment of topical drug products, a similar experimental design to crossover is used, in which Test and Ref formulations are tested in the same subject at the same time, on contralateral body sites. This study design is known as a bilateral paired comparison. In this design, the application sites are sufficiently separated to avoid radial diffusion of the tested formulation to the other site which is analogous to the washout period in crossover design for oral dosage forms. Therefore, a washout period is not necessary to be scheduled between the first and second periods of the treatments [352].

In DPK approach, usually two distinct studies are required for BE assessment, a pilot and a pivotal study [368]. A pilot study is conducted with the RLD product to validate the methodology and to optimize the sampling scheme. Within a pivotal study, the BE between the test and reference products is determined by comparing their PK profiles.

The underlying principle in the DPK approach is that the amount of drug recovered from the SC is directly correlated with the amount reaching the target site in deeper layers [306, 351, 369]. Regardless of how far through the skin layers (SC, epidermis, dermis), the drug needs to penetrate; it needs to pass through the SC first before reaching deeper skin layers. As a consequence, the amounts and the PK profile of the drug in the SC may theoretically be expected to be related to its amounts and PK profile in deeper layers such as the epidermis and dermis [156, 370]. This assumption is analogous to the one in traditional PK-endpoint studies for systemically delivered drug products, where the concentrations of a drug in blood, serum, or plasma are in equilibrium with its concentrations in the site of action [156, 357]. Therefore, two formulations that produce comparable SC PK profiles may be bioequivalent just as two oral formulations are judged bioequivalent if they produce comparable plasma concentration-time profiles [156].

4.2.1.1 TEWL in DPK

Stratum corneum is known for its efficient barrier function against water loss [371-373]. Therefore, water diffusivity across the SC is considered as the classic measure of barrier integrity and is expressed as Trans Epidermal Water Loss (TEWL). Differently said, TEWL is the flux (*J*) of water (g m^{-2} h^{-1}) across the SC and can be written in terms of Fick's First Law of Diffusion [10]:

$$TEWL = J = \frac{(K\,D)\Delta C}{L} = Kp\,\Delta C \qquad \text{(Equation 12)}$$

where K is the SC-viable tissue partition coefficient of water (which may be expected to be 0.06 [374]; D is the diffusion coefficient of water in the SC of thickness L (μm); and ΔC is the water concentration difference across the membrane (tissue concentration minus the one in the atmosphere above the skin surface, i.e., ΔC~ 55 M ≈1 g·cm^{-3}). The *Kp* (= $\frac{K\,D}{L}$) is called the permeability coefficient of water across the SC. As mentioned earlier, this equation assumes that the SC is the main barrier to water loss, and that it provides a homogeneous barrier to water diffusion, justified by Kalia et al. [375].

It has been shown that removal of the SC by sequential tape stripping causes a significant increase in TEWL [376, 377]. The baseline TEWL ($TEWL_0$) across unstripped SC of thickness L could be given by Fick's First Law of Diffusion as it was previously indicated in **Equation 12**:

$$TEWL_0 = J_0 = \frac{K\,D}{L}\Delta C \qquad \text{(Equation 13)}$$

The TEWL progressively increases as a function of the absolute depth (μm) of SC removed. After TS has removed a depth x of SC, the TEWL will have increased to a new value given by:

$$TEWL_x = J_x = \frac{K\,D}{(L-x)}\Delta C \qquad \text{(Equation 14)}$$

The value of baseline TEWL ($TEWL_0$) is usually ≤10 g.m^{-2}.h^{-1}. This value reaches 80-100 g.m^{-2}.h^{-1} when the complete removal of the SC occurs [378]. Complete removal of SC is not desired as it can be painful and may cause discomfort or even lesion in the skin. Therefore, during the tape stripping process, TEWL is measured in order to stop stripping before complete removal of SC.

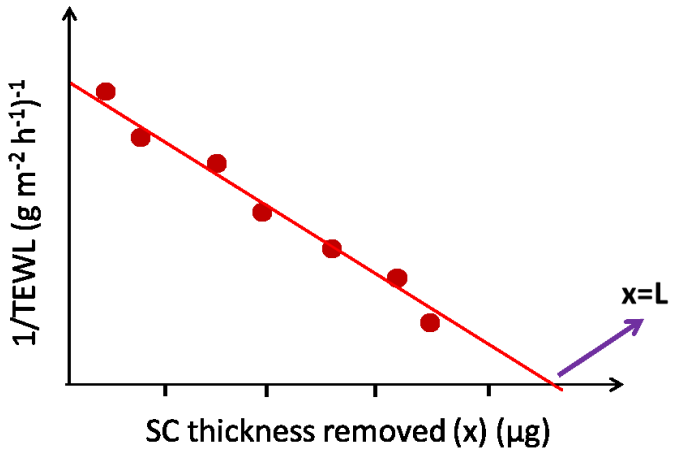
Implementation of TEWL measurement in the DPK approach is not limited to determining when to stop tape stripping, but also it is used to determine the total thickness of the SC which is briefly described in **Section 4.2.1.1.1**.

4.2.1.1.1 TEWL measurement in determining full SC thickness

The full thickness of the unstripped SC (L) can be deduced from the TEWL measurements during the sequential tape stripping process [365, 379]. To date, different approaches have been employed to determine the full thickness of SC.

The full thickness of SC (L) most frequently is obtained by the *Linear Model* approach. In the *Linear Model* approach, the SC is assumed to behave as a homogeneous diffusion barrier to water, i.e., D is independent of x, and therefore, D at x is equal to D at L; K is also independent of x and equals 0.06, determined earlier [374]. Then the inversion of **Equation 14** yields a linear relationship between $1/J_x$ (i.e. $TEWL_x^{-1}$) and the depth of SC removed (x). Extrapolation of the linear regression to 1/TEWL = 0 yields x = L. The x-axis intercept of the linear relationship between $TEWL_x^{-1}$ and x yields the full thickness of unstripped SC (L) (**Figure 16**) [179, 375, 379].

Figure 16. Determination of full thickness of the *stratum corneum* using Trans Epidermal Water Loss. The full thickness (L) is determined from linear regression of the plot of 1/TEWL versus SC thickness removed (x) assuming K = 0.06 and ΔC = 1 g.cm^{-3}. The Full thickness is equal to the intercept on the ordinate axis of the plot. TEWL, Trans Epidermal Water Loss; SC, *Stratum corneum*.



Alternatively, different *Non-Linear Models* have also been developed to estimate the full thickness of the SC (L) [380]. The details of *Non-Linear Models* are beyond the scope of this thesis.

4.2.1.2 DPK History

The DPK approach was evolved from a series of studies by Rougier et al. [370, 381] and was introduced in a Draft Guidance from the FDA in June 1998 [368]. Over the following four years, a number of concerns were raised, especially with respect to the reproducibility of tape stripping and its applicability in BE assessment [357, 369]. These concerns peaked when two different laboratories investigated whether the skin stripping could correctly demonstrate BE and distinguish non-bioequivalence, and found contradictory results in comparative studies using commercially available approved generic tretinoin gel (Spear Pharmaceuticals, Randolph, NJ, USA), its RLD Retin-A® (Valeant Pharmaceuticals North America LLC, Bridgewater, NJ, USA), and non-bioequivalent tretinoin gel (Avita®; Mylan Pharmaceuticals Inc., Morgantown, WV, USA). In the study conducted by Pershing et al. research group [382], the tretinoin concentrations in the SC were equivalent from the RLD and approved generic gel products, while it was significantly higher for RLD than non-bioequivalent formulation. In another study conducted by the Franz research group [383], however, tretinoin concentrations in the SC were lower for RLD than non-bioequivalent formulation. This discrepancy in the two study results eventually led to the withdrawal of the FDA Draft Guidance in May 2002 that recommended tape stripping as a method for topical BE assessment [382, 384, 385].

The apparent conflict in these results was subsequently determined to be attributable to differences in the design and methodology between the two studies, most notably the area of skin which was stripped outside the dosing region. Differences in lateral spreading for different formulations of tretinoin drug products influenced the amount of recovered tretinoin from tape stripping [385]. Since then, the FDA has been critically reevaluating DPK, with a view to improving sensitivity and reducing complexity, and validating the approach.

In 2007, the FDA's Critical Path Initiative has, one more time, suggested tape stripping as one of the potential approaches for the BE assessment of topical dermatological products. Many efforts have been directed to the refinement of tape stripping study design and DPK data analysis. None has been successful yet in adopting tape stripping back as a reliable and robust method for BE assessment of topical drug products for regulatory purposes.

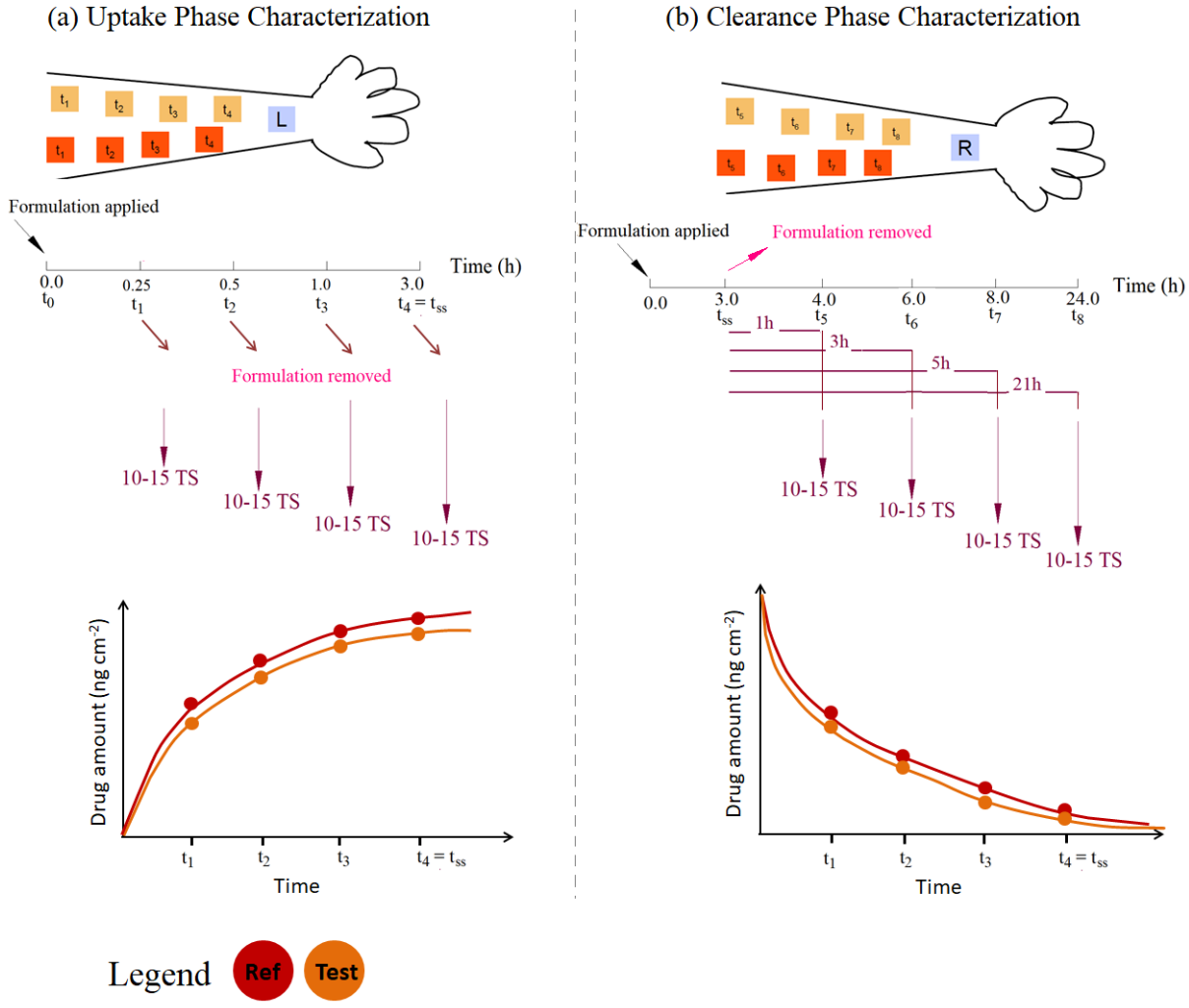
Among the tested methods, the *Relative-Depth* and *Two-Time* methods have been studied most widely. In the following subsections, the DPK approach which was recommended by the withdrawn FDA Guidance along with the *Relative-Depth* and *Two-Time* methods are described.

4.2.1.3 Different DPK methods

4.2.1.3.1 FDA Proposed method

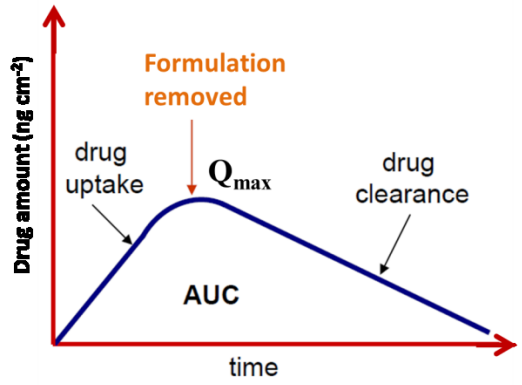
In traditional DPK approach, indicated as per the 1998 FDA Guidance [368], tape stripping should be conducted in no less than eight sites for each of the test and reference formulations: four sites on one arm in the uptake phase corresponding to four different drug exposure periods of time (dose duration), and four sites on the contralateral arm in the clearance phase corresponding to different post-drug removal time points. The general procedure for the *FDA Proposed* method of tape stripping is briefly illustrated in **Figure 17**.

Figure 17. Schematic representation of the traditional DPK approach for BE assessment between the test and reference formulations. (a) In the uptake phase, the SC is tape stripped immediately after each treatment time, and the drug level in the barrier is determined. (b) In the clearance phase, after the maximum treatment time, the SC is subsequently stripped after progressively longer periods post-removal of the formulations. TS, tape stripping.



In this approach, both the uptake and clearance phase of the drug in the SC should be characterized. The DPK profile consists of the total amount of drug in the SC per unit area (ng cm^{-2}) as a function of time (**Figure 18**) [12, 13, 156]. Classical PK measures of maximum drug quantity per unit area ($ng.cm^{-2}$) in the SC (Q_{max}), time to reach this maximum (t_{max}), and the AUC are deduced from the PK profile, in a similar fashion to PK measurements for an orally administered drug [12, 382]. The Q_{max} is simply the maximum value of SC drug content in the time course. The AUC is defined to be the integral of SC drug content per unit of surface area as a function of time since application and is calculated over the 0- to the last measured content (AUC_{0-t}) by the trapezoidal approximation [363, 386].

Figure 18. DPK profile as per the 1998 FDA Guidance



In the *FDA Proposed* method, the BE is recommended to be assessed by comparing the Q_{max} (also referred to as "C_{max}" in the literature) and AUC between Test and Ref.

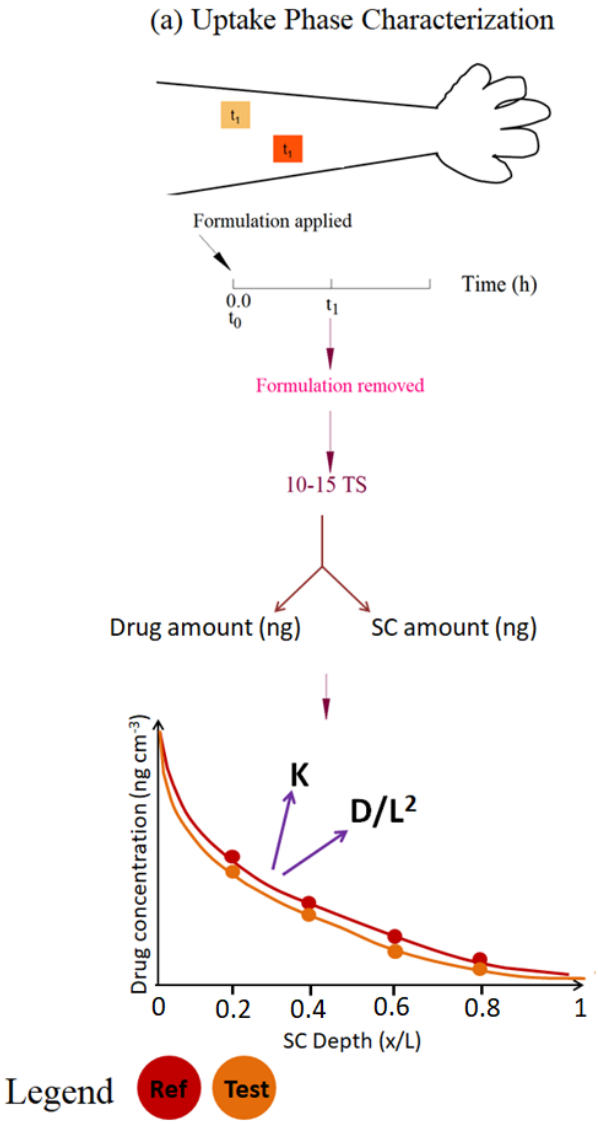
4.2.1.3.2 Relative-Depth method

In the *Relative-Depth* method, tape stripping is conducted only at one dose duration for each of the test and reference formulations in the uptake phase. The uptake of the drug into the SC is normalized by expressing drug concentration in the SC in terms of amount per unit volume or weight of SC [12, 179, 387].

In this method, the DPK profile consists of drug concentration (drug amount per volume of SC [mol/L]) vs. normalized depth of the SC removed (x/L), where x and L represent the thickness of SC removed and the full thickness of the unstripped SC, respectively [12, 179, 371]. Therefore, this method requires quantification of the mass of SC

removed in each layer (ng) and converting it to the SC thickness [365, 379]. The general procedure for the *Relative-Depth* method is briefly illustrated in **Figure 19.**

Figure 19. Schematic representation of the *Relative-Depth* method for BE assessment between the test and reference formulations. In the uptake, the SC is tape stripped immediately after the only studied dose duration. TS, tape stripping.



The most straightforward procedure to quantify the SC amount removed is to weigh each tape strip before and after SC removal and to determine the amount/mass (m; ng) of tissue removed from the difference between these weights. As the area (A; cm^2) of SC stripped is known, and the density (ρ) of the SC has been published (~0.8-1.3 g cm^{-3}) [378], it is possible to convert this mass into a depth, in other words, into the distance from the surface

of the SC (x; cm). The following equation is used to convert the mass into a depth [12, 375, 379]:

$$x = \frac{m}{A\,\rho} \qquad \text{(Equation 15)}$$

As described in **Section 4.2.1.1.1**, the full thickness of the unstripped SC (L) is deduced from the TEWL measurements using either *Linear Model* approach or *Non-Linear Models*. However, no single method has achieved universal acceptance yet.

Once the SC thickness removed (x) and the full thickness of the SC (L) are obtained, the SC concentration (C_x) versus normalized depth (x/L) profile can be generated. The C_x vs. x/L profile is then fitted to the appropriate solution of Fick's Second Law of Diffusion which assumes that the applied drug concentration (C_v) remains constant for the treatment period (t) and that the viable epidermis is a perfect sink for the drug. The third boundary condition is that the SC contains no drug at t = 0. Fitting the SC concentration–depth profile permits K and D/L^2 parameters to be deduced (**Figure 19**). K is SC-vehicle partition coefficient of the drug, reflecting the relative affinity of the drug for the SC compared to the applied formulation, and is related to the extent of absorption. D/L^2 is the drug diffusivity across the SC of thickness L. The ratio D/L^2 has units of $time^{-1}$ and can be considered as a first-order rate constant for drug transport/diffusion across the SC [179, 387, 388].

In the *Relative-Depth* method, the D/L^2 and K parameters are used as the rate and extent of absorption or exposure for the BE assessment [179, 371, 387].

4.2.1.3.3 Two-Time method

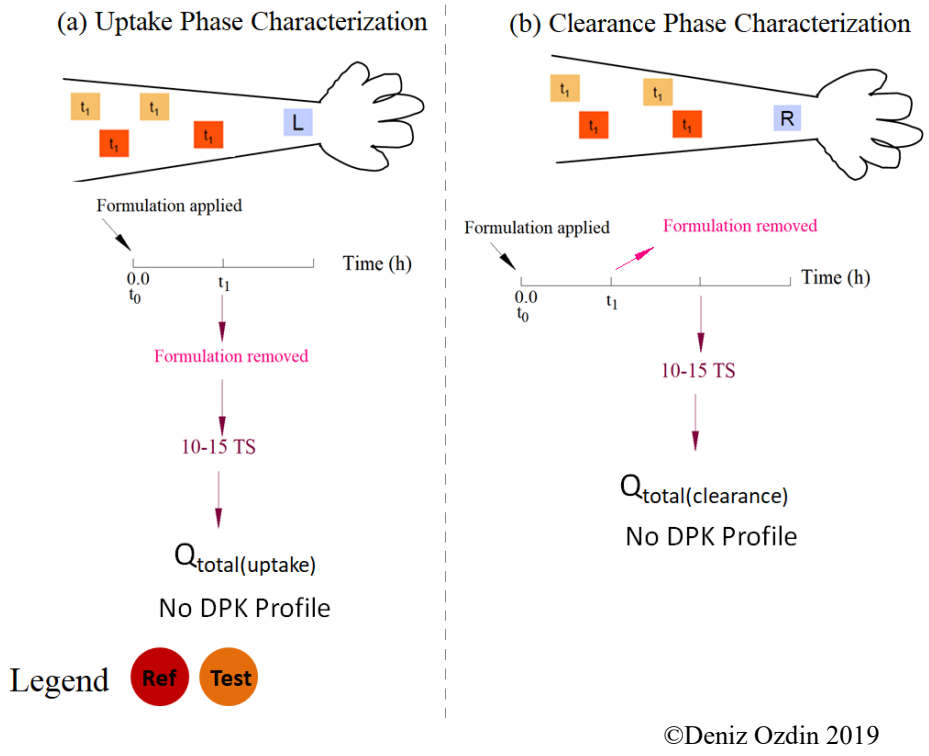
In the *Two-Time* method, tape stripping is conducted in four sites for each of the test and reference formulations: duplicate application on one arm for one dose duration in the uptake phase, and duplicate application on the contralateral arm in the clearance phase corresponding to the same dose duration post-drug removal time point [389-391]. The general procedure for the *Two-Time* method is briefly illustrated in **Figure 20**.

The *Two-Time* method is relatively a simpler DPK approach in comparison to the *Relative-Depth* method, as it does not require SC amount quantification on each strip and numerous assumptions. However, in this method, neither a DPK profile can be generated nor the rate of absorption or exposure can be calculated. Instead, the total amount of drug per unit

area in the SC (Q_{total}) is determined as the sum of the drug in all of the tape strip samples normalized by their sample area (ng cm^{-2}) [363, 364, 386].

In this method, BE is assessed using the ratios (Test/Ref) of total drug amounts in uptake ($Q_{total(uptake)}$) and clearance phase ($Q_{total(clearance)}$) [33, 47].

Figure 20. Schematic representation of the *Two-Time* method for BE assessment between the test and reference formulations. (a) In the uptake phase, the SC is tape stripped immediately after one dose duration. (b) In the clearance phase, the SC is stripped after a single period post-removal of the formulation. TS, tape stripping.



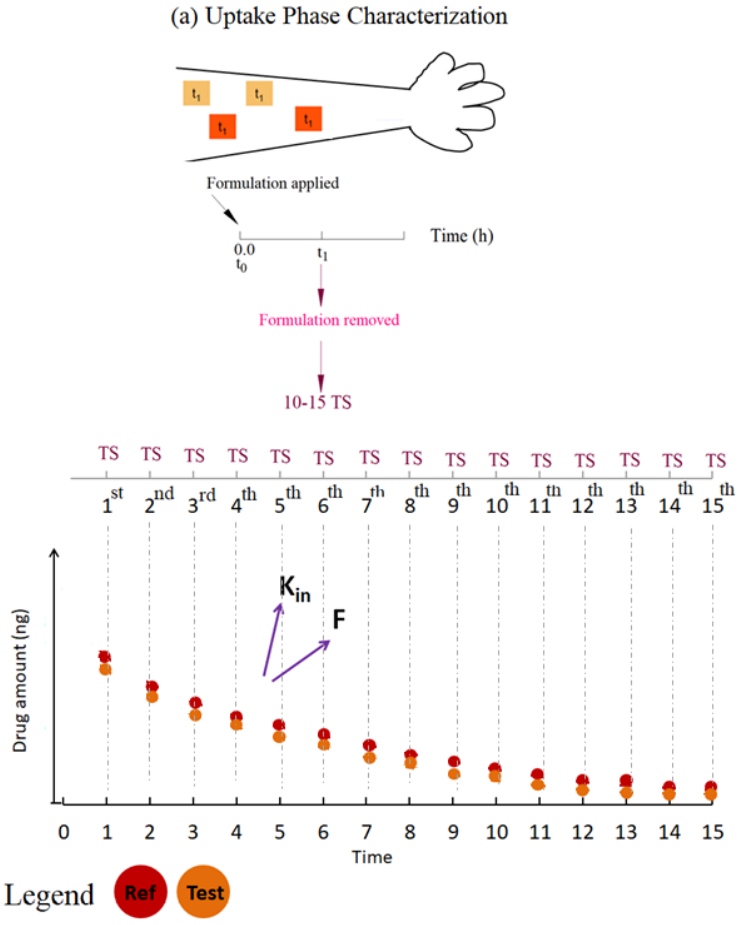
4.2.1.2.3. Our proposed method

In the Article #3 of this thesis, we present a novel DPK approach, within which tape stripping is conducted only at one dose duration for each of the test and reference formulations in the uptake phase. This dose duration corresponds to the DD_{50} which is primarily estimated from an E_{max} curve within a pilot dose duration study. Conducting tape stripping at DD_{50} during the uptake phase renders the procedure to be more discriminative of formulation differences. This method is comprised of duplicate applications of each formulation at the tested DD_{50}.

The DPK profile in our method is generated by plotting the drug amount (ng) versus time. Different than previously described methods, the time points on the DPK profile in our method do not represent different studied dose durations. Instead, each time point corresponds to each of the tape strips, all of which were taken at the same dose duration, DD_{50}. This is based on the fact that each stripping approximately takes 30 seconds. The general procedure for our proposed method is briefly illustrated in **Figure 21.**

In our proposed method, population CPT modeling is used to fit the DPK data and estimate the rate (K_{in}) and extent (F_S) of input into the skin (analogous to absorption). This method enables one to assess BE by comparing the direct measures of rate (K_{in}) and extent (F_S) of exposure. The reader is referred to **Chapter 4** for the details of this method.

Figure 21. Schematic representation of our proposed DPK approach for BE assessment between the test and reference formulations. In the uptake, the SC is tape stripped immediately after the sole studied dose duration, DD_{50}. TS, tape stripping.



5 Compartmental Analysis

Compartmental analysis, also known as the model-based approach, is an alternative approach to traditional non-compartmental analysis for the characterization of PK and/or PD of a drug by implementing mathematical and statistical models. Compartmental analysis is based on non-linear regression. In contrast with linear regression, where data are being fitted with a straight line defined by a slope and intercept, nonlinear regression depends on equations whose partial derivatives involve other model parameters [392].

Various types of CPT analyses exist, ranging from individual analysis to population PK modeling. The primary objectives of any modeling evaluation is to develop a model that can describe the PK and/or PD of a drug by predicting concentration values (or whatever observation is being studied) that are as close as possible to the observed values, and estimating the associated parameters such as clearance and volume of distribution of a drug [392, 393].

Models are usually the simplified representations of systems. Models provide a basis for describing and understanding the time-course of drug exposure and response after the administration of different doses of a drug to individuals. The fundamental building block of models is a hypothetical compartment in which the drug is well mixed and kinetically homogenous (and can, therefore, be described in terms of a single representative concentration at any time point). However, it does not necessarily represent any particular region of the body [394, 395]. Movement between compartments is comprised of rate constants, which are often labeled as K_{ij} (where i and j are different compartments). Compartmental analyses attempt to find the simplest model that explains best the observed concentrations while remaining true to being physiologically relevant [396].

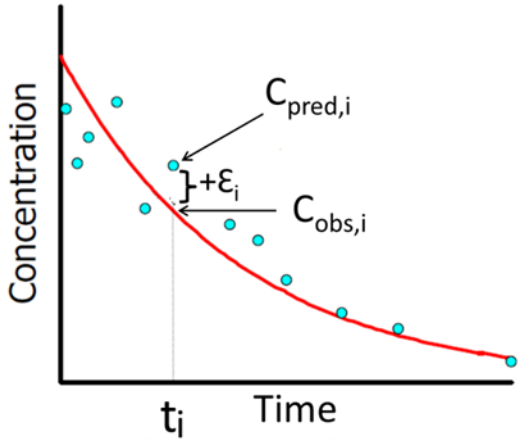
Generally, two types of models exist, PK models and PK/PD models. PK models describe the relationship between drug concentration and time, where drug concentration is the dependent, and time is the independent variable. PK/PD models include a measure of PD effect of a drug and are vital for linking PK information (e.g., concentrations, dose, or exposure measures such as AUC, C_{max}) to measures of pharmacological response and clinical outcomes. In PK/PD models, the independent variable is not time, but rather, a metric describing drug exposure (e.g., dose, AUC, C_{max}). The E_{max} models, which were described in **Section 2.1** are examples of PK/PD models [113].

In CPT analysis, a model is defined by integrated, matrix, and/or partial differential equations and then it is fitted to the PK and/or PD data to estimate the PK and/or PD parameters along with their variability and error components depending on the type of analysis (individual or population analysis). As mentioned earlier, the main purpose in developing a model is to minimize the differences between the model-predicted values and observations. The difference between predictions and observations (represented by $\mathcal{E}_i$) arises from the error that is always inherent in data, whether due to the collection procedures themselves, or due to analytical assays. Unlike NCPT analysis, which does not estimate $\mathcal{E}_i$, CPT analyses take these errors into consideration and try to find predicted concentrations to minimize the individual $\mathcal{E}_i$. In the simplest way, $\mathcal{E}_i$ can be demonstrated as below [397]:

$$C_{obs,i} = C_{pred,i} + \mathcal{E}_i \qquad \text{(Equation 16)}$$

where $C_{obs,i}$ is the i^{th} observed concentration in the individual, $C_{pred,i}$ is the i^{th} predicted concentration in the individual, and $\mathcal{E}_i$ is the difference between the predicted and observed i^{th} concentrations at time t_i (**Figure 22**).

Figure 22. The error Ɛi between predicted and observed concentration



In the following sections we introduce a function which is used to describe the difference between the model-predicted values and observations (**Section 5.1**), and the statistical solvers (estimation methods) for minimizing this difference (**Section 5.2**)

5.1 Objective Function

Estimating the "best parameters" for a model is the pivotal purpose of the modeling. The difference between each pair of observed (e.g., C_{obs}) and predicted (e.g., C_{pred}) values yields the residual ($C_{obs} - C_{pred}$). The best parameters achieve the lowest value of the sum of the squares of the residuals ($\sum(C_{obs} - C_{Pred})^2$). This sum of squares is considered as the Objective Function (OF) [394].

Objective function is a numerical value that can be used to guide the process of parameter estimation and to compare models in a sequential model building process. In some fashion, the OF quantifies the fit of the model to the data. Changes in the value of the OF following iterative changes in parameter values provides a stopping criterion, identifying the point where changes to model parameters no longer lead to essential improvement in model fit. The OF is computed with each iteration of the parameter estimation and continues until a minimum value of the objective function (MOF) is reached. The MOF for a particular model and data set is associated with the "best fit" parameter values [394, 397]. Different statistical solvers (estimation methods) to find the OF are described in the next section.

5.2 Estimation Methods to Minimizing Objective Function

The Ordinary Least Squares (OLS), Weighted Least Squares (WLS), Extended Least Squares (ELS), Bayesian (Maximum a Posteriori Probability – MAP), and Maximum Likelihood (ML) are the statistical solvers (estimation methods) to minimize the difference between the observations and model predictions [392, 393].

5.2.1 Least Squares Methods

The OLS method minimizes the squared errors between the observed ($C_{obs,i}$) and predicted ($C_{pred,i}$) concentrations and yields a value of the Objective Function (O_{OLS}) obtained in (**Equation 17**):

$$O_{OLS} = \sum_{i=1}^{n}(C_{obs,i} - C_{Pred,i})^2 \qquad \text{(Equation 17)}$$

Although it is a simple formula, OLS is not ideal if there is a wide range of concentrations. Because O_{OLS} is inherently biased to favor model estimates that provide better predictions for larger observations compared to smaller ones. As a result, higher concentrations will have a greater impact on the OLS function than lower ones. To address

this limitation, a weighting factor is often incorporated into the OLS function which gives rise to WLS and ELS solvers [398, 399].

5.2.2 Bayesian Method

The Bayesian method uses an Objective Function (OF) that takes into consideration the results of the individual and population. With this method, if an individual has many observations, the algorithm will add more weight to the individual's observations and the impact from the population parameter values will be minimal while the opposite is true when an individual has fewer observations. In this case, more weight will be given to the population PK parameter values and the subject's individual parameter estimates will tend to more closely resemble the population values [400, 401].

5.2.3 Maximum Likelihood Method

In the ML method, the observed data are approximated by a model function consisting of the parameters being estimated. This approach maximizes the probability of obtaining the observed data by estimating the best possible parameter estimates, which is known as log-likelihood (LL) [402]. The maximum likelihood estimation approach constitutes a large family of methods used in the population analysis, such as Iterative Two-Stage (ITS) and non-linear mixed effect population modeling methods [166, 403, 404].

5.3 Individual Analysis

Individual analysis involves the development of a model that only predicts the concentrations and PK parameters for each individual separately without considering any data from the other subjects in the analysis. Obviously, this analysis does not take into consideration the inter-individual variability and only has one error component, which represents the difference between the predicted and observed concentration (previously introduced as $\mathcal{E}_i$). Individual analyses could theoretically use different models to fit data from different subjects in a given situation (e.g., a one CPT model for some subjects and a two CPT model for others). Different algorithms have been developed for individual analysis. The reader is referred to the literature for further information [392].

5.3.1 Components of Individual Models

Traditional PK models for individual analysis are constructed of two main components, the structural part which is defined by fixed-effect parameters, and the statistical part which is commonly referred to as variance model.

5.3.1.1 Structural Model

Structural models are functions that describe the time course of a measured concentration or response, and can be represented as algebraic or differential equations. The number of compartments, their order, and their connections to each other are parts of the structural model.

Through the model fitting process, point estimates of PK (or PD) parameters are obtained. These parameters are called fixed-effect parameters and represent structural elements such as volume of distribution (V_d), clearance (Cl), elimination rate constant (K_{el}), and absorption rate constant (K_a) in a PK model. The standard nomenclature for a fixed-effect parameter is THETA (θ).

5.3.1.2 Variance Model

The variance model in the individual analysis includes only one level of random-effect which accounts for the variance of the differences between observations and the model predictions (i.e., residual error, denoted as $\mathcal{E}_i$).

Individual analysis is not the focus of this thesis and will not be described any further.

5.4 Population Analysis

Population (PK) analysis, or population (PK) modeling, is the study of the PK similarity and differences between individuals from measurements of drug concentrations in biological fluids of HVs or patients. Population modeling was first introduced in 1972 by Sheiner et al. [405]. Because of its superior robustness, the population approach is the preferred analysis when performing compartmental analyses.

The analysis of sparse data was a prime motivating factor for the development of population analysis. For traditional NCPT analysis, a sufficient number of samples from the studied population must be collected, which requires a rigid and extensive sampling design,

and this is mostly feasible in healthy adult volunteers [395, 406]. In practice, however, studied populations are often the patient groups from whom it is most difficult to obtain data. Using population analysis enables the study of sparse PK data from special populations such as the pediatric population, hepatic impairment patients, and critically ill patients that are likely to differ in PK disposition from the typical healthy adult volunteers [405].

The PK data inherently is associated with different levels of variability. The variability may arise from different sources and is generally considered under two main categories, inter-individual and intra-individual variabilities. Different characteristics of patients such as weight, age, renal function, and many other (patho)physiological factors contribute to the inter-individual variability. Even when a drug is administered to the same individual on two different occasions, the PK profile will not be exactly the same and there will be a certain extent of variability. This type of variability is called Inter-Occasion Variability (IOV) and can arise from different sources such as, but not limited to, the administered dose, collection procedures, bioanalytical assays, etc. Population analysis is distinguished from individual analysis in that the model not only predicts concentration values and PK parameters in a targeted population, but it also determines the dispersion of PK parameters by taking into consideration the inter-individual variability as well as the residual error (which includes intra-subject variability and measurement errors). Describing the variation of the PK parameters adds parameters to be estimated in population analysis.

In summary, population analysis describes the behavior of the whole population as well as the behavior of each individual within this population by partitioning of variability into inter-individual, intra-individual, inter-occasion, and residual sources [394, 407]. As a result, a proper population analysis not only predicts the results of the subjects that were analyzed but enables us to make inferences on the population and future outcomes.

5.4.1 Components of Population Models

Population models typically include all the components of the models in the individual analysis (structural and variance models), with the addition of the models that account for the magnitude and sometimes the sources of variability in structural parameters between individuals. Population models may also have covariate models that describe the influence of factors such as demographics or disease on the individual time course of the response, and explain a part of inter-individual variability [393]. In the projects within the context of this

thesis covariate models were not used, and therefore, they will not be discussed any further in this thesis.

5.4.1.1 Structural Model

With population models, fixed-effect parameters of the structural model define the typical values, or central tendencies, of the structural model parameters for the population. Thus, THETA is an estimate of the typical value, or central tendency, of a parameter (e.g., k_a) in the population [394, 397].

5.4.1.2 Variance Models

For population models, there are two levels of random-effects. The first level is at the parameter level and accounts for the variations in structural model parameters between individuals. The second level is at the observation (e.g., concentration) level for PK models.

5.4.1.2.1 Level 1 random-effects

First level random-effect parameters quantify the magnitude of unexplained variability in structural parameters (i.e., parameter-level random error). Level 1 random-effect may describe the magnitude of the differences in the values of fixed-effect parameters between subjects (i.e., inter-individual variability) or the differences between occasions within a subject (i.e., inter-occasion variability). The vector containing the individual subject estimates of the Level 1 random-effect parameters is denoted as η_i, where i stands for the individual subject. The Level 1 random-effect parameter that is estimated is the variance of the distribution of η_i values which is termed ETA and is output in the omega matrix (Ω) when using NONMEM® which is a Non-linear Mixed Effects Modeling tool.

If a population model is constructed with the following fixed and Level 1 random effects,

Ka = THETA(1)*EXP(ETA(1))

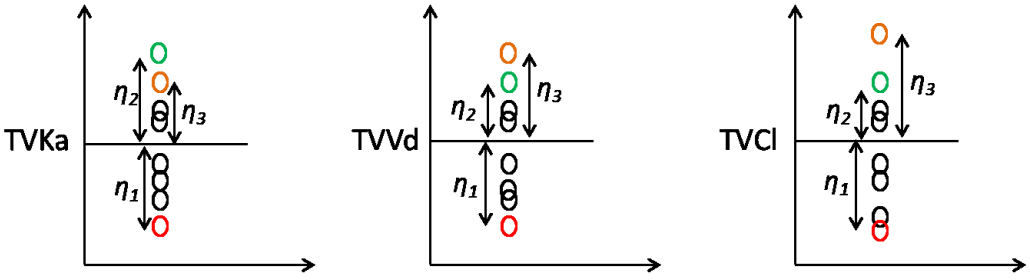
Vd = THETA(2)*EXP(ETA(2))

Cl = THETA(2)*EXP(ETA(3))

then each individual subject may have one ETA vector for each of the PK parameters. For instance, individual subject #1 will have $\eta_{1,Ka}$, $\eta_{1,Vd}$, and $\eta_{1,Cl}$ vectors and individual

subject #n will have $\eta_{n,Ka}$, $\eta_{n,Vd}$, and $\eta_{n,Cl}$ vectors. Level 1 random-effects parameter is illustrated in **Figure 23.**

Figure 23. Demonstration of inter-individual variability (η_i) for estimated PK parameters from population compartmental analysis. The η_i quantifies the magnitude of the difference between individual parameters and the typical value of the population.



TVKa: Typical value of population for Ka (THETA(1))
TVVd: Typical value of population for Vd (THETA(2))
TVCl: Typical value of population for Cl (THETA(3))

- Estimated Ka value for Subject 1
- Estimated Ka value for Subject 2
- Estimated Ka value for Subject 3
- Estimated Vd value for Subject 1
- Estimated Vd value for Subject 2
- Estimated Vd value for Subject 3
- Estimated Cl value for Subject 1
- Estimated Cl value for Subject 2
- Estimated Cl value for Subject 3

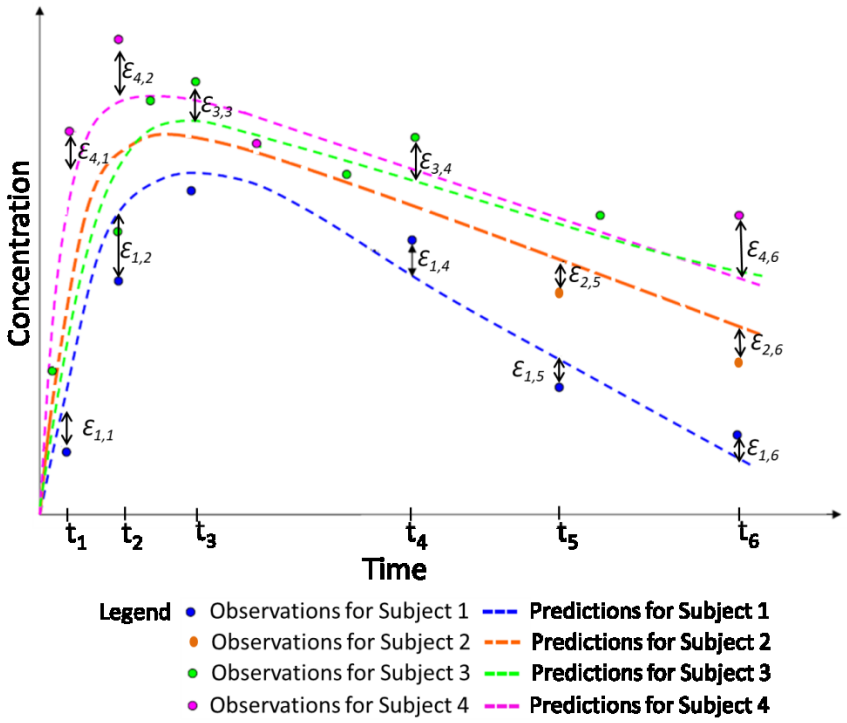
5.4.1.2.2 Level 2 random-effects

Second level random-effect parameters quantify the magnitude of the error in model predictions. In other words, they quantify the magnitude of unexplained differences between the observed values of the dependent variable (e.g., concentration) and their predicted values from the model (i.e., observation-level random error). Level 2 random-effects parameter exists at each time point of the PK profile for each individual subject and is denoted as $\mathcal{E}_{i,j}$, where i and j stand for individual subject and time point, respectively. For a concertation-time profile in the i^{th} individual, $\mathcal{E}_{i,j}$ can be defined as in **Equation 18**.

$$C_{obs,i} = C_{pred,i} \pm \mathcal{E}_{i,j} \qquad \text{(Equation 18)}$$

As per this equation, the observation-level random errors for individual subject #1 at time points 1, 2 and 3 are denoted as $\mathcal{E}_{1,1}$, $\mathcal{E}_{1,2}$, and $\mathcal{E}_{1,3}$, respectively, and for individual subject #2 as $\mathcal{E}_{2,1}$, $\mathcal{E}_{2,2}$, and $\mathcal{E}_{2,3}$. Level 2 random-effects parameter is illustrated in **Figure 24**.

Figure 24. Demonstration of observation-level random error, $\mathcal{E}_{i,j}$, on PK profiles from population compartmental analysis



The distribution of differences between the observed and predicted concentrations, $\mathcal{E}_{i,j}$, is called the residual variability. The Level 2 random-effect parameter that is estimated is the variance of the residual variability and is denoted as σ^2. The variance of the residual variability (σ^2) is output in the sigma matrix (Σ) when using NONMEM®.

Residual variability may arise due to the errors in the measuring or recording of the dependent or independent variables, or due to the use of an inappropriate structure of the model. The errors in the values of the dependent variable (e.g., the concentration values) may be due to errors in the sample collection, storage, or bioanalytical processes, and the errors in the values of the independent variable may arise from inaccurate recording of time of dosing, time of sample collection, or inaccurate measuring of a covariate such as body weight or height.

5.4.2 Estimation Methods in Population Analysis

Various types of estimation methods are available for population modeling such as the naïve-pooled data approach, Two-Stage approaches such as Standard Two-Stage (STS) and Global Two-Stage (GTS), and the most widely used one, non-linear mixed effect modeling approach [408, 409]. In the following subsections some of these methods are described with more emphasis on two types of the non-linear mixed effect modeling method, the First Order Conditional Estimation (FOCE) and the Maximum likelihood Expectation Maximization (MLEM) methods.

5.4.2.1 The Naïve-Pooled Data Approach

Sheiner and Beal [410] proposed the naïve-pooled data approach in which all data from all individuals are considered as arising from one unique individual and the data is fitted with least squares method. Naïve pooled data does not take into account inter-individual variability when estimating population parameters. As a result, population estimates poorly correlate with the observed data, and may not accurately represent the study population. With development of improved methods of estimation, naïve-pooled data is not a method of choice anymore [406, 411].

5.4.2.2 The Two-Stage Approach

The Two-Stage approach offers some improvement over the naïve-pooled data approach. With this approach, individual parameters are estimated in the first stage by separately fitting each subject's data using the same structural model. Once the parameters are obtained across individuals, population parameters will be estimated in the second stage. Different types of the Two-Stage approach exist, such as Standard Two-Stage (STS) and Global Two-Stage (GTS) [392, 411].

In the STS approach, PK parameters and residual variability are determined for each subject individually, and the population values are directly obtained from the individual results by simply taking the mean and variances of the individual results [165]. The advantage of the STS approach is its simplicity, but previous studies have shown that this approach tends to overestimate parameter dispersion [165, 408].

The GTS approach estimates expectations for the mean and variance through an iterative process [407, 408].

5.4.2.3 Iterative Two-Stage

The Iterative Two-Stage (ITS) method could be considered as the refined Two-Stage method that relies on repeated fittings of individual data. The ITS method utilizes a mixture of ML and MAP statistical solvers [408, 412].

As the name implies, the ITS method is implemented in two stages. In the first stage, the population model is used for Bayesian estimation of the individual parameters for all individuals, irrespective of the number of samples supplied by each individual. In the second stage, these new individual parameters are used to recalculate newer and more probable population parameters. Steps one and two are subsequently repeated until there is little to no difference between the new and old prior distributions (e.g., until the algorithm "converges") [392, 411]. A method close to ITS is the Expectation-Maximization (EM) method [413] which will be explained in **Section 5.4.2.4.1.**

5.4.2.4 The Non-Linear Mixed Effect Modeling Approach

The non-linear mixed effect modeling approach was developed by Sheiner et al. [405]. In non-linear mixed effect modeling, all data are modeled simultaneously while retaining individual information, in order to obtain estimates of population mean, inter-individual variability, and to quantify the sources of variability, even if the data is sparse and imbalance [393, 395, 407, 409, 410, 414-416].

Several estimation methods for non-linear mixed effect modeling have been improved over the last decades. Estimation methods for non-linear mixed effect modeling fall under two main categories, Expectation Maximization (EM)-like methods and First Order Conditional Estimation (FOCE)-like methods. Both EM-like and FOCE-like methods fit the data in an iterative fashion but in a different order. The details will be described in the following subsections.

Along with the estimation methods and the relevant algorithms, different software packages incorporating these algorithms have also emerged. The most commonly used software packages are NONMEM® and ADAPT-5® [166, 417].

NONMEM® stands for "Nonlinear Mixed-Effect Modeling". NONMEM® originally developed by L. B. Sheiner and S. L. Beal, and the NONMEM Project Group at the University of California [401]. The versions until 6.2 were available by the University of

California at San Francisco (UCSF). Starting from version 7.0, this software program is available by ICON Development Solutions. NONMEM® incorporates different algorithms for population analysis, including classical likelihood methods such as First-Order (FO) and First-Order Conditional Estimation (FOCE). The ITS, and Monte Carlo EM and Markov Chain Monte Carlo Bayesian methods have also been incorporated in NONMEM® version 7 [417].

ADAPT-5® stands for "Application Design for Analytical Processing Technologies" version 5.0.049 and above. ADAPT-5® is a well-established program that has been developed under the direction of David Z. D'Argenio in collaboration with Alan Schumitzky and Xiaoning Wang [418]. The program is supported by the Biomedical Simulations Resource (BMSR) in the Department of Biomedical Engineering at the University of Southern California, under support from the National Institute for Biomedical Imaging and Bioengineering and the National Center for Research Resources of the National Institutes of Health (NIH). ADAPT-5® incorporates Maximum likelihood Expectation Maximization (MLEM) algorithm, as well as other estimation methods such as ITS, STS, and naïve-pooled data modeling, each with WLS, ML, and MAP estimators [417]. In the following subsections the EM-like and FOCE-like methods are described in more detail.

5.4.2.4.1 Expectation Maximization

The Expectation Maximization (EM) method can be viewed as an extension of ITS where both random and fixed effects are included in the model. This algorithm was first implemented in the software P-PHARM® [403, 419]. Over the last decades, different types of EM algorithms have been developed and implemented in software packages. Some examples include Maximum likelihood Expectation Maximization (MLEM) in ADAPT-5®, Monte Carlo Parametric EM (MCPEM) in S-ADAPT®, NONMEM® and Phoenix®; Stochastic Approximation EM (SAEM) in Monolix® and NONMEM®; Monte Carlo Importance Sampling Parametric EM (IMP) in NONMEM®; and Quasi-Random Parametric EM (QRPEM) in Phoenix® NLME (Non-Linear Mixed Effect). It is of worth to mention that Kinetica® incorporates the EM algorithm that was originally in P-Pharm® [166, 404].

EM-like methods compute ML with an iterative approach that involves two repetitive steps: an expectation step (E-step) and a maximization step (M-step). In the E-step, parameter variables are estimated using the latest predicted parameter values and the observed data

(Bayesian estimation of the individual parameters). In the M-step, parameter values are estimated and updated to maximize the log-likelihood function in the E-step (estimation of population parameters). These new values are then reused for the subsequent iterations until the model converges (**Figure 25a**) [418, 420]. In the MLEM algorithm, ML is combined with an EM algorithm [421, 422].

EM-like methods use importance sampling-based estimation method, instead of linear approximation, therefore, the parameters obtained are true/exact ML estimates [420, 423].

5.4.2.4.2 First Order Conditional Estimation

As initially described by Lindstrom and Bates (28), the FOCE algorithm implements the estimation of maximum likelihood (ML) to solve the nonlinear problems. Therefore, the population mean estimates from FOCE-like methods are based on model approximation and not true ML estimators [424].

In FOCE method, inter-individual variability is estimated simultaneously with the population mean and the residual variability [392, 413].

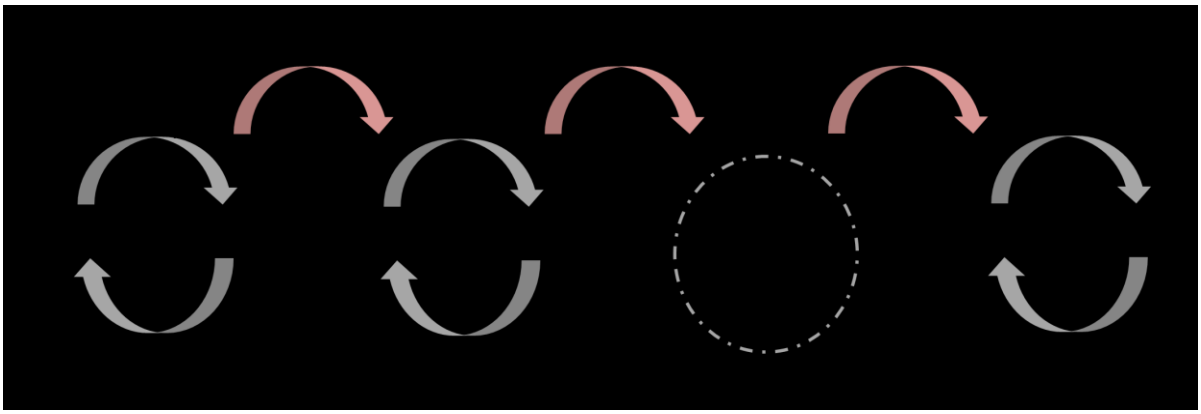
FOCE and similar methods first obtain population mean estimates, followed in a second step with individual data estimates in an iterative fashion. Unlike the EM-like methods, once the second step is accomplished, the iteration will not be repeated and the estimated values will not be updated (**Figure 25b**). Therefore, the difference between population and individual fits is usually larger than in EM-like methods.

In FOCE-like methods, the MOF is sought out by linearization of the model through a series of first order Taylor series expansions of the error model [165, 425].

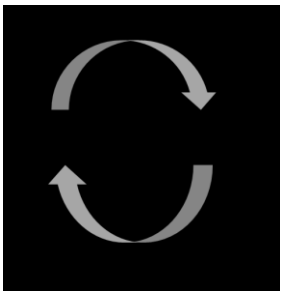
The FOCE and similar algorithms such as FO and Laplacian FOCE are implemented in NONMEM®. Other FOCE-like methods such as extended least squares FOCE-I and Lindstron–Bates FOCE have also been developed and are incorporated in other software packages such as Phoenix® NLME [417].

Figure 25. Comparison of iterative processes of EM versus FOCE methods. Due to the repetitive iterations in the EM method, the model convergence with EM usually requires longer time than with FOCE. E-step, expectation step; M-step, maximization step.

(a) EM



(b) FOCE



5.4.3 Applications of Population Analysis

Population PK analysis was initially developed as a tool to allow the extraction of useful PK data from sparse observational data [410]. Its implementation has soon been expanded to different stages of drug development and approval processes. The development of CPT analysis has dramatically enhanced the ability to pool data for analysis from different studies, subjects, and experimental conditions; to simulate new circumstances of drug product use; to evaluate the effect of demographic factors (e.g., weight, age, drug polymorphism), formulation, disease variables, and use of concomitant medication on inter-individual variability; to establish the dose-response relationship.

An important shift towards the use of population analysis in the drug development process of new drugs began in the 1990s. While regulatory submissions rarely included

population PK approaches in the 1980s, presently almost every new drug application to the FDA includes a population PK component in the submission. The use of population modeling in extending information from adult indications to pediatric indications, drug approval, and labeling decisions has widely been acknowledged [426-430].

The implementation of population CPT analysis in BE assessment has also shown significant progress over the last few years. Some examples include the use of population modeling in dose duration-response relationship establishment for BE assessment of topical corticosteroids [155]; BE assessment between two transdermal formulations of nitroglycerin [174]; model-based approach for BE assessment of two different formulations of alendronate [176]; dose-scale approach for BE assessment of inhaler type generic products such as albuterol sulfate [115] and locally acting oral dosage forms such as orlistat capsules [202]; and BE assessment between two acyclovir cream formulations based on tape striping data [173]. For other examples and the details of the methods, the reader is referred to a book chapter published by Colucci et al. [431] and other literature data [138, 432, 433].

6 Research Hypothesis

Theoretically, any generic drug that is bioequivalent to its reference counterpart is expected to be interchangeable with it. Therefore, the accuracy and reliability of BE study results are pivotal in ensuring the "*sameness*" between the generic and reference products in terms of clinical safety and efficacy. Enormous advancements in the field of bioequivalence and the standards set by regulatory agencies provide confidence to clinicians and patients that the quality of generic drug products and their clinical outcomes are indeed equivalent to reference products. Nevertheless, there are still controversies and the fields which are not yet studied sufficiently in the practice of bioequivalence. The objective of this thesis was to discover some of the controversial issues and challenges in the current practice of bioequivalence, to investigate the reliability of the bioequivalence study results when controversies present, and eventually, to propose novel alternative approaches when appropriate.

One of the most controversial issues in the current practice of bioequivalence is the extrapolation of pivotal bioequivalence study results from one population to another, such as from HVs to patients, from one ethnic population to another one. This is based on the premise that (a) PK BE studies are commonly conducted in a crossover design and, therefore, subjects act as their own control, and that (b) BE is assessed as the ratio of BA from a test versus a reference product. Therefore, the prevailing assumption is that even if the BA of Ref is different between two populations, the BA of Test would alter similarly, and the Test/Ref ratio would remain the same across the two populations. We hypothesis that due to the number of factors that have not been considered to date - such as the significance of transporters and CYP enzymes in governing drug BA pre-absorption and post-absorption, genetic polymorphism in transporters and CYP enzymes, and different types of interactions between transporters/enzymes and xenobiotics (food constituents, formulation excipients, API) - the prevailing assumption may not always hold true. As a consequence, BE study results may not be extrapolated from one population to another.

Challenges in the context of BE are not always limited to the pivotal studies where the performance of a generic product is compared to that of Reference, but could also be in preliminary pilot studies. In BE assessment of topical dermatological corticosteroids, response versus dose duration relationship should be characterized in a pilot study in order to determine

the population DD_{50}, the dose duration at which the PD response of the generic product is compared to its reference in the subsequent pivotal BE study. Therefore, any uncertainty or imprecision in estimated DD_{50} can influence the outcomes of BE assessment. The response versus dose duration relationship is commonly characterized using non-linear mixed effect modeling methods as it is also recommended by the US FDA 1995 Guidance [155]. Nevertheless, the FDA Guidance, the only regulatory Guidance document for BE assessment of topical dermatological corticosteroids available worldwide, does not specify which type of non-linear mixed effect modeling method could or could not be used. Given the availability of different types of non-linear mixed effect modeling algorithms, each sponsor could choose a different one.

It is also of value to recall that non-linear mixed effect modeling algorithms incorporate parametric methods for estimating the central tendency of population data. Therefore, certain distribution assumption should be made prior to the analysis. Nevertheless, the FDA 1995 Guidance does not recommend a particular type of distribution (normal versus ln-normal) to be assumed.

We hypothesize that (a) due to the different estimation methods and statistical solvers implemented in different non-linear mixed effect algorithms, employing different non-linear mixed effect algorithms in characterization of response versus dose duration relationship may yield to different DD_{50} estimates, rendering the BE study results unreliable. Additionally (b) due to the different shapes of normal and ln-normal distributions, the values of their central tendency vary. As a consequence, assuming different distribution profiles prior to population analysis may result in different population mean estimates of DD_{50}.

Another challenge in today's practice of BE, is the BE assessment of topical dermatological products, where conventional PK-endpoint studies are not applicable with few exceptions. A PK approach analogous to conventional PK-endpoint studies for topical drug products is quantification of drug in the outermost layer of the skin by sequential removal of microscopic layers of SC, called tape stripping. Nevertheless, due to the limitations and complexity associated with this method, presently it is not accepted for regulatory purposes. The complexities of tape stripping method mainly arise from study design procedure as well as sophisticated calculations and numerous assumptions in estimating the PK parameters for BA and BE assessment. The limitation of this method, on the other hand, is mainly due to the irrelevance of suggested PK parameters in reflecting topical BA and in BE assessment.

The three articles presented in the next chapters of this thesis will address the above-mentioned challenges that exist in the context of BE with the following objectives:

1. To investigate whether we can conduct PK BE studies in one geographical or ethnic population and extrapolate the results to another (Article #1).
2. To investigate whether using different non-linear mixed effect methods and different distribution assumptions yield to different population estimates, and whether additional pre-specifications are required when implementing compartmental approach in BE assessment of topical corticosteroids (Article #2).
3. To suggest a novel PK-based approach for BE assessment of topical dermatological drug products by implementing population compartmental analysis (Article #3).

While finding the results confirmatory on the current practice of BE can improve our confidence in generic drug products, obtaining contradictory results would raise a flag to practice BE with more caution, initiating reconsiderations in relevant fields.

Chapter 2

2 Influence of Different Populations on Pharmacokinetic Bioequivalence Results: Can We Extrapolate Bioequivalence Results from One Population to Another?

2.1 Preface

The first research project undertaken for this thesis aimed to investigate whether there is any concern in extrapolating BE study results from one population to another one. At present conducting pharmacokinetic BE studies in one population and extrapolating the results to another is a common practice. For instance, pharmacokinetic BE studies are mainly conducted in HVs, although the targeted population is patients. This is based on the assumption that the use of HVs should minimize both inter- and intra-subject variability, and therefore conducting BE studies in HVs would be more discriminative of formulation differences. However, the extrapolation of BE study results is not only done from HVs to patients, but also from one geographical or ethnic population to another.

Over the last 20 years, an ever-increasing proportion of pharmacokinetic BE studies for European and North American generic submissions appeared to have been performed in geographical or ethnic populations other than the intended ones, mainly due to the lowers cost of these studies outside North America and Europe. Regulatory agencies from these regions, the US FDA's OGD, HC, and EMA, currently accept pharmacokinetic BE studies that are performed in other regions. For instance, PK BE studies are now mostly conducted in India for the US, Canada, and European generic submissions. This is not the case, however, for regulatory agencies of Japan, Mexico, and Russia. Russia and Mexico require that pharmacokinetic BE studies be conducted in their own respective countries, and Japan requires that these studies be conducted in Japanese subjects whether they live in Japan or not.

The extrapolation of BE study results is, therefore, an accepted practice in many countries although the PK profiles and BA of a drug in different populations are known to be different. To date, many studies, animal models and clinical studies, have shown that the PK profile and BA of a drug alter in different physiological conditions [434-440], and physiological conditions per se change between different populations due to many factors such as, but not limited to, diseased conditions, genetic polymorphism, and different levels of expression and activity of CYP metabolizing enzymes and transporters. Nevertheless, the influence of altered physiological conditions on relative BA of drug products has conventionally been considered negligible. This is based on the premise that (a) PK BE studies are commonly conducted in a crossover design and, therefore, subjects act as their own control, and that (b) BE is assessed as the ratio of BA from a test versus a reference

product. Therefore, the prevailing assumption is that even if the BA of Ref is different between two populations, the BA of Test would alter similarly, and the Test/Ref ratio would remain the same across the two populations. For instance, if the Ref has lower or higher BA with food in population 1 but not in population 2, it will be the same for the Test, and therefore, the Test/Ref ratio will not change between the two populations.

In this research project, we hypothesized that the current assumptions would hold true if the absorption was unidirectional, which is often not the case. As described in **Section 1.2.1.1,** due to the presence of influx and efflux transporters at intestinal level, absorption is not unidirectional. Once a drug is absorbed from the intestinal lumen into the enterocyte, it can be ejected back into the lumen. This process can repeat multiple times and eventually determines the fraction of drug that is absorbed.

Moreover, the level of the expression and activity of transporters and CYP enzymes is influenced by different intrinsic and extrinsic factors. Formulation excipients and food ingredients as extrinsic, and polymorphism as intrinsic factors can influence the function of transporters and enzymes significantly. An increasing number of studies demonstrated the inhibitory and inductive effect of commonly used excipients on CYP enzymes and transporters [271-281, 283, 284], as described in **Section 3.7.2**. In addition, the presence versus the absence of an excipient but also different concentrations or different combinations of excipients can influence CYP enzymes and transporters differently [233, 271, 441]. Similarly, the influence of different food ingredients on transporters and CYP enzymes has been widely documented [181, 239, 241, 242, 246-250], as described in **Section 3.7.1**. Furthermore, the level of expression and activity of transporters and CYP enzymes have been reported to be different between different ethnic or racial populations [296, 304, 330], as described in **Section 3.7.3**. In a population with a lower level of expression or activity of transporters and CYP enzymes, the disposition of drug substances will be less regulated by these transporters and enzymes. Consequently, the impact of any interaction between enzymes or transporters with extrinsic factors (i.e., food ingredients and excipients) on drug BA is expected to be smaller.

The outstanding question is how this may affect the outcomes of bioequivalence studies. In order to extrapolate BE results from one population to another, two formulations assessed as bioequivalent in one population must be bioequivalent in another one as well. For two products to be bioequivalent in two different populations, they need to be bioequivalent

under both fasted and fed conditions in those two populations which requires having a similar food effect in both populations. The first article will test whether the food effect for the same drug product is the same between two geographical or ethnic populations.

2.2 Article #1

Influence of Different Populations on Pharmacokinetic Bioequivalence Results: Can We Extrapolate Bioequivalence Results from One Population to Another?

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To be submitted

2.2.1 Abstract

Purpose: Over the last 20 years, an ever-increasing proportion of pharmacokinetic bioequivalence studies for European/North American generic submissions appeared to have been conducted in geographical/ethnic populations other than the targeted ones. The results of pharmacokinetic bioequivalence studies have traditionally been considered to be insensitive to the population studied. Several recent studies have suggested that this may not necessarily be true. The objective of this study was, therefore, to investigate whether there may be concerns or not to the current practice of extrapolating bioequivalence study results from one ethnic population to another.

Methods: In order to extrapolate bioequivalence results from one population to another, two formulations assessed as bioequivalent under Fasted and Fed in one population must be bioequivalent in another one under both conditions. Unfortunately, BE study results between a generic and its Ref product are only filed using one population. Instead of comparing BE study results between two populations for the same generic and reference products, which are not available, we compared the Food-Effect for the same Ref product between two populations. This is based on the rationale that if two products are bioequivalent under both fasted and fed conditions in two populations, even if there are PK differences in the product exposures between these two populations, the Food-Effect of both Test and Ref products will remain constant. Food-Effects (Fed/Fasted ratios) were, therefore, calculated using pharmacokinetic data from publicly available regulatory resources and compared between two geographical/ethnic populations using the same Ref for each studied drug product. Meta-analyses were conducted.

Results: Significant statistical differences were found in Food-Effect results between two populations for nine out of the ten available studied products (90%). For three of these nine products, the observed differences may be clinically relevant.

Conclusions: These results suggest that BE results from one population may not always be representative of what we may find in another.

Keywords: Bioequivalence, Food Effect, Ethnicity, Polymorphism, Excipients, Transporters, CYP enzymes

2.2.2 Introduction

The bioavailability of orally administered drug products can be altered under different (patho)physiological conditions. To date, different studies in animal models and human subjects have revealed that altered pathophysiological conditions can affect not only the bioavailability of drug products (1-4), but also bioequivalence (BE) study outcomes. Drug products that were found to be bioequivalent in one condition, have been shown to not always be bioequivalent in altered physiological conditions (5-8). The US Food and Drug Administration (FDA) and other regulatory agencies require demonstration of BE under both Fasted and Fed conditions for generic submissions (9-11), as formulations may perform differently under these different conditions, and therefore two formulations assessed to be bioequivalent under Fasted conditions may not necessarily be under Fed conditions. Regulatory agencies typically recommend a high-fat, high-caloric meal in Food Effect (FE) bioavailability studies for NDAs and Fed bioequivalence studies for ANDAs (12), as BE would then be investigated in potentially extreme conditions (i.e., fasting and high-fat conditions). A high-fat, high-caloric meal would likely provide maximal gastrointestinal perturbation, rendering the physiological conditions more discriminative at detecting formulation differences.

The population for which the drug is intended to be marketed has to be part of the population(s) studied for pivotal Phase III studies. A new drug, therefore, cannot be marketed in one population without pivotal data obtained from the same population. In contrast, generic drugs are currently marketed for populations after being tested in different populations, an important factor for companies to ensure the lower cost of their generic products. It is not surprising, therefore, to learn that most generic products have been for a while now tested in Indian populations for submissions to “higher-cost” regions such as Canada, the USA, and Europe. Over the last 15 years, an ever-increasing proportion of Fasted and Fed PK BE studies for European/North American generic submissions appear to have been conducted outside of Europe/North America, especially in India. Extrapolation of BE results from one population to another has been historically considered to be a non-issue for many decades now, and is actually the reason for which BE studies are typically conducted in Healthy Volunteers (HVs) instead of patients in the first place. This is based on the premise that (a) PK BE studies are usually conducted in a crossover fashion and, therefore, subjects will act as their own control, and so even if they would present different PK characteristics,

these differences would apply equally to both crossover periods and therefore products, and that (b) bioequivalence involves the assessment of the relative bioavailability of a Test formulation in one period versus a Reference (Ref) one in another period of the study, and is expressed as a ratio.

We are noting, however, that these hypotheses would only hold true if the following two conditions are met: (a) absorption would be a unidirectional passage of drug molecules from intestinal lumen into the portal vein, meaning that once absorbed an active ingredient or moiety cannot come back in the gut lumen, and (b) excipients in Test and Ref formulations are exactly the same qualitatively and quantitatively (Q1/Q2) or if they are different they cannot produce a different effect on metabolizing enzymes and transporters in the gut wall between the two formulations.

Regarding the unidirectional passage of drug molecules, influx and efflux transporters localized in the enterocytes membranes have been shown to be key determinants of drug absorption, regulating the transport of drug molecules from extracellular to the intracellular environment and vice versa (13, 14). Once a drug is absorbed from the intestinal lumen into the enterocyte, efflux transporters at the apical membrane of enterocytes may drive it from inside the cell back into the lumen, thus "reversing" its absorption through the gut wall and subsequently into the portal vein. This process can be repeated multiple times predominantly in the small intestine and eventually determines the fraction of drug that will be absorbed. Therefore, contrary to the main general assumption, absorption is not always *unidirectional* and an active ingredient or moiety that is absorbed can be "de-absorbed" and come back in the gut lumen.

As for the excipients, an oral generic formulation does not generally have to contain the same inactive ingredients as a Ref one (16). Excipients are traditionally used in part to facilitate drug release and dissolution which are essential precursor steps for drug absorption, and their impact on bioavailability and therefore BE outcomes has been traditionally assumed to be negligible. Accumulating evidence is showing that many excipients can impact bioavailability and BE outcomes, not only by modulating drug release and dissolution, but also by their inhibitory/inductive effect on CYP enzymes and transporters that are present in the gut wall and elsewhere (17-19). Indeed, many studies have revealed that the presence of some excipients in one formulation, but not in another, had unexpected impact on the bioavailability of the drug product, and hence caused non-bioequivalence (20-22).

In summary, none of the two necessary conditions are met to support the traditional view that the studied population should not impact BE results. Therefore, bioequivalence outcomes between two populations need to be further studied.

There is evidence in the literature that the level of expression and the prevalence of functional variants of transporters and CYP enzymes can be different in varying populations. For example, transporters and CYP enzymes have been reported to be markedly lower in an Asian/Indian population compared to a North American/Caucasian one (23, 26-29). Consequently, if true, transporters/enzymes may theoretically play a less important role in drug disposition in an Indian population, and the impact of any interaction between enzymes/transporters, food and/or excipients on drug bioavailability may be expected to be smaller. Should gut transporters and/or enzymes be involved in FE, one could then hypothesize that a different FE would be expected in an Indian population versus a North American one for some products.

Many studies have also demonstrated that the large FE observed for some drug products cannot be solely explained by physicochemical interactions, and that the major reasons behind the observed FEs were, in fact, the interactions between food constituents and transporters/CYP enzymes (30-34).

Using the above discussed concept that drugs can be absorbed and then de-absorbed by transporters, the presence of food constituents and excipients affecting differently between populations the activity of transporters and enzymes in the gut wall may alter the bioavailability and BE of Test and Ref products between populations. Therefore, contrary to the historical common viewpoint that populations do not matter in the assessment of bioequivalence, it may be possible that changes in physiological factors between populations would result in different Test/Ref ratios between different populations. As a consequence, the BE study results may potentially be different between two populations.

A hypothetical situation is depicted in **Figure 1**, where bioequivalence of the same Test and Ref product is investigated in two different populations that differ in the level of expression or activity of transporters/enzymes. For simplicity purposes Population 1 (Pop 1) is assumed to have no enzymes/transporters, while Population 2 (Pop 2) has a high level of activity/expression of enzymes/transporters. In addition, we assume that the FE is only due to the interaction between food constituents and transporters, and no physicochemical factor is

involved. In Pop 1, if Test and Ref are assessed to be bioequivalent under Fasted conditions, they will readily be assessed as bioequivalent under Fed. In Pop 2, the same Test and Ref would be found to be bioequivalent under Fasted conditions, but not necessarily under Fed, due to the possible interaction between enzymes/transporters and food. Therefore, Test and Ref administered in Pop 2 may or may not meet bioequivalence criteria under Fed in such relatively extreme conditions in comparison to Pop 1. In summary, the depicted situation demonstrates the possibility of obtaining (1) no or lower FE in Pop 1 versus larger FE in Pop 2, as well as (2) different BE results in different populations.

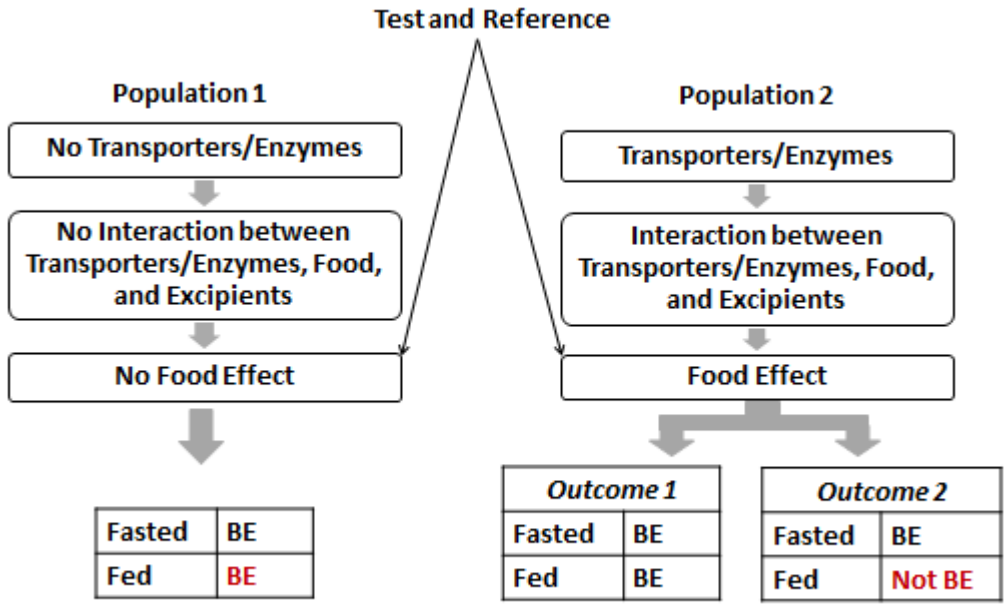


Figure 1. The hypothetical outcomes of performing pharmacokinetic bioequivalence (BE) studies in a population with different levels of expression or activity of CYP enzymes and/or transporters (Population 1) than the one intended to be marketed for (Population 2), and extrapolation of the bioequivalence study results. This figure illustrates that two formulations found to be bioequivalent in Population 1 (with no food effect) under both fasted and fed conditions, may not be bioequivalent in Population 2 (with food effect) under fed conditions (Outcome 2), due to the potential interaction between enzymes/transporters, food, and excipients.

On the other hand, for two products to be bioequivalent in two different populations, they need to be bioequivalent under both Fasted and Fed conditions in both populations which requires having the same FE in both populations. This can be better comprehended by an

example where the same Test and Ref products are given in two different populations under both Fasted and Fed conditions. Due to the influence of altered physiological conditions, the products bioavailabilities are different under Fed versus Fasted, and also in Pop 1 versus Pop 2. For instance, the bioavailabilities (AUC) of Test and Ref are 100 ng h mL^{-1} under Fasted versus 200 ng h mL^{-1} under Fed in Pop 1 while 50 ng h mL^{-1} under Fasted versus 100 ng h mL^{-1} under Fed in Pop 2. In this case, the ratio of Fed/Fasted in each population is equal to 0.5, implying the same FE in both populations (**Figure 2**). This example explains that if Test and Ref products are bioequivalent under both Fasted and Fed conditions in two populations, the FE for these products has to be also the same between the two populations. Otherwise, the Fed BE study conducted in Pop 1 will not be applicable to Pop 2, and as a consequence, extrapolating BE results from one population to another may not be possible.

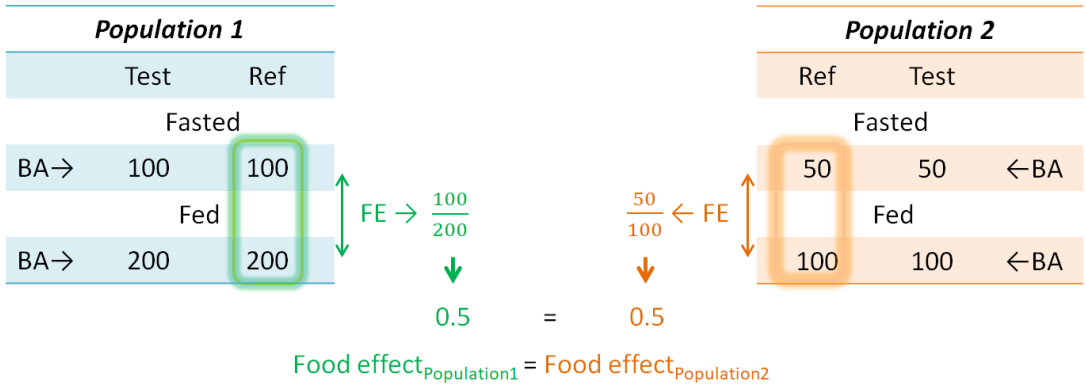


Figure 2. An example illustrating the same food effect between two ethnic/geographical populations despite different bioavailabilities of drug products under altered physiological conditions (Fed vs. Fasted; Population 1 vs. Population 2) when they are bioequivalent in both populations (Fed vs. Fasted; Population 1 vs. Population 2). BA, Bioavailability; Ref, Reference.

The main objective of this project is therefore to investigate whether BE study results from one ethnic population can be extrapolated to another one (different in ethnicity, but both consisted of HVs). In order to extrapolate bioequivalence results from one population to another, two formulations assessed as bioequivalent in one population must be bioequivalent in another one as well. In this retrospective study, it was impossible to compare BE study results of the same generic products versus the Ref in two different populations as generic firms only conduct their pivotal BE studies once in one population. Using the above discussed concept that for two products to be bioequivalent in two different populations, the FE also has

to be the same, we compared the FE results -instead of BE study results- for a given Ref product between two different populations. FE results in different populations can be derived as some sponsors conduct their BE studies in one population (e.g., in North America) while others conduct them in another one (e.g., India) for the same Ref products. The FE results are also a better measure as PK results may vary from one population to the next, so the mean exposure results (i.e., AUC and C_{max}) cannot be compared across populations. Comparing FE results, which are the exposure ratios of Fed to Fasted for the same Ref products, allow for appropriate comparisons between two populations. In this study, FE results for several Ref drug products between two or more different geographical/ethnic HV populations were calculated and compared using data from BE studies publicly available from different generic submissions.

2.2.3 Method

Data Extraction

A literature search for drug products with Fasted and Fed BE data was employed using Health Canada's Drug Product Database Online Query (35). All available product monographs by different manufacturers for oral pharmaceutical dosage forms (i.e. immediate-release and modified-release tablets and capsules) of each Active pharmaceutical ingredient (API) were screened. Only those providing the complete list of required information were included for FE calculations. The required information was extracted from *Part II* (*Scientific Information*), *Clinical Trials* subsection of product monographs, *Comparative Bioavailability Studies* (*Fasted and Fed*) in ANDSs, and only for Ref (US Reference Listed Drug or Canadian Reference Product). In parallel, the US FDA online database for FDA Approved Drug Products, Drugs@FDA (36); FDA's Clinical Pharmacology, Biopharmaceutics and Statistical Reviews in NDA of each drug were reviewed for FE bioavailability study results. When available, Fed and Fasted data were also extracted from FE bioavailability studies in NDAs.

Required information consisted of the following:

1. Population geometric means of ln-transformed AUC_{0-t} and C_{max}
2. Inter-individual variability (inter-CV%) of each PK parameter from both Fasted and Fed Comparative Bioavailability Studies
3. Studied population

4. Subject numbers
5. Date and site of the study

The labels of US Reference Listed Drug (RLD) and monographs of Canadian Reference Products were compared to ensure that they were exactly the same products when data from both US and Canadian Reference drug products were to be used.

The data was publicly available for two populations. Indian population was selected as Population 1 for which the studies were conducted in India, and North American population was selected as Population 2 for which the studies were conducted in Canada or in the US.

Assessment of Food Effect for Each ANDA/ANDS Study

Effect of food (FE) on bioavailability was assessed by calculating Fed/Fasted ratios of population geometric means (GMR) of ln-transformed PK parameters ($AUC_{0\text{-}t}$ and C_{max}) and the 90% Confidence Intervals (CIs) for this ratio using $AUC_{0\text{-}t}$ and C_{max} of Ref from *Comparative Bioavailability Studies (Fasted and Fed)* in the same ANDS. Since the Fasted and Fed PK data were collected from two independent groups of subjects with unbalanced sample sizes, the equation of CI was adapted to an unbalanced parallel design *via* a pooled standard deviation estimate (37):

$$\text{CI} = \text{Exp}\left[\left(\text{Ln}\frac{\mu_{fed}}{\mu_{fasted}}\right) \pm t_{1-\alpha,\, n_{fed}+n_{fasted}-2} \cdot SD_{pooled}\sqrt{\frac{n_{fed}+n_{fasted}}{n_{fed} \cdot n_{fasted}}}\right]$$

where μ_{fed} and μ_{fasted} are population geometric means of PK parameter under Fed and Fasted respectively, $\frac{\mu_{fed}}{\mu_{fasted}}$ represents the point estimate (PE), and $t_{1-\alpha,\, n_{fed}+n_{fasted}-2}$ denotes the critical value of the *t* distribution with $n_{fed} + n_{fasted} - 2$ degrees of freedom at the $1-\alpha$ probability level. The α = 0.05 was chosen based on the Two One-Sided Tests (TOST) procedure (38). SD_{pooled} is the pooled standard deviation which is calculated as the square root of the pooled error variance ($\sigma^2{}_{pooled}$) estimate:

$$SD_{pooled} = \sqrt{\sigma^2{}_{pooled}} = \sqrt{\frac{[(n_{fed}-1)\sigma^2{}_{fed}]+[(n_{fasted}-1)\sigma^2{}_{fasted}]}{(n_{fed}+ n_{fasted}- 2)}}$$

where $\sigma^2{}_{fed}$ and $\sigma^2{}_{fasted}$ are the variance estimates associated with the Fed and Fasted treatments, respectively. The variance associated with each treatment was calculated from the inter-individual variability (CV%) provided for Ref in that treatment. To convert

between a variance (σ^2) on the log scale and a CV on the observed scale, the following relation was applied:

$\sigma^2 = \text{Ln}\ (CV^2+1)$

Assessment of Summary Food Effect in Each Population

Fixed-effect model meta-analysis was performed to assess the summary FE for each drug in each region/population in terms of GMRs (Fed/Fasted) for C_{max} and $AUC_{0\text{-}t}$ and the 90% CI for the ratios, using the FEs calculated from all ANDS studies available in that population. Meta-analytic computations were performed manually (39) using Microsoft Excel 2010®.

Provided that the PK of the drug substance was linear, studies with different dose strengths for the same Ref product were included in the meta-analysis for the calculation of the summary FE. When more than one Ref was available for a drug, a literature search was performed to investigate the bioequivalence between the Ref products. Only the same (bioequivalent) Ref products were included in the same meta-analysis for the calculation of the summary FE.

Comparison of Food Effect between North American and Indian Populations for Significance

The summary FEs for the same Ref products were compared between the North American and Indian populations for a significant difference from both statistical and clinical standpoints.

Statistical significance: A subgroup analysis was performed to compare the summary FEs between Indian and North American subgroups for statistical significance using a Q-test based on analysis of variance. The difference in summary FE between the two populations was concluded to be statistically significant when $P<0.05$ for either of the PK parameters.

Clinical significance: A difference of ≥40% between the FEs of the North American and Indian populations ($\%\text{Diff}_{\text{inter-ethnic}}$) for either of the PK parameters was considered to be of possible clinical relevance. When food impacted exposure in the same direction in both populations, the difference was calculated as:

$\%\text{Diff}_{\text{inter-ethnic}} = |\%\text{FE}_{North\ American} - \%\text{FE}_{Indian}|$

When food impacted exposure in two different directions, the difference was calculated as:

$$\%\text{Diff}_{\text{inter-ethnic}} = |\%\text{FE}_{North\ American}| + |\%\text{FE}_{Indian}|$$

Between-Study Variability in Food Effect

Between-study variability in FEs of different studies in each population was assessed for each study drug using heterogeneity measures, tau-squared (τ^2) and I^2 indices. I^2 of 25%, 50%, and 75% were considered as low, moderate, and high heterogeneity, respectively (39). The heterogeneity measure (I^2) was then compared between the two populations.

2.2.4 Results

Figure 3 summarizes the study steps for meeting the objective (FEs calculations in two populations and their comparison), as well as for evaluating and comparing heterogeneity in each population. A total of 27 drug candidates with available Fasted and Fed BE studies in the literature were selected based on pre-established search criteria; however, only nine drug active ingredients (10 drug products) had all the required information for evaluating FE in two different geographical/ethnic populations and were therefore included in the data analysis. Data from 53 Abbreviated New Drug Submissions (ANDS) and six New Drug Applications (NDA) were included in this analysis. A list of the nine drug active ingredients, all available respective Ref products, and reported FE in the product labels is provided in **Table 1**. The table also includes which CYP enzymes and/or transporters are known at this time to be involved in the metabolism and disposition of these particular drugs (40-42).

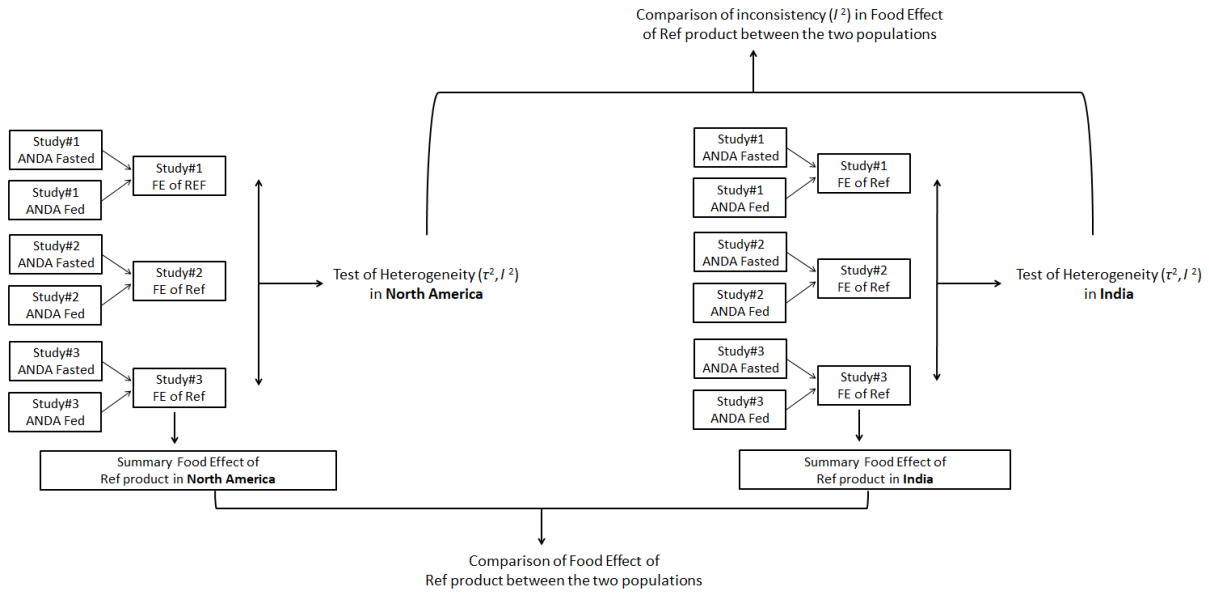


Figure 3. Study flow diagram. ANDS, Abbreviated New Drug Submission; τ^2, measure of heterogeneity (Between studies variance); I^2, measure of heterogeneity (degree of inconsistency in %).

Detailed FE results (point estimates and 90% confidence intervals) are summarized by drug, ANDS, and region in **Table 2** for $AUC_{0\text{-}t}$ and **Table 3** for C_{max}, and only for Ref products which had the required information available for summary FE calculations in both populations, enabling the comparison of FEs between them. All other Ref products with summary FE in one population, but not the other, were only used in the heterogeneity analysis. In North American population 11.1% and 44.4% of the studies were associated with heterogeneity above the level of significance ($I^2 \geq 75\%$) in terms of the $AUC_{0\text{-}t}$ and C_{max}, respectively, while 71.4% of the studies in Indian populations had significant between-study variability in terms of both exposure measures (**Table 4**).

When the FEs for different Ref products of a drug were similar (e.g., Nexium® 20 and 40 mg tablets of esomeprazole; Prilosec® 20 mg capsule, Losec® 20 mg tablet and capsule of omeprazole), for information purposes, the summary FE was calculated one more time by including all Ref products of that drug (**Table 5**, **Table 6**).

Table 7 specifies which Ref Products were used for the comparison of FEs between the two populations and summarizes the overall FEs (i.e., the ones calculated in this study and those from the Ref product labels) per drug and population, and the comparison between the two populations (statistical and clinical relevance).

For nine out of the ten drug products, the FEs were significantly different between North American and Indian populations statistically (Q-test; $P<0.05$) and for three of these nine the difference could be of possible clinical relevance ($\%Diff_{inter\text{-}ethnic}$ in FE≥40%).

Table 1 Included drugs overview

API	Reference Product	CYP Enzyme/Transporter [ψ]	Designated FE on the label (range)
Amiodarone	Cordarone® 200 mg Tab[ab]	Major substrate of CYP3A, CYP2C8 Minor substrate of CYP2D6, CYP1A2 P-gp/ABCB1	AUC increase x 2.4 (1.7-3.6) C_{max} increase x 3.8 (2.7-4.4)
Carbamazepine	Tegretol® CR 400 mg Tab[b]	Major substrate of CYP3A4 Minor substrate of CYP2C8	No significant FE
Diltiazem	Cardizem® SR 120 mg Cap[ab] Cardizem® CD 300 mg Cap[ab] Cardizem® CD 360 mg Cap[a] Tiazac® XC 360 mg Tab[b] Tiazac® ER 360 mg Cap[b]	Major substrate of CYP3A4 Minor substrate of CYP2C9, CYP2D6 P-gp/ABCB	No significant FE on extent of absorption No information in relation to C_{max}
Verapamil	Isoptin® SR 240 mg Tab[ab]	Major substrate of CYP3A4 Minor substrate of CYP1A2, CYP2C8, CYP2C9, CYP2C18, CYP2B6, CYP2E1	AUC decrease by 1-8% C_{max} decrease by 15%[$]
Esomeprazole	NEXIUM® 40 mg Tab[ab] NEXIUM® 20 mg Tab[ab]	Major substrate of CYP2C19 Minor substrate of CYP3A4	AUC decrease by 43.7%† C_{max} decrease by 68.3%†
Omeprazole	Prilosec® 20 mg Cap[a] Losec® 20 mg Cap[b] Losec® 20 mg Tab[b]	Major substrate of CYP2C19 Minor substrate of CYP2A6, CYP2C9, CYP2D6, CYP3A4	No FE
Lansoprazole	Prevacid® 30 mg Cap[ab]	Major substrate of CYP2C19, CYP3A4 Minor substrate of CYP2C9	AUC decrease by 50-70% C_{max} decrease by 50-70%
Pantoprazole Na	Pantoloc®40 mg Tab[b]	Major substrate of CYP2C19 Minor substrate of CYP2D6, CYP3A4	No FE
Pantoprazole Mg	Tecta®40 mg Tab[b]	Major substrate of CYP2C19 Minor substrate of CYP2D6, CYP3A4	No FE
Rabeprazole	Pariet™/® 20 mg Tab[b]	Major substrate of CYP2C19, CYP3A4	No FE

API, Active pharmaceutical ingredient; FE, Food effect; Tab, Tablet; Cap, Capsule; SR, Sustained release; CD, Controlled Delivery; CR, Controlled release; Na, Sodium; Mg, Magnesium.

[ψ] Extracted from literature [21-23].

[a] US Reference Listed Drug (RLD) Product

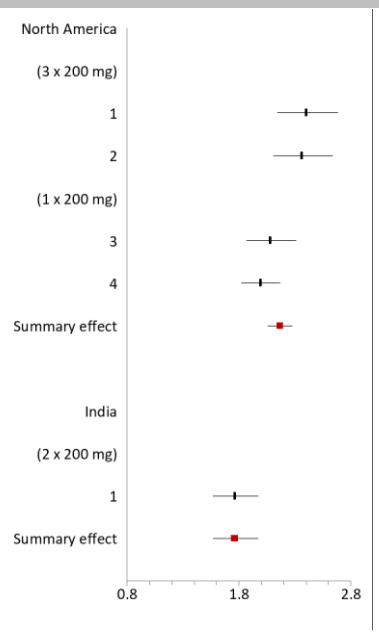
[b] Canadian Reference Product

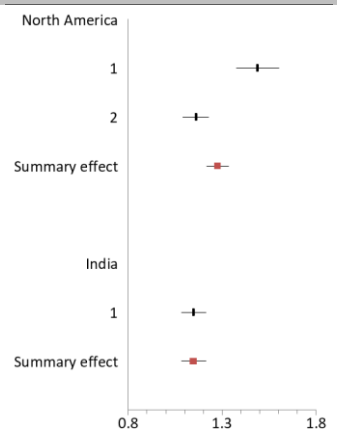
[$] As per label by FDA, C_{max} decreased by 100% in the presence of food.

†The magnitude of decrease in the AUC and C_{max} is derived from esomeprazole (NEXIUM®) 20 mg Tab Clinical Pharmacology and Biopharmaceutics Review, NDA 207920. As per monograph, NEXIUM® may be taken with or without food.

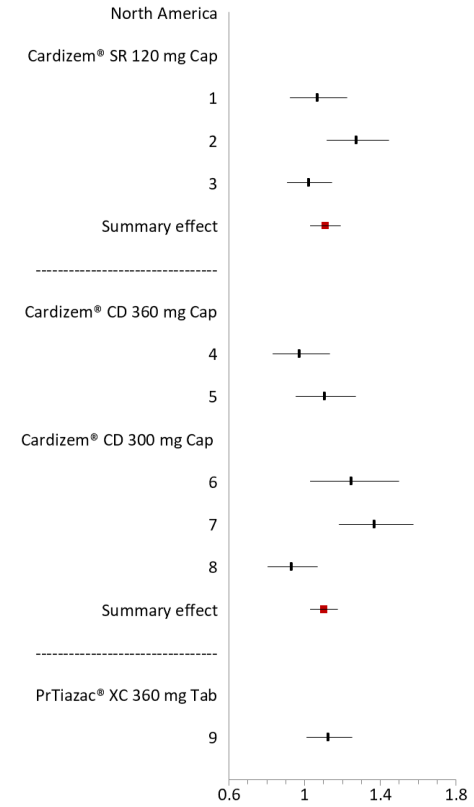
Table 2 Summary Food Effect in North America and India represented as Point Estimate (PE) and 90% Confidence Intervals (CI) for AUC_{0-t}.

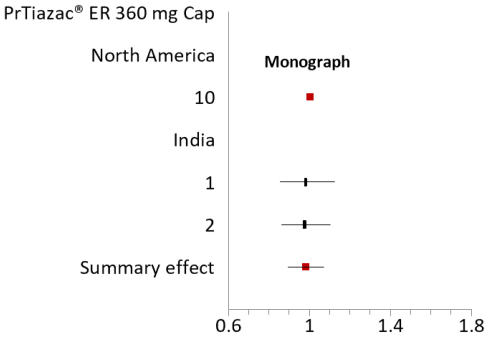
		AUC_{0-t}		
Drug	**Study**	**PE (Fed/Fasted)**	**90% CI**	**Food Effect**
Amiodarone (Cordarone® 200 mg Tab)				
	North America			
(3 x 200 mg)				
	1	2.40	[2.14, 2.68]	
	2	2.36	[2.11, 2.64]	
(1 x 200 mg)				
	3	2.08	[1.87, 2.31]	
	4	1.99	[1.82, 2.17]	
	Summary effect	**2.16**	**[2.06, 2.28]**	
Summary statistics: P= 0.069, τ^2= 0.005, I^2=57.63%				
	India			
(2 x 200 mg)	1	1.76	[1.57,1.97]	
	Summary effect	**1.76**	**[1.57,1.97]**	
Summary statistics: P= NA, τ^2= NA, I^2= NA				
Q-test for subgroup differences: $Q_{Between}$= 7.65, P= 0.0057				
Carbamazepine (Tegretol® CR 400 mg Tab) (1 x 400 mg)				
	North America			
	1	1.49	[1.38, 1.60]	
	2	1.16	[1.09, 1.23]	
	Summary effect	**1.27**	**[1.22, 1.33]**	
Summary statistics: P< 0.0001, τ^2= 0.029, I^2=94.80%				
	India			
	1	1.15	[1.08, 1.21]	
	Summary effect	**1.15**	**[1.08, 1.21]**	
Summary statistics: P= NA, τ^2= NA, I^2= NA				
Q-test for subgroup differences: $Q_{Between}$= 5.81, P= 0.01				





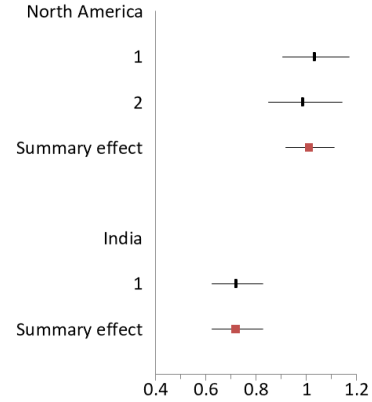
Drug	Study	PE (Fed/Fasted)	90% CI	Food Effect
		AUC_{0-t}		
Diltiazem				
	North America			
Cardizem® SR 120 mg Cap (1 x 120 mg)				
	1	1.06	[0.92, 1.23]	
	2	1.27	[1.12, 1.44]	
	3	1.02	[0.91, 1.15]	
	Summary effect	1.11	[1.03, 1.19]	
Summary statistics: P= 0.09, τ^2=0.008 , I^2= 58.46%				
Cardizem® CD 360 mg Cap (1 x 360 mg)				
	4	0.97	[0.83, 1.13]	
	5	1.10	[0.95, 1.27]	
Cardizem® CD 300 mg Cap (1 x 300 mg)				
	6	1.24	[1.03, 1.50]	
	7	1.36	[1.18, 1.57]	
	8	0.93	[0.81, 1.07]	
	Summary effect	**1.10**	**[1.05, 1.16]**	
Summary statistics: P= 0.009, τ^2= 0.019, I^2=70.65%				
Tiazac® XC 360 mg Tab (1 x 360 mg)				
	9	1.01	[1.12, 1.25]	
Tiazac® ER 360 mg Cap (1 x 360 mg)				
	North America			
	10	No Food Effect		
	India			
	1	0.98	[0.85, 1.13]	
	2	0.98	[0.86, 1.10]	
	Summary effect	**0.98**	**[0.89, 1.07]**	
Summary statistics: P= 0.965, τ^2=0 , I^2=0%				

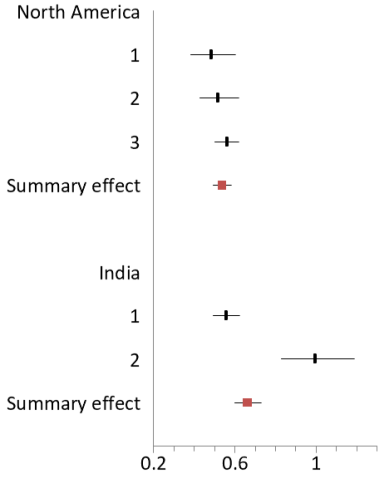




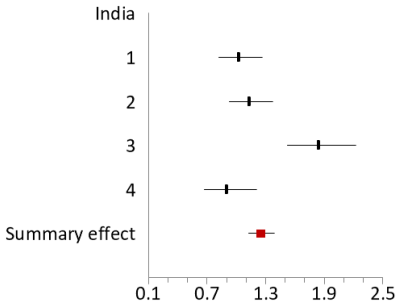
No PK bioequivalence study was available in North American population. As per monograph, the extent of diltiazem absorption was not affected by food. Therefore, (Fed/Fasted) PE for AUC_{0-t} in this population was shown to be unity (PE=1).

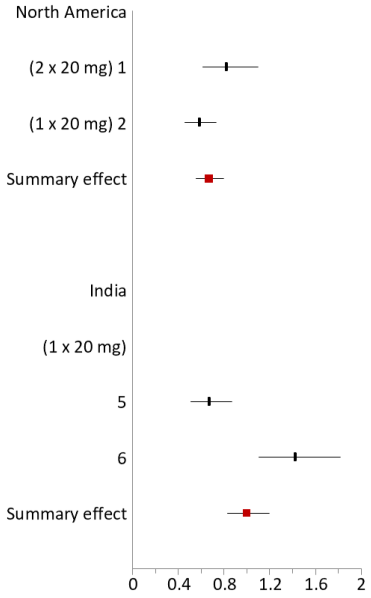
AUC_{0-t}				
Drug	**Study**	**PE (Fed/Fasted)**	**90% CI**	**Food Effect**
Verapamil (Isoptin® SR 240 mg Tab) (1 x 240 mg)				
	North America			
	1	1.03	[0.91, 1.17]	
	2	0.99	[0.85, 1.14]	
	Summary effect	**1.01**	**[0.92, 1.11]**	
Summary statistics: P= 0.697, τ^2= 0 , I^2= 0%				
	India			
	1	0.72	[0.62, 0.83]	
	Summary effect	**0.72**	**[0.62, 0.83]**	
Summary statistics: P=NA, τ^2= NA, I^2= NA				
Q-test for subgroup differences: $Q_{Between}$= 11.32, P=0.0008				
Esomeprazole (NEXIUM® 40 mg Tab) (1 x 40 mg)				
	North America			
	1	0.48	[0.38, 0.60]	
	2	0.51	[0.43, 0.62]	
	3	0.56	[0.50, 0.62]	
	Summary effect	**0.54**	**[0.49, 0.58]**	
Summary statistics: P= 0.573, τ^2= 0, I^2= 0%				
	India			
	1	0.56	[0.49, 0.63]	
	2	0.99	[0.83, 1.19]	
	Summary effect	**0.66**	**[0.60, 0.73]**	
Summary statistics: P<0.0001, τ^2= 0.159, I^2= 95.00%				
Q-test for subgroup differences: $Q_{Between}$= 7.06, P= 0.008				

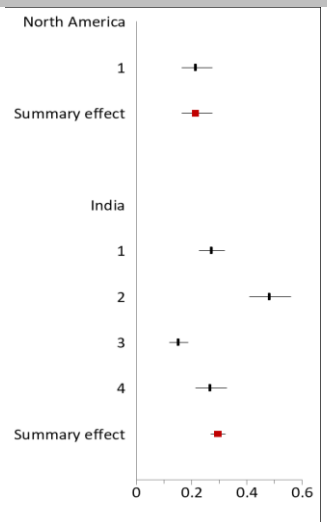




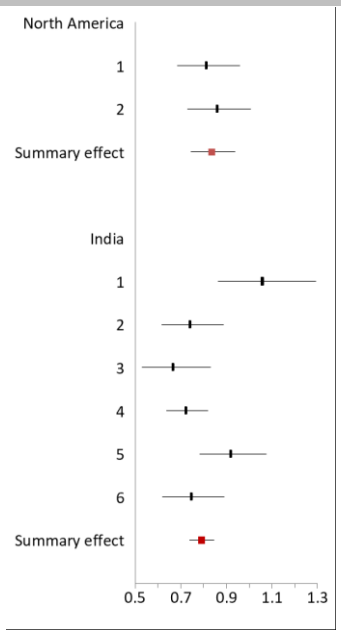
Drug	Study	PE (Fed/Fasted)	90% CI	Food Effect
		AUC_{0-t}		
Omeprazole				
Losec® 20 mg Tab (1 x 20 mg)				
	India			
	1	1.02	[0.82, 1.27]	
	2	1.13	[0.92, 1.38]	
	3	1.84	[1.52, 2.22]	
	4	0.90	[0.67, 1.21]	
	Summary effect	**1.25**	**[1.12, 1.39]**	
Summary statistics: P=0.0004, τ^2= 0.087, I^2= 83.30				
Losec® 20 mg Cap				
	North America			
(2 x 20 mg)				
	1	0.82	[0.61, 1.10]	
(1 x 20 mg)				
	2	0.58	[0.46, 0.73]	
	Summary effect	**0.67**	**[0.56, 0.80]**	
Summary statistics: P= 0.116, τ^2= 0.036, I^2=59.60%				
	India			
(1 x 20 mg)				
	5	0.66	[0.51, 0.87]	
	6	1.42	[1.10, 1.82]	
	Summary effect	**1.00**	**[0.83, 1.19]**	
Summary statistics: P= 0.001, τ^2= 0.264, I^2= 91.61				
Q-test for subgroup differences: $Q_{Between}$= 6.82, P= 0.009				
Lansoprazole (Prevacid® 30 mg Cap) (1 x 30 mg)				
	North America			
	1	0.21	[0.17, 0.27]	
	Summary effect	**0.21**	**[0.17, 0.27]**	
Summary statistics: P= NA, τ^2= NA, I^2=NA				
	India			
	1	0.27	[0.23, 0.32]	
	2	0.48	[0.41, 0.56]	
	3	0.15	[0.12, 0.19]	
	4	0.27	[0.22, 0.33]	
	Summary effect	**0.30**	**[0.27, 0.32]**	
Summary statistics: P<0.0001, τ^2= 0.212, I^2= 94.49%				
Q-test for subgroup differences: $Q_{Between}$= 4.04, P= 0.04				

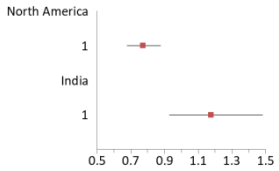


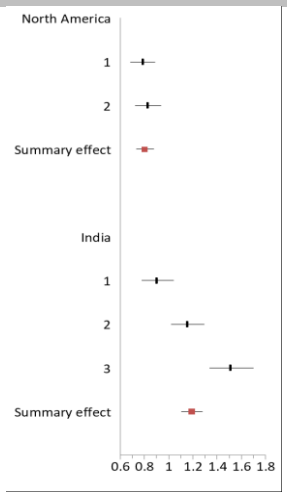




AUC_{0-t}				
Drug	**Study**	**PE (Fed/Fasted)**	**90% CI**	**Food Effect**
Pantoprazole Na (Pantoloc® 40 mg Tab) (1 x 40 mg)				
	North America			
	1	0.81	[0.69, 0.96]	
	2	0.86	[0.73, 1.01]	
	Summary effect	**0.84**	**[0.74, 0.94]**	
Summary statistics: P=0.684, τ^2= 0, I^2= 0%				
	India			
	1	1.06	[0.87, 1.29]	
	2	0.74	[0.62, 0.89]	
	3	0.67	[0.53, 0.83]	
	4	0.72	[0.64, 0.82]	
	5	0.92	[0.78, 1.08]	
	6	0.74	[0.62, 0.89]	
	Summary effect	**0.79**	**[0.74, 0.85]**	
Summary statistics: P=0.035, τ^2= 0.015, I^2= 58.26%				
Q-test for subgroup differences: $Q_{Between}$= 0.45, P= 0.504				
Pantoprazole Mg (Tecta® 40 mg Tab) (1 x 40 mg)				
	North America			
	1	0.77	[0.68, 0.88]	
	India			
	1	1.17	[0.93, 1.48]	
Rabeprazole (Pariet™/Pariet® 20 mg Tab) (1 x 20 mg)				
	North America			
	1	0.78	[0.69, 0.89]	
	2	0.82	[0.72, 0.94]	
	Summary effect	**0.80**	**[0.73, 0.88]**	
Summary statistics: P= 0.64, τ^2= 0, I^2=0%				
	India			
	1	0.90	[0.78, 1.04]	
	2	1.15	[1.02, 1.30]	
	3	1.51	[1.34, 1.70]	
	Summary effect	**1.19**	**[1.11, 1.28]**	
Summary statistics: P<0.0001, τ^2= 0.056, I^2= 90.60%				
Q-test for subgroup differences: $Q_{Between}$= 31.63, P= 0.00001				



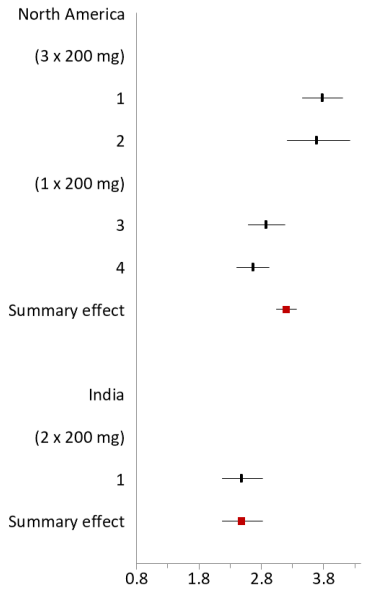


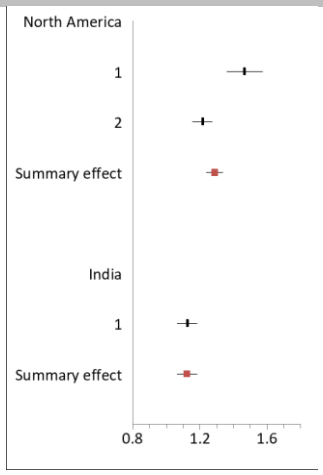


The vertical straight lines denote the GMRs of AUC_{0-t} (Fed/Fasted) for each study and the lines on either side the 90% confidence intervals. The summary effect is represented by a red square on the bottom line of each population. The *P*-value in summary statistics of each population corresponds to the variance of studies within that subgroup population and demonstrates whether the variance within subgroup is statistically significant. The *P*--value corresponding to $Q_{Between}$ on the bottom line of forest plot for each drug demonstrates whether the summary effect is the same for studies in North American as for the studies in India. PE, Point Estimate; GMR, Geometric Mean Ratio; CI, Confidence interval; NA, Not Applicable; Na, Sodium; Mg, Magnesium; τ^2, measure of heterogeneity (between-studies variance); I^2, measure of heterogeneity (degree of inconsistency in %).

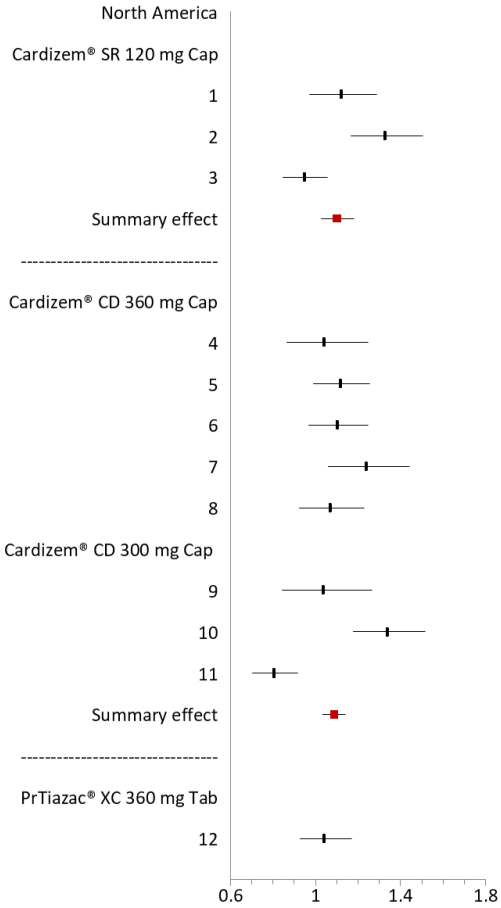
Table 3 Summary Food Effect in North America and India represented as Point Estimate (PE) and 90% Confidence Intervals (CI) for C_{max}.

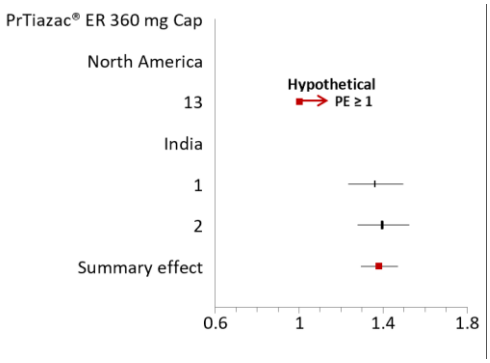
		C_{max}		
Drug	**Study**	**PE (Fed/Fasted)**	**90% CI**	**Food Effect**
Amiodarone (Cordarone® 200 mg Tab)				
	North America			
(3 x 200 mg)				
	1	3.77	[3.46, 4.11]	
	2	3.68	[3.22, 4.22]	
(1 x 200 mg)				
	3	2.87	[2.59, 3.18]	
	4	2.66	[2.41, 2.93]	
	Summary effect	**3.20**	**[3.04, 3.36]**	
Summary statistics: *P* <0.0001, τ^2= 0.03, I^2= 88.64%				
	India			
(2 x 200 mg)	1	2.48	[2.17, 2.83]	
	Summary effect	**2.48**	**[2.17, 2.83]**	
Summary statistics: *P*= NA, τ^2= NA, I^2= NA				
Q-test for subgroup differences: $Q_{Between}$= 9.27, *P*= 0.0023				
Carbamazepine (Tegretol® CR 400 mg Tab) (1 x 400 mg)				
	North America			
	1	1.46	[1.36, 1.57]	
	2	1.21	[1.16, 1.27]	
	Summary effect	**1.29**	**[1.24, 1.34]**	
Summary statistics: *P*= 0.0003, τ^2= 0.016, I^2= 92.31%				
	India			
	1	1.12	[1.07, 1.18]	
	Summary effect	**1.12**	**[1.07, 1.18]**	
Summary statistics: *P*= NA, τ^2= NA, I^2= NA				
Q-test for subgroup differences: $Q_{Between}$= 11.86, *P*= 0.0006				





Drug	Study	PE (Fed/Fasted)	90% CI	Food Effect
		C_{max}		
Diltiazem				
	North America			
Cardizem® SR 120 mg Cap (1 x 120 mg)				
	1	1.12	[0.97, 1.29]	
	2	1.33	[1.17, 1.51]	
	3	0.95	[0.85, 1.06]	
	Summary effect	1.10	[1.03, 1.18]	
Summary statistics: P= 0.003, τ^2= 0.026, I^2= 82.36%				
Cardizem® CD 360 mg Cap (1 x 360 mg)				
	4	1.04	[0.87, 1.25]	
	5	1.12	[0.99, 1.26]	
	6	1.10	[0.97, 1.25]	
	7	1.24	[1.06, 1.44]	
	8	1.07	[0.93, 1.23]	
Cardizem® CD 300 mg Cap (1 x 300 mg)				
	9	1.03	[0.84, 1.27]	
	10	1.34	[1.18, 1.52]	
	11	0.80	[0.71, 0.92]	
	Summary effect	**1.09**	**[1.05, 1.14]**	
Summary statistics: P= 0.0008, τ^2= 0.018, I^2= 71.98%				
PrTiazac® XC 360 mg Tab (1 x 360 mg)				
	12	1.04	[0.93, 1.17]	
PrTiazac® ER 360 mg Cap (1 x 360 mg)				
	North America			
	13	Slightly >1		
	India			
	1	1.36	[1.24, 1.50]	
	2	1.40	[1.28, 1.53]	
	Summary effect	**1.38**	**[1.29, 1.47]**	
Summary statistics: P= 0.728, τ^2= 0, I^2= 0%				

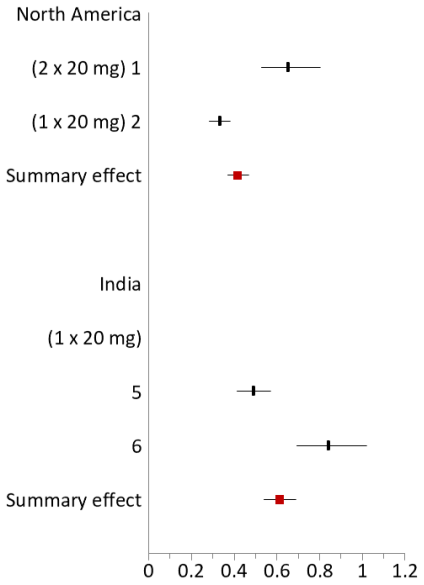




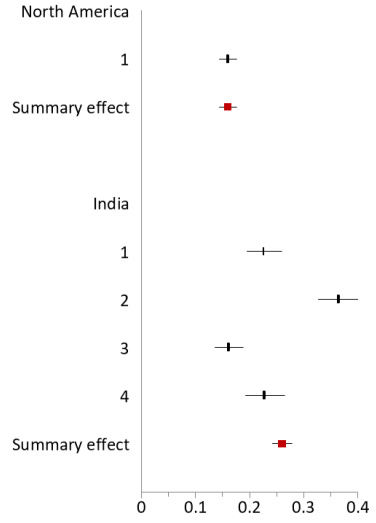
No information regarding the effect of food on C_{max} was available in the North American population. As per monograph, in the presence of food t_{max} occurred slightly earlier. Since earlier t_{max} appears in parallel with increased C_{max}, the PE (Fed/Fasted) for C_{max} was assumed to be slightly larger than unity.

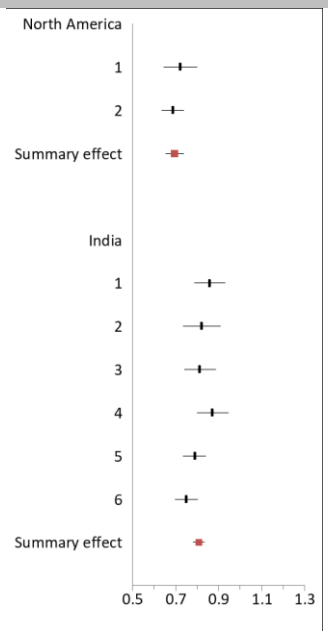
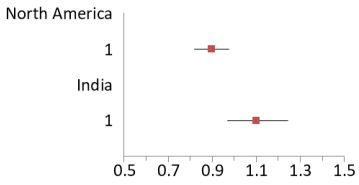
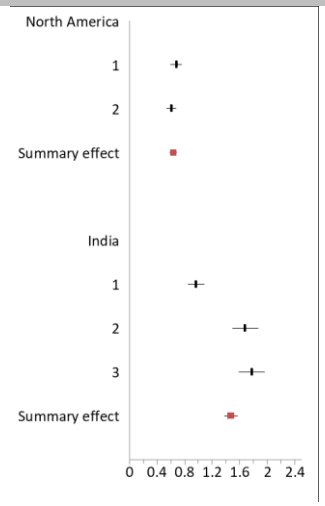
Drug	Study	PE (Fed/Fasted)	90% CI	Food Effect
		C_{max}		
Verapamil (Isoptin® SR 240 mg Tab) (1 x 240 mg)				
	North America			
	1	0.76	[0.66, 0.88]	
	2	0.64	[0.54, 0.77]	
	Summary effect	**0.71**	**[0.64, 0.80]**	
Summary statistics: P= 0.224, τ^2= 0.005, I^2= 32.50%				
	India			
	1	0.50	[0.43, 0.58]	
	Summary effect	**0.50**	**[0.43, 0.58]**	
Summary statistics: P=NA, τ^2= NA, I^2= NA				
Q-test for subgroup differences: $Q_{Between}$= 10.44, P= 0.001				
Esomeprazole (NEXIUM® 40 mg Tab) (1 x 40 mg)				
	North America			
	1	0.41	[0.34, 0.49]	
	2	0.34	[0.29, 0.40]	
	3	0.42	[0.38, 0.46]	
	Summary effect	**0.40**	**[0.37, 0.43]**	
Summary statistics: P= 0.171, τ^2= 0.006, I^2= 43.42				
	India			
	1	0.42	[0.38, 0.46]	
	2	0.67	[0.59, 0.77]	
	Summary effect	**0.49**	**[0.45, 0.53]**	
Summary statistics: P<0.0001, τ^2=0.112, I^2= 95.68%				
Q-test for subgroup differences: $Q_{Between}$= 9.23, P= 0.0024				
Omeprazole				
Losec® 20 mg Tab (1 x 20 mg)				
	India			
	1	1.32	[1.15, 1.52]	
	2	1.10	[0.97, 1.25]	
	3	1.74	[1.55, 1.95]	
	4	0.97	[0.80, 1.17]	
	Summary effect	**1.33**	**[1.24, 1.42]**	
Summary statistics: P <0.00001, τ^2= 0.059, I^2= 89.37				

		C_{max}		
Drug	**Study**	**PE (Fed/Fasted)**	**90% CI**	**Food Effect**
Losec® 20 mg Cap				
	North America			
(2 x 20 mg)				
	1	0.65	[0.53, 0.81]	
(1 x 20 mg)				
	2	0.33	[0.28, 0.38]	
	Summary effect	**0.42**	**[0.37, 0.47]**	
Summary statistics: P <0.0001, τ^2= 0.219, I^2= 94.83%				
	India			
(1 x 20 mg)				
	5	0.49	[0.41, 0.57]	
	6	0.84	[0.69, 1.02]	
	Summary effect	**0.61**	**[0.54, 0.69]**	
Summary statistics: P= 0.0003, τ^2= 0.139, I^2= 92.39				
Q-test for subgroup differences: $Q_{Between}$= 13.74, P= 0.0002				



Lansoprazole (Prevacid® 30 mg Cap) (1 x 30 mg)				
	North America			
	1	0.16	[0.14, 0.18]	
	Summary effect	**0.16**	**[0.14, 0.18]**	
Summary statistics: P= NA, τ^2= NA, I^2= NA				
	India			
	1	0.22	[0.19, 0.26]	
	2	0.36	[0.33, 0.40]	
	3	0.16	[0.14, 0.19]	
	4	0.23	[0.19, 0.27]	
	Summary effect	**0.26**	**[0.24, 0.28]**	
Summary statistics: P<0.0001, τ^2= 0.129, I^2= 94.71%				
Q-test for subgroup differences: $Q_{Between}$= 43.03, P<0.0001				



Drug	Study	PE (Fed/Fasted)	90% CI	Food Effect
		C_{max}		
Pantoprazole Na (Pantoloc® 40 mg Tab) (1 x 40 mg)				
	North America			
	1	0.72	[0.64, 0.80]	
	2	0.68	[0.64, 0.74]	
	Summary effect	**0.70**	**[0.65, 0.74]**	
Summary statistics: P= 0.543, τ^2= 0, I^2= 0%				
	India			
	1	0.86	[0.79, 0.93]	
	2	0.82	[0.74, 0.91]	
	3	0.81	[0.74, 0.89]	
	4	0.87	[0.80, 0.95]	
	5	0.79	[0.74, 0.84]	
	6	0.75	[0.70, 0.80]	
	Summary effect	**0.81**	**[0.78, 0.83]**	
Summary statistics: P=0.204, τ^2= 0.001, I^2= 30.89%				
Q-test for subgroup differences: $Q_{Between}$= 12.66, P<0.0004				
Pantoprazole Mg (Tecta® 40 mg Tab) (1 x 40 mg)				
	North America			
	1	0.89	[0.82, 0.97]	
	India			
	1	1.10	[0.97, 1.25]	
Rabeprazole (Pariet™/Pariet® 20 mg Tab) (1 x 20 mg)				
	North America			
	1	0.67	[0.60, 0.76]	
	2	0.60	[0.54, 0.67]	
	Summary effect	**0.63**	**[0.59, 0.69]**	
Summary statistics: P=0.261, τ^2=0.001 , I^2= 20.88				
	India			
	1	0.96	[0.85, 1.09]	
	2	1.67	[1.50, 1.87]	
	3	1.77	[1.59, 1.96]	
	Summary effect	**1.47**	**[1.38, 1.57]**	
Summary statistics: P<0.00001, τ^2= 0.098, I^2= 95.44%				
Q-test for subgroup differences: $Q_{Between}$=184.18, P= 0.00001				

The vertical straight lines denote the GMRs of C_{max} (Fed/Fasted) for each study and the lines on either side the 90% confidence intervals. The summary effect is represented by a red square on the bottom line of each population. The *P*-value in summary statistics of each population corresponds to the variance of studies within that subgroup population and demonstrates whether the variance within subgroup is statistically significant. The *P*-value corresponding to $Q_{Between}$ on the bottom line of forest plot for each drug demonstrates whether the summary effect is the same for studies in North American as for the studies in India. PE, Point Estimate; GMR, Geometric Mean Ratio; CI, Confidence interval; NA, Not Applicable; Na, Sodium; Mg, Magnesium; τ^2, measure of heterogeneity (between-studies variance); I^2, measure of heterogeneity (degree of inconsistency in %).

Table 4 Heterogeneity (I^2%) of Food Effect from clinical PK bioequivalence studies conducted in North American and Indian populations

API	**n_{NA}**	**n_{India}**	**Drug Product**	**I^2 (North American)**		**I^2 (Indian)**	
				AUC_{0-t}	C_{max}	AUC_{0-t}	C_{max}
Amiodarone	4	1	Cordarone® 200 mg Tab	57.63	88.64	-	-
Carbamazepine	2	1	Tegretol® CR 400 mg Tab	94.8	92.31	-	-
Diltiazem	3	-	Cardizem® SR 120 mg Cap	58.46	82.36	-	-
	5	-	Cardizem® CD 300 & 360 mg Cap	70.65	71.98	-	-
	1	2	Tiazac® ER 360 mg Cap	-	-	0	0
Verapamil	2	1	Isoptin® SR 240 mg Tab	0	32.5	-	-
Esomeprazole$^{\psi}$	3	2	Nexium® PR 40 mg Tab	0	43.42	95	95.68
Omeprazole$^{\psi}$	2	2	Losec® 20 mg Cap	59.6	94.83	91.61	92.39
	4	-	Losec® 20 mg Tab	-	-	83.3	89.37
Lansoprazole	1	4	Prevacid® 30 mg Cap	-	-	94.49	94.71
Pantoprazole Na$^{\psi}$	2	6	Pantoloc®40 mg Tab	0	0	58.26	30.89
Rabeprazole$^{\psi}$	2	3	Pariet™/® 20 mg Tab	0	20.88	90.6	95.44
Common products with High heterogeneity$^{\psi}$*				0	1	3	3
Total number of common products$^{\psi}$				4	4	4	4
% of Common products with high heterogeneity ($I^2 \geq 75\%$)$^{\psi}$				0%	25%	75%	75%
All products with High heterogeneity*				1	4	5	5
Total number of products				9	9	7	7
% of all products with high heterogeneity ($I^2 \geq 75\%$)				11.1%	44.4%	71.4%	71.4%

* I^2 values ≥ 75% are considered as high heterogeneity, indicating that most of the observed variance is due to the real difference in underlying true effects (food effect) between studies rather than random error.

$ Common products represents the products with available heterogeneity data in both populations

n_{NA}; Number of ANDSs in North American populations; n_{India}, Number of ANDSs in Indian populations

Table 5 Summary statistics of esomeprazole food effect using all available ANDS studies with Nexium® 20 and 40 mg tablets

AUC_{0-t}

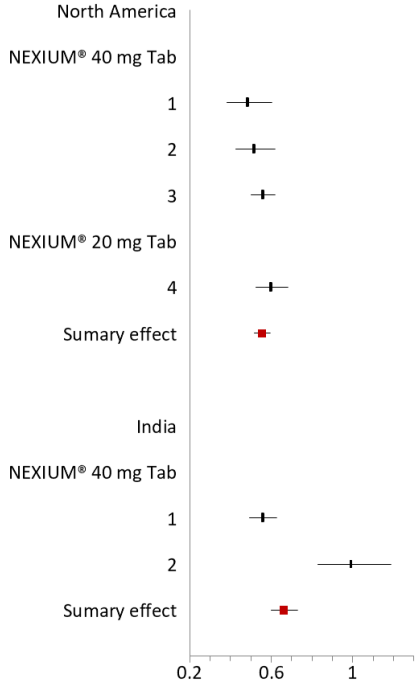
North America		
NEXIUM® 40 mg Tab (1 x 40 mg)		
1	0.48	[0.38, 0.60]
2	0.51	[0.43, 0.62]
3	0.56	[0.50, 0.62]
NEXIUM® 20 mg Tab (1 x 20 mg)		
4	0.60	[0.52, 0.68]
Summary effect	**0.55**	**[0.52, 0.60]**

Summary statistics: P= 0.502, τ^2= 0, I^2= 0%

India		
NEXIUM® 40 mg Tab (1 x 40 mg)		
1	0.56	[0.49, 0.63]
2	0.99	[0.83, 1.19]
Summary effect	**0.66**	**[0.60, 0.73]**

Summary statistics: P<0.0001, τ^2= 0.159, I^2= 95.00%

Q-test for subgroup differences: $Q_{Between}$= 5.85, P= 0.016



C_{max}

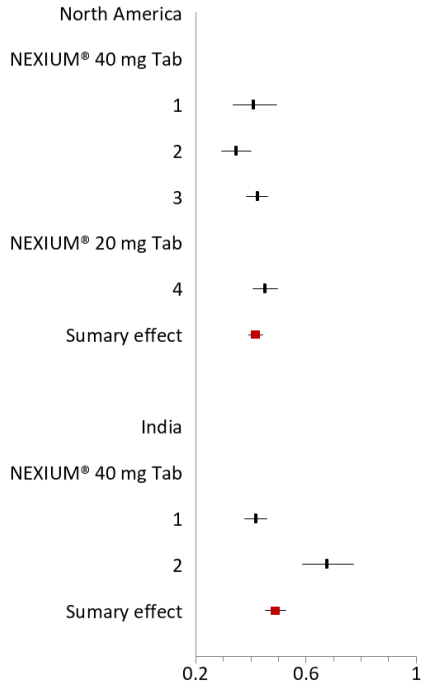
North America		
NEXIUM® 40 mg Tab (1 x 40 mg)		
1	0.41	[0.34, 0.49]
2	0.34	[0.29, 0.40]
3	0.42	[0.38, 0.46]
NEXIUM® 20 mg Tab (1 x 20 mg)		
4	0.45	[0.41, 0.50]
Summary effect	**0.42**	**[0.39, 0.44]**

Summary statistics: P= 0.115, τ^2= 0.006, I^2= 49.42%

India		
NEXIUM® 40 mg Tab (1 x 40 mg)		
1	0.42	[0.38, 0.46]
2	0.67	[0.59, 0.77]
Summary effect	**0.49**	**[0.45, 0.53]**

Summary statistics: P<0.0001, τ^2=0.112, I^2= 95.68%

Q-test for subgroup differences: $Q_{Between}$= 6.96, P= 0.0083

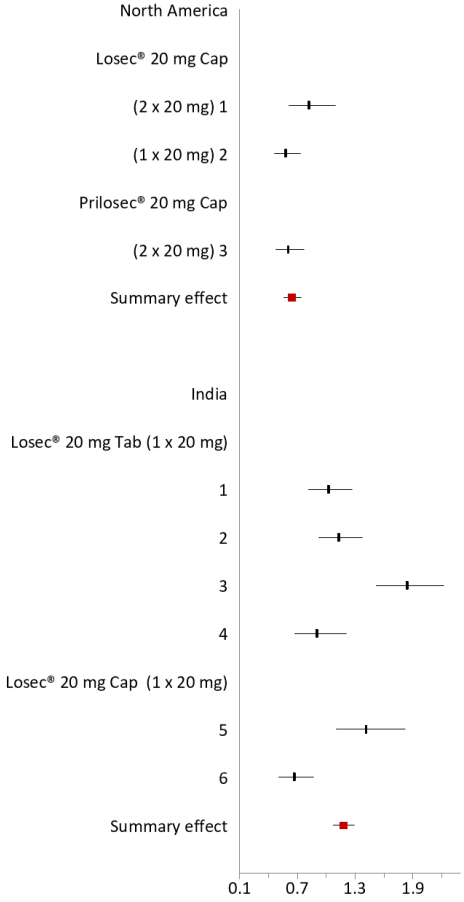


The vertical straight lines denote the GMRs of AUC_{0-t} (Fed/Fasted) for each study and the lines on either side the 90% confidence intervals. The summary effect is represented by a red square on the bottom line of each population. The *P*-value in summary statistics of each population corresponds to the variance of studies within that subgroup population and demonstrates whether the variance within subgroup is statistically significant. The *P*-value corresponding to $Q_{Between}$ on the bottom line of forest plot for each drug demonstrates whether the summary effect is the same for studies in North American as for the studies in India. PE, Point Estimate; CI, Confidence interval; τ^2, measure of heterogeneity (between-studies variance); I^2, measure of heterogeneity (degree of inconsistency in %).

Table 6 Summary statistics of omeprazole food effect using all available ANDS studies with Prilosec® 20 mg capsule, Losec® 20 mg tablet and capsule

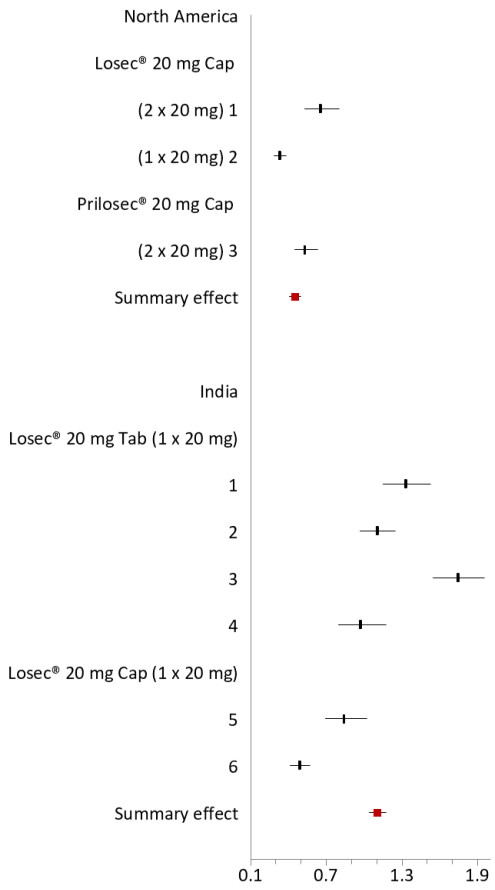
AUC_{0-t}

North America		
Losec® 20 mg Cap (2 x 20 mg)		
1	0.82	[0.61, 1.10]
Losec® 20 mg Cap (1 x 20 mg)		
2	0.58	[0.46, 0.73]
Prilosec® 20 mg Cap (2 x 20 mg)		
3	0.61	[0.48, 0.77]
Summary effect	**0.64**	**[0.56, 0.74]**
Summary statistics: P= 0.256, τ^2= 0.008 , I^2= 26.62%		
India		
Losec® 20 mg Tab (1 x 20 mg)		
1	1.02	[0.82, 1.27]
2	1.13	[0.92, 1.38]
3	1.84	[1.52, 2.22]
4	0.90	[0.67, 1.21]
Losec® 20 mg Cap (1 x 20 mg)		
5	1.42	[1.10, 1.82]
6	0.66	[0.51, 0.87]
Summary effect	**1.18**	**[1.07, 1.29]**
Summary statistics: P<0.0001, τ^2= 0.108, I^2= 84.86		
Q-test for subgroup differences: $Q_{Between}$= 34.0, P<0.0001		



C_{max}

North America		
Losec® 20 mg Cap (2 x 20 mg)		
1	0.65	[0.53, 0.81]
Losec® 20 mg Cap (1 x 20 mg)		
2	0.33	[0.28, 0.38]
Prilosec® 20 mg Cap (2 x 20 mg)		
3	0.53	[0.45, 0.63]
Summary effect	**0.45**	**[0.41, 0.50]**
Summary statistics: P<0.0001 , τ^2= 0.116 , I^2= 95.39%		
India		
Losec® 20 mg Tab (1 x 20 mg)		
1	1.32	[1.15, 1.52]
2	1.10	[0.97, 1.25]
3	1.74	[1.55, 1.95]
4	0.97	[0.80, 1.17]
Losec® 20 mg Cap (1 x 20 mg)		
5	0.84	[0.69, 1.02]
6	0.49	[0.41, 0.57]
Summary effect	**1.11**	**[1.04, 1.17]**
Summary statistics: P<0.0001, τ^2= 0.191, I^2= 95.96%		
Q-test for subgroup differences: $Q_{Between}$= 165.76, P<0.0001		



The vertical straight lines denote the GMRs of AUC_{0-t} (Fed/Fasted) for each study and the lines on either side the 90% confidence intervals. The summary effect is represented by a red square on the bottom line of each population. The *P*-value in summary statistics of each population corresponds to the variance of studies within that subgroup population and demonstrates whether the variance within subgroup is statistically significant. The *P*-value corresponding to $Q_{Between}$ on the bottom line of forest plot for each drug demonstrates whether the summary effect is the same for studies in North American as for the studies in India. PE, Point Estimate; CI, Confidence interval; τ^2, measure of heterogeneity (between-studies variance); I^2, measure of heterogeneity (degree of inconsistency in %).

Table 7 Overall summary of calculated and labeled food effects for the studied drug products and the comparison (statistical and clinical) between North American and Indian populations

API	Drug Product	PK Metric	n_{NA}	n_{India}	Label	FE in North America	FE in India	$Difference_{inter-ethnic}$ in FE		
						Magnitude [a]	Magnitude [a]	Difference in % [b]	Statistical significance [c]	Possible Clinical relevance? [d]
Amiodarone	Cordarone® 200 mg Tab	AUC_{0-t}	4	1	↑	+116%	+76%	40%	Yes	Yes
		C_{max}				+220%	+148%	72%		
Carbamazepine	Tegretol® CR 400 mg Tab	AUC_{0-t}	2	1	No FE	+27%	+15%	12%	Yes	No
		C_{max}				+29%	+12%	17%		
Diltiazem	Tiazac® ER 360 mg Cap	AUC_{0-t}	1	2	No FE	-	-2%	-	No	No
		C_{max}				-	+38%	-	-	
Verapamil	Isoptin® SR 240 mg Tab	AUC_{0-t}	2	1	↓	+1%	-28%	29%	Yes	No
		C_{max}				-28%	-50%	22%		
Esomeprazole	NEXIUM® PR 40 mg Tab	AUC_{0-t}	3	2	↓	-46%	-34%	12%	Yes	No
		C_{max}				-60%	-51%	9%		
Omeprazole	Losec® 20 mg Cap	AUC_{0-t}	2	2	No FE	-33%	0%	33%	Yes	No
		C_{max}				-58%	-39%	20%		
Omeprazole	Losec® 20 mg Tab	AUC_{0-t}	-	4	No FE	-	+25%	-	-	-
		C_{max}				-	+33%	-		
Lansoprazole	Prevacid® 30 mg Cap	AUC_{0-t}	1	4	↓	-79%	-71%	8%	Yes	No
		C_{max}				-84%	-74%	10%		
Pantoprazole Na	Pantoloc®40 mg Tab	AUC_{0-t}	2	6	No FE	-16%	-21%	4%	No	No
		C_{max}				-30%	-19%	11%	Yes	
Pantoprazole Mg	Tecta® 40 mg Tab	AUC_{0-t}	1	1	No FE	-23%	+17%	40%	Yes	Yes
		C_{max}				- 11%	+10%	21%		No
Rabeprazole	Pariet™/® 20 mg Tab	AUC_{0-t}	2	3	No FE	-20%	+19%	39%	Yes	No
		C_{max}				-37%	+47%	84%		Yes

↑ and ↓ arrows denote increased and decreased exposure with food, respectively.
n_{NA}; Number of ANDSs in North America; n_{India}, Number of ANDSs in India; PE, Point Estimate; Diff, Difference; FE, Food effect; $Difference_{inter-ethnic}$, inter-ethnic difference.

[a] Magnitude of the FE is given in terms of the percentage of the difference between Fed and Fasted (% Difference= $\frac{\text{Fed} - \text{Fasted}}{\text{Fasted}}$ x 100).

[b] When food impacted exposure in the same direction in both populations, the difference in their FE was calculated as: $\%\text{Difference}_{\text{inter-ethnic}} = |\%\text{FE}_{North\ American} - \%\text{FE}_{Indian}|$. When food impacted exposure in two different directions, the difference in FE between two populations was calculated by summing the absolute value of %FE in each population: $\%\text{Difference}_{\text{inter-ethnic}} = |\%\text{FE}_{North\ American}| + |\%\text{FE}_{Indian}|$.

[c] Statistically significant difference in summary food effect between the two geographical/ethnic subgroups was concluded based on subgroup analysis (Q-test, $P<0.05$). When there was a statistically significant difference "*Yes"*, and when there was not "*No"* was assigned.

[d] Possible clinical relevance was concluded when $\%\text{Difference}_{\text{inter-ethnic}}$ in FE ≥40%. When there was a clinically significant difference "*Yes"*, and when there was not "*No"* was assigned.

2.2.5 **Discussion**

This study aimed to investigate if two formulations assessed as being bioequivalent in one geographic/ethnic population would also be bioequivalent in another one. FEs were calculated and compared between populations for the same Ref products. At this point in time we could only find data available for two different populations, North American and Indian.

The most striking differences in FEs between the two populations were observed with **rabeprazole** and **amiodarone**. For **rabeprazole**, an increased exposure with food was observed in India, while a decrease was observed in North America. The difference in FEs between the two regions was found to be of both statistical ($P<0.05$) and possible clinical significance ($\%\text{Diff}_{\text{inter-ethnic}}\geq 40\%$). The observed FE on C_{max} was more apparent in both populations with Point Estimates (PEs: Fed/Fasted ratios) falling completely out of the 80-125% usual equivalence limits. High between-study variability was only observed for the Indian population rabeprazole studies (I^2= 90% for $AUC_{0\text{-}t}$, 95% for C_{max}). Two of the three available rabeprazole submissions using Indian populations (#2 and #3) were in general agreement with each other, as significant increases in C_{max} were observed with PEs falling above the usual 125% upper limit. Submission#1 with no FE on C_{max} was however in contradiction with the other two. In contrast, very low to negligible between-study variability in FEs were observed for the submissions using North American populations (I^2= 20.9% for C_{max} and I^2= 0% for $AUC_{0\text{-}t}$). As such, the significant differences observed in the FEs for rabeprazole between these two populations do not seem to be due to between-study variability. For **amiodarone**, increased exposure with food was observed in both populations; however, the difference in FEs between the two populations were still statistically significant ($P<0.05$) and of possible clinical relevance (40% and 72% larger increase in North America than in India in terms of $AUC_{0\text{-}t}$ and C_{max}, respectively).

Similarly to rabeprazole, a difference in the direction of the FE between the two populations was also observed for **pantoprazole-magnesium**. An increase in exposure in terms of AUC_{0-t} with food was observed in Indian populations, while a decrease was observed in North American ones. This difference in AUC_{0-t} was statistically significant ($P<0.05$) and possibly clinically relevant.

To date, many interactions between pharmaceutical excipients and transporters/CYP enzymes have been documented. For the studied drug products, Tween 80 (43), SLS (20, 22), and PEG (19, 44-47) in lansoprazole (PREVACID® Cap); magnesium-stearate (48, 49) in esomeprazole (NEXIUM® Tab) and amiodarone (CORDARONE® Tab); and PEG in esomeprazole (NEXIUM® Tab) and omeprazole (LOSEC® Cap) are known for their interaction with enzymes and transporters. CYP3A4 inhibition by polysorbates (Tweens®) has also been demonstrated (43, 50), with inhibition of human cDNA expressed CYP3A4 at concentrations of 0.005% and above (43). In an in vitro human colon and liver cell lines study, magnesium-stearate was shown to decrease CYP3A4 mRNA expression by more than 40% (48). Similarly, common pharmaceutical excipients have been shown to inhibit or at least attenuate P-gp function by more than fivefold (17). Cosolvents (e.g., PEG 400) (51), the Cremophor® class of pharmaceutical excipients (e.g., Cremophor EL) (52, 53), Tween® 20, and Tween® 80 have been identified as P-gp inhibitors (52, 54) and were found to enhance the transfer of P-gp substrate, digoxin, across the intestinal mucosa in different in vitro cell models by ≈2 folds (54). In the meantime, lower level of expression and lower functional abundance of CYP3A4 (55-57), CYP2C19 (26-28, 58, 59), CYP2D6 (60-62), CYP2C9 (23), and P-gp transporters (29) in Asians/Indians than in Caucasians/North Americas has been reported. The fourfold larger omeprazole AUC in Asian patients compared to Caucasian ones, indicated in the label (63), is also in support of this information.

Mannitol (64) in rabeprazole (PARIET® Tab) formulation, and magnesium-stearate (48, 49) in rabeprazole and amiodarone (CORDARONE® Tab) formulations are among the excipients with potential to influence drug bioavailability and BE. Rabeprazole has been reported to be metabolized by polymorphically expressed CYP2C19 and CYP3A4 enzymes. Similarly, amiodarone is a major substrate of CYP3A, but also of CYP2D6 and P-gp (42, 65). The differences observed in the rabeprazole and amiodarone FEs between the North American and Indian populations may be hypothesized to be partly attributed to the different prevalence of the variants of CYP enzymes and P-gp transporters between the two

populations, and to the influence of excipients on bioavailability through the interaction with CYP enzymes and transporters, but this would of course need to be verified by further studies.

For five of the study drugs, where food impacted exposure in the same direction in both populations, the effect of food on exposure in terms of both AUC_{0-t} and C_{max} in North American populations were numerically larger than in the Indian one. For instance, 33% and 20% larger decrease in omeprazole AUC_{0-t} and C_{max} with food, respectively, was observed in the North American versus Indian populations. Omeprazole, esomeprazole, and lansoprazole have been reported to be mainly metabolized by CYP2C19 and also by CYP3A4 enzymes; omeprazole and pantoprazole are reported to be also metabolized by CYP2D6 to a lesser extent. Although not widely investigated, some studies suggest that proton pump inhibitors (PPIs) are also substrates of P-gp transporters (66). The larger FE observed in North American populations versus the Indian ones could be hypothesized to be partly due to the reportedly lower levels of expression/function of CYP enzymes/P-gp transporters in Indians, but this would also need to be verified in further studies.

The excipients mentioned above are only some of the examples that have been previously indicated to have an impact on the function of transporters and enzymes. Excipients can influence BA, and therefore may impact BE outcomes via different mechanisms and depending on many factors such as the type and concentrations of the excipients relevant to the pharmaceutical formulation, different combinations of excipients in formulation, and PK and physicochemical characteristics of incorporated API (20, 22, 67, 68). For instance, drugs with high permeability are often assumed to be less susceptible to excipient influence on their BA than drugs with low permeability, and therefore the influence of excipients on BE between their formulations is expected to be of no significance (69). The published data may contradict this assumption, however, because it shows that the influence of excipients on BE outcomes is unpredictable. A clinical study with remarkable importance in undermining the traditional assumptions is the BE study of a highly permeable, highly soluble drug, risperidone's oral solutions (20). In that study a manufacturer developed two oral test solutions of risperidone containing 50 and 7 mg/ml of sorbitol in addition to the same qualitative and quantitative excipients included in the Ref product (Risperdal® 1 mg/ml oral solution). Both Test solutions failed to show BE to their Ref despite low intra-subject variability and sufficient power of the study. The results of this study showed that; (1) commonly used excipients can impact not only the BA and BE outcomes for drugs with low

permeability, but also those of drugs with high permeability and high solubility, and (2) the impact of excipients on BA and BE should be expected even when they are used in solutions, where the release of the drug substance from the drug product is self-evident. There are several other examples demonstrating the unpredictable impact of excipients on BE outcomes (20, 21, 67, 72).

In the evaluation of heterogeneity, less than 11.1% and 44.4% of the studies conducted with the North American population were associated with significant inconsistency ($I^2 \geq 75\%$) in FE results in terms of the $AUC_{0\text{-}t}$ and C_{max}, respectively, while 71.4% of the studies conducted with Indian populations had significant inconsistency for both exposure measures. Larger between-study variabilities (I^2) for all PPIs were found in studies conducted in Indian populations (**Table 4**). Greater prevalence of polymorphism in CYP enzymes has been reported in Indian versus North American populations (26-28, 58, 59). This may be a reason explaining the higher heterogeneity seen in Indian populations, but this would also need to be confirmed by further data.

Due to the large observed between-study variability in some studies, especially in those conducted in Indian populations, one may question whether the observed inter-ethnic differences in FEs were the true differences in PEs (Fed/Fasted) or they could simply be confounded by between-study variability. In order to account for between-study variability, we also conducted a random-effects model meta-analysis to calculate the summary FEs for each study drug product (data not shown). The results were in general agreement with the fixed-effect model meta-analysis. The same inter-ethnic differences were concluded for all study drugs from a clinical significance standpoint. It suggested that the observed inter-ethnic differences in FEs cannot be due to the between-study variability. A comparison of summary FEs calculated from fixed-effect and random-effects model meta-analyses for each drug in each population is provided in **Table 8**.

Table 8 Comparison of summary food effects for the studied drug products when fixed-effect and random-effects model meta-analyses were implemented

API	Drug Product	PK Metric	Fixed-Effect Model		Random-Effects Model		Difference$_{inter-ethnic}$ in FE		
			FE in North America	FE in India	FE in North America	FE in India			
			Magnitude [a]	Magnitude [a]	Magnitude [a]	Magnitude [a]	Difference in % (Random-Effects Model) [b]	Statistical significance [c]	Possible Clinical relevance? [d]
Amiodarone	Cordaron® 200 mg Tab	AUC_{0-t}	+116%	+76%	+118%	+76%	42%	Yes	Yes
		C_{max}	+220%	+148%	+220%	+148%	72%		
Carbamazepine	Tegretol® CR 400 mg Tab	AUC_{0-t}	+27%	+15%	+31%	+15%	16%	No*	No
		C_{max}	+29%	+12%	+33%	+12%	21%		
Diltiazem	Tiazac® ER 360 mg Cap	AUC_{0-t}	-	-2%	-	-2%	-	No	No
		C_{max}	-	+38%	-	+36%	-	-	
Verapamil	Isoptin® SR 240 mg Tab	AUC_{0-t}	+1%	-28%	+1%	-29%	29%	Yes	No
		C_{max}	-28%	-50%	-28%	-50%	21%		
Esomeprazole	Nexium® PR 40 mg Tab	AUC_{0-t}	-46%	-34%	-48%	-27%	21%	No*	No
		C_{max}	-60%	-51%	-61%	-47%	14%		
Omeprazole	Losec® 20 mg Cap	AUC_{0-t}	-33%	0%	-42%	4%	38%	Yes	No
		C_{max}	-58%	-39%	-67%	-36%	31%		
Lansoprazole	Prevacid® 30 mg Cap	AUC_{0-t}	-79%	-71%	-79%	-73%	6%	Yes	No
		C_{max}	-84%	-74%	-84%	-77%	7%		
Pantoprazole Na	Pantoloc® 40 mg Tab	AUC_{0-t}	-16%	-21%	-16%	-20%	4%	No	No
		C_{max}	-30%	-19%	-30%	-19%	11%	Yes	
Pantoprazole Mg	Tecta® 40 mg Tab	AUC_{0-t}	-23%	+17%	-23%	+17%	40%	Yes	Yes
		C_{max}	- 11%	+10%	- 11%	+10%	21%		No
Rabeprazole	Pariet™/® 20 mg Tab	AUC_{0-t}	-20%	+19%	-9%	+17%	26%	No*	No
		C_{max}	-37%	+47%	-12%	+31%	43%		Yes

↑ and ↓ arrows denote increased and decreased exposure with food, respectively.

n_{NA}; Number of ANDSs in North American population; n_{India}, Number of ANDSs in Indian population; PE, Point Estimate; Diff, Difference; FE, Food effect; $Difference_{inter\text{-}ethnic}$, inter-ethnic difference.

*When the results were different from fixed-effect model meta-analysis.

[a] Magnitude of the FE is given in terms of the percentage of the difference between Fed and Fasted (% Difference= $\frac{\text{Fed} - \text{Fasted}}{\text{Fasted}}$ x 100).

[b] When food impacted exposure in the same direction in both populations, the difference in their FE was calculated as: $\%Difference_{inter\text{-}ethnic} = |\%FE_{North\ American} - \%FE_{Indian}|$. When food impacted exposure in two different directions, the difference in FE between two populations was calculated by summing the absolute value of %FE in each population: $\%Difference_{inter\text{-}ethnic} = |\%FE_{North\ American}| + |\%FE_{Indian}|$.

[c] Statistically significant difference in summary food effect between the two geographical/ethnic subgroups was concluded based on subgroup analysis (Q-test, $P<0.05$). When there was a statistically significant difference "*Yes"*, and when there was not "*No"* was assigned.

[d] Possible clinical relevance was concluded when $\%Difference_{inter\text{-}ethnic}$ in FE ≥40%. When there was a clinically significant difference "*Yes"*, and when there was not "*No"* was assigned.

The examples in this study suggest that possible differences in FEs between geographical/ethnic populations may exist for certain drugs, regardless of the underlying mechanisms. Although unlikely because their specific caloric breakdown to protein, carbohydrate, and fat contents are the same, some may attribute these inter-ethnic differences in FEs to the different meal contents between the North American versus Indian studies. When conducted in India or North America, BE studies for the purpose of generic submission to the North American regulatory agencies (i.e., the FDA and Health Canada (HC)) must comply with the standards sets in terms of meal contents by the US FDA and HC. Minor differences in the test meal are expected to have no impact on FE and are accepted by regulatory agencies. As per the FDA Guidance (73) substitutions in the test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity. In summary, the observed differences in FEs between the two populations are unlikely to be due to a difference in meal contents between the studies conducted in North America and India.

Our study suggested possible differences in FE of nine drug products between two populations. If these results can be confirmed by others, then this means there are likely differences to be found among the thousands of other drug products that are marketed. The FEs observed for the Ref products using North American populations were in general different than those using Indian populations, implying that two formulations that are assessed as bioequivalent in one population may not necessarily be bioequivalent in another one. This is in contradiction with the traditional view that BE outcomes should not differ between populations when a study is using a crossover design.

One of the common extrapolations in the current practice of BE is the extrapolation of BE outcomes from healthy populations to patients. It is based on the assumption that (1) the use of HVs should minimize both inter- and intra-subject variability, and that (2) the equivalence observed between two products under healthy conditions can be extrapolated to the disease conditions. However, findings in the literature are sometimes at odds with these assumptions. To date, different studies in human subjects and animal models have revealed that BE or PK equivalence observed under healthy conditions may not always translate to equivalence under experimentally altered conditions or disease conditions (2-8, 74-77). For example, levothyroxine is a drug whose bioavailability is affected by altered gastrointestinal conditions, which might be present in a patient population. Several studies have documented that the elevated gastric pH, either due to impaired gastric acid secretion (77) or gastric pH altering drugs, such as PPIs (3, 4, 6, 74), reduced the oral bioavailability of levothyroxine significantly. In a levothyroxine PK study (two-way crossover) conducted in human subjects (n=15) (6) levothyroxine capsules (Tirosint® capsule) and levothyroxine tablets (Synthroid® tablet) were assessed as being PK equivalent under fasted conditions prior to the intravenous administration of esomeprazole, while they were found to be not PK equivalent under altered gastric pH conditions caused by prior intravenous administration of esomeprazole. The PK studies (two-way crossover) of omeprazole in 40 (7) and 23 (8) human subjects also demonstrated that differences between two formulations may remain hidden or nonsignificant under one condition, while they would be accentuated under another.

In conclusion, we suggest that readily extrapolation of BE study results from one population/region to another may not always be appropriate. We acknowledge that the detected discrepancies in our calculated FEs between the two populations may not always be clinically relevant. Nevertheless, we also found the inter-ethnic differences in FE which may be of clinical significance for drugs with fatal side-effects such as amiodarone (65) and verapamil (78). Further studies and research in this field should be undertaken.

2.2.6 References

1. Jamali F, Aghazadeh-Habashi A. Rapidly dissolving formulations for quick absorption during pain episodes: ibuprofen. International journal of clinical pharmacology and therapeutics. 2008;46(2):55-63.
2. Jamali F, Kunz-Dober CM. Pain-mediated altered absorption and metabolism of ibuprofen: an explanation for decreased serum enantiomer concentration after dental surgery. British journal of clinical pharmacology. 1999;47(4):391-6.
3. Pabla D, Akhlaghi F, Zia H. A comparative pH-dissolution profile study of selected commercial levothyroxine products using inductively coupled plasma mass spectrometry. European journal of pharmaceutics and biopharmaceutics. 2009;72(1):105-10.
4. Sachmechi I, Reich DM, Aninyei M, Wibowo F, Gupta G, Kim PJ. Effect of proton pump inhibitors on serum thyroid-stimulating hormone level in euthyroid patients treated with levothyroxine for hypothyroidism. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2007;13(4):345-9.
5. Aghazadeh-Habashi A, Jamali F. Pharmacokinetics of meloxicam administered as regular and fast dissolving formulations to the rat: influence of gastrointestinal dysfunction on the relative bioavailability of two formulations. European journal of pharmaceutics and biopharmaceutics. 2008; 70(3):889-94.
6. Seng Yue C, Benvenga S, Scarsi C, Loprete L, Ducharme MP. When Bioequivalence in Healthy Volunteers May not Translate to Bioequivalence in Patients: Differential Effects of Increased Gastric pH on the Pharmacokinetics of Levothyroxine Capsules and Tablets. Journal of pharmacy & pharmaceutical sciences: a publication of the Canadian Society for Pharmaceutical Sciences. 2015;18(5):844-55.
7. Elkoshi Z, Behr D, Mirimsky A, Tsvetkov I, Danon A. Multiple-Dose Studies can be a More Sensitive Assessment for Bioequivalence than Single-Dose Studies : The Case with Omeprazole. Clinical drug investigation. 2002;22(9):585-92.
8. Vaz-da-Silva M, Loureiro AI, Nunes T, Maia J, Tavares S, Falcao A, et al. Bioavailability and bioequivalence of two enteric-coated formulations of omeprazole in fasting and fed conditions. Clinical drug investigation. 2005;25(6):391-9.
9. Health Canada, Guidance Document - Comparative Bioavailability Standards: Formulations Used for Systemic Effects, 2018. Last Accessed 5 April 2019, at https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/bio/comparative-bioavailability-standards-formulations-used-systemic-effects.pdf

10. US-FDA, CDER: Guidance for Industry, Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA. Last accessed 7 February 2019, at: https://www.fda.gov/media/87219/download.

11. EMA Guideline on the Investigation of Bioequivalence. August 2010. Last access 6 March 2019 at https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf.

12. Cornell RC, Stoughton RB. Correlation of the vasoconstriction assay and clinical activity in psoriasis. Arch Dermatol. 1985;121(1):63-7.

13. Hilal-Dandan R, Brunton LL. Membrane Transporters and Drug Response. Goodman and Gilman's Manual of Pharmacology and Therapeutics, 2e. New York, NY: McGraw-Hill Education; 2016.

14. Scherrmann JM. Transporters in absorption, distribution, and elimination. Chemistry & biodiversity. 2009;6(11):1933-42.

15. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proceedings of the National Academy of Sciences of the United States of America. 1987;84(21):7735-8.

16. Kanfer I, Shargel L. Introduction-Bioequivalence Issues. In: Kanfer I, Shargel L, editors. Generic drug product development : bioequivalence issues. New York: Informa Healthcare; 2008. p. 1-14.

17. Zhang W, Li Y, Zou P, Wu M, Zhang Z, Zhang T. The Effects of Pharmaceutical Excipients on Gastrointestinal Tract Metabolic Enzymes and Transporters—an Update. The AAPS Journal. 2016;18(4):830-43.

18. Goole J, Lindley DJ, Roth W, Carl SM, Amighi K, Kauffmann JM, et al. The effects of excipients on transporter mediated absorption. International journal of pharmaceutics. 2010;393(1-2):17-31.

19. Buggins TR, Dickinson PA, Taylor G. The effects of pharmaceutical excipients on drug disposition. Advanced drug delivery reviews. 2007; 59(15):1482-503.

20. Garcia-Arieta A. Interactions between active pharmaceutical ingredients and excipients affecting bioavailability: impact on bioequivalence. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences. 2014;65:89-97.

21. Chen ML, Straughn AB, Sadrieh N, Meyer M, Faustino PJ, Ciavarella AB, et al. A modern view of excipient effects on bioequivalence: case study of sorbitol. Pharmaceutical research. 2007;24(1):73-80.

22. Vaithianathan S, Haidar SH, Zhang X, Jiang W, Avon C, Dowling TC, et al. Effect of Common Excipients on the Oral Drug Absorption of Biopharmaceutics Classification System Class 3 Drugs Cimetidine and Acyclovir. Journal of pharmaceutical sciences. 2016;105(2):996-1005.
23. Limdi NA, McGwin G, Goldstein JA, Beasley TM, Arnett DK, Adler BK, et al. Influence of CYP2C9 and VKORC1 1173C/T genotype on the risk of hemorrhagic complications in African-American and European-American patients on warfarin. Clinical pharmacology and therapeutics. 2008;83(2):312-21.
24. Chen M-L. Fundamentals of Bioequivalence. In: Yu LX, Li BV, editors. FDA Bioequivalence Standards. New York, NY: Springer New York; 2014. p. 29-53.
25. Cordery SF, Pensado A, Chiu WS, Shehab MZ, Bunge AL, Delgado-Charro MB, et al. Topical bioavailability of diclofenac from locally-acting, dermatological formulations. International journal of pharmaceutics. 2017;529(1-2):55-64.
26. Yasuda S, Zhang L, Huang S-M. The Role of Ethnicity in Variability in Response to Drugs: Focus on Clinical Pharmacology Studies. Clinical Pharmacology & Therapeutics. 2008;84(3):417-23.
27. Sohn DR, Kobayashi K, Chiba K, Lee KH, Shin SG, Ishizaki T. Disposition kinetics and metabolism of omeprazole in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation recruited from an Oriental population. The Journal of pharmacology and experimental therapeutics. 1992;262(3):1195-202.
28. Chang M, Tybring G, Dahl ML, Gotharson E, Sagar M, Seensalu R, et al. Interphenotype differences in disposition and effect on gastrin levels of omeprazole--suitability of omeprazole as a probe for CYP2C19. British journal of clinical pharmacology. 1995;39(5):511-8.
29. Balram C, Sharma A, Sivathasan C, Lee EJD. Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic-genotypic correlates. British journal of clinical pharmacology. 2003;56(1):78-83.
30. Kenyon CJ, Brown F, McClelland GR, Wilding IR. The use of pharmacoscintigraphy to elucidate food effects observed with a novel protease inhibitor (saquinavir). Pharmaceutical research. 1998;15(3):417-22.
31. Li LY, Amidon GL, Kim JS, Heimbach T, Kesisoglou F, Topliss JT, et al. Intestinal metabolism promotes regional differences in apical uptake of indinavir: coupled effect of P-glycoprotein and cytochrome P450 3A on indinavir membrane permeability in rat. The Journal of pharmacology and experimental therapeutics. 2002;301(2):586-93.
32. Ducharme MP, Warbasse LH, Edwards DJ. Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. Clinical pharmacology and therapeutics. 1995;57(5):485-91.

33. Gupta SK, Manfro RC, Tomlanovich SJ, Gambertoglio JG, Garovoy MR, Benet LZ. Effect of food on the pharmacokinetics of cyclosporine in healthy subjects following oral and intravenous administration. Journal of clinical pharmacology. 1990;30(7):643-53.

34. Gupta SK, Benet LZ. High-fat meals increase the clearance of cyclosporine. Pharmaceutical research. 1990;7(1):46-8.

35. Veng-Pedersen P, Tillman LG. Center of gravity of drug level curves: a model-independent parameter useful in bioavailability studies. J Pharm Sci. 1989; 78(10):848-54.

36. US FDA Drug Product Database for FDA Approved Drug Products. 2018 [Available from: https://www.accessdata.fda.gov/scripts/cder/daf/.

37. Fuglsang A. Sequential bioequivalence approaches for parallel designs. The AAPS journal. 2014;16(3):373-8.

38. Schuirmann DJ. A comparison of the Two One-Sided Tests Procedure and the Power Approach for assessing the equivalence of average bioavailability. Journal of Pharmacokinetics and Biopharmaceutics. 1987;15(6):657-80.

39. Michael Borenstein, Larry V. Hedges, Julian P. T. Higgins, Rothstein HR. Introduction to Meta-Analysis: John Wiley & Sons, Ltd.; 2009.

40. Handbook of Drug-Nutrient Interactions. 2 ed. J.I. Boullata, Armenti VT, editors: Humana Press; 2010.

41. FDA, Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers. Last accessed 28 June 20192 at: https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers.

42. Evidence-based clinical decision support resource from Wolters Kluwer: www.UptoDate.com.

43. Mountfield RJ, Senepin S, Schleimer M, Walter I, Bittner B. Potential inhibitory effects of formulation ingredients on intestinal cytochrome P450. International journal of pharmaceutics. 2000;211(1-2):89-92.

44. Basit AW, Podczeck F, Newton JM, Waddington WA, Ell PJ, Lacey LF. Influence of polyethylene glycol 400 on the gastrointestinal absorption of ranitidine. Pharmaceutical research. 2002;19(9):1368-74.

45. Ashiru DA, Patel R, Basit AW. Polyethylene glycol 400 enhances the bioavailability of a BCS class III drug (ranitidine) in male subjects but not females. Pharmaceutical research. 2008;25(10):2327-33.

46. Singh P, Guillory JK, Sokoloski TD, Benet LZ, Bhatia VN. Effect of inert tablet ingredients on drug absorption. I. Effect of polyethylene glycol 4000 on the intestinal absorption of four barbiturates. Journal of pharmaceutical sciences. 1966;55(1):63-8.

47. Ren X, Mao X, Si L, Cao L, Xiong H, Qiu J, et al. Pharmaceutical excipients inhibit cytochrome P450 activity in cell free systems and after systemic administration. European journal of pharmaceutics and biopharmaceutics. 2008;70(1):279-88.

48. Tompkins L, Lynch C, Haidar S, Polli J, Wang H. Effects of Commonly Used Excipients on the Expression of CYP3A4 in Colon and Liver Cells. Pharmaceutical research. 2010;27(8):1703-12.

49. Richards RM, Xing JZ, Mackay KM. Excipient interaction with cetylpyridinium chloride activity in tablet based lozenges. Pharmaceutical research. 1996;13(8):1258-64.

50. Bravo Gonzalez RC, Huwyler J, Boess F, Walter I, Bittner B. In vitro investigation on the impact of the surface-active excipients Cremophor EL, Tween 80 and Solutol HS 15 on the metabolism of midazolam. Biopharmaceutics & drug disposition. 2004;25(1):37-49.

51. Ashiru-Oredope DA, Patel N, Forbes B, Patel R, Basit AW. The effect of polyoxyethylene polymers on the transport of ranitidine in Caco-2 cell monolayers. International journal of pharmaceutics. 2011;409(1-2):164-8.

52. Hanke U, May K, Rozehnal V, Nagel S, Siegmund W, Weitschies W. Commonly used nonionic surfactants interact differently with the human efflux transporters ABCB1 (p-glycoprotein) and ABCC2 (MRP2). European journal of pharmaceutics and biopharmaceutics. 2010;76(2):260-8.

53. Zhao G, Huang J, Xue K, Si L, Li G. Enhanced intestinal absorption of etoposide by self-microemulsifying drug delivery systems: roles of P-glycoprotein and cytochrome P450 3A inhibition. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences. 2013;50(3-4):429-39.

54. Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. International journal of pharmaceutics. 2004;278(1):119-31.

55. Xie HG, Kim RB, Wood AJ, Stein CM. Molecular basis of ethnic differences in drug disposition and response. Annual review of pharmacology and toxicology. 2001;41:815-50.

56. Kim K, Johnson JA, Derendorf H. Differences in drug pharmacokinetics between East Asians and Caucasians and the role of genetic polymorphisms. Journal of clinical pharmacology. 2004;44(10):1083-105.

57. Simeprevir Clinical Pharmacology and Biopharmaceutics Review. NDA 205-123. Last accessed 16 December 2018, at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/205123Orig1s000ClinPharmR.pdf.

58. Benet LZ. The drug transporter-metabolism alliance: uncovering and defining the interplay. Molecular pharmaceutics. 2009;6(6):1631-43.

59. Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. The Journal of biological chemistry. 1994;269(22):15419-22.

60. Relling MV, Cherrie J, Schell MJ, Petros WP, Meyer WH, Evans WE. Lower prevalence of the debrisoquin oxidative poor metabolizer phenotype in American black versus white subjects. Clinical Pharmacology & Therapeutics. 1991;50(3):308-13.

61. Evans WE, Relling MV, Rahman A, McLeod HL, Scott EP, Lin JS. Genetic basis for a lower prevalence of deficient CYP2D6 oxidative drug metabolism phenotypes in black Americans. The Journal of clinical investigation. 1993;91(5):2150-4.

62. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics. 2002;3(2):229-43.

63. Endrenyi L, Csizmadia F, Tothfalusi L, Balch AH, Chen ML. The duration of measuring partial AUCs for the assessment of bioequivalence. Pharmaceutical research. 1998;15(3):399-404.

64. Adkin DA, Davis SS, Sparrow RA, Huckle PD, Wilding IR. The effect of mannitol on the oral bioavailability of cimetidine. Journal of pharmaceutical sciences. 1995;84(12):1405-9.

65. HC, Cordarone® (Amiodarone HCl) Tablet Label [Online]. Last Accessed 27 March 2019, at: https://pdf.hres.ca/dpd_pm/00033875.PDF.

66. Pauli-Magnus C, Rekersbrink S, Klotz U, Fromm MF. Interaction of omeprazole, lansoprazole and pantoprazole with P-glycoprotein. Naunyn-Schmiedeberg's archives of pharmacology. 2001;364(6):551-7.

67. Garcia-Arieta A, Gordon J. Bioequivalence requirements in the European Union: critical discussion. Aaps j. 2012;14(4):738-48.

68. Vaithianathan S, Haidar SH, Zhang X, Jiang W, Avon C, Dowling TC, et al. Reply to "On the Effect of Common Excipients on the Oral Absorption of Class 3 Drugs". Journal of pharmaceutical sciences. 2016;105(4):1355-7.

69. US-FDA, CDER: Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations. July 2002. Last accessed 13 March 2019 at https://www.fda.gov/downloads/Drugs/Guidances/ucm154838.

70. CDER FDA. Inactive Ingredient Search for Approved Products. Last accessed 7 February 2019, at http://www.accessdata.fda.gov/scripts/cder/iig/index.Cfm.

71. World Health Organization. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. WHO. In: WHO Technical Report Series, Vol. 937, WHO Press, Geneva. 2006. WHO Expert Committee on Specifications for Pharmaceutical Preparations, pp. 347–390.

72. Van Os S, Relleke M, Piniella PM. Lack of bioequivalence between generic risperidone oral solution and originator risperidone tablets. International journal of clinical pharmacology and therapeutics. 2007;45(5):293-9.

73. Health Canada. Guidance Document: Conduct and Analysis of Comparative Bioavailability Studies. Last accessed 25 Oct 2019 at: https://www.canada.ca/content/dam/hc-sc/documents/services/drugs-health-products/drug-products/applications-submissions/guidance-documents/bioavailability-bioequivalence/conduct-analysis-comparative.pdf. 2018.

74. Trifiro G, Parrino F, Sultana J, Giorgianni F, Ferrajolo C, Bianchini E, et al. Drug interactions with levothyroxine therapy in patients with hypothyroidism: observational study in general practice. Clinical drug investigation. 2015; 35(3):187-95.

75. Meredith P. Bioequivalence and other unresolved issues in generic drug substitution. Clinical therapeutics. 2003;25(11):2875-90.

76. Volans GN. Migraine and drug absorption. Clinical pharmacokinetics. 1978;3(4):313-8.

77. Centanni M, Gargano L, Canettieri G, Viceconti N, Franchi A, Delle Fave G, et al. Thyroxine in goiter, Helicobacter pylori infection, and chronic gastritis. The New England journal of medicine. 2006; 354(17):1787-95.

78. HC, PrIsoptin® SR Verapamil HCl Tablets Drug Product Monograph, 2016. [Online]. Last accessed 30 March 2019, at: http://www.mylan.ca/-/media/mylanca/documents/english/product%20pdf/pdfs%20dec%202015/isoptin-pm-2016.01.08.pdf

Chapter 3

3 Revisiting FDA's 1995 Guidance on Bioequivalence Establishment of Topical Dermatologic Corticosteroids: New Research Based Recommendations

3.1 Preface

Challenges in the context of BE are not always limited to the pivotal studies where the performance of a generic product is compared to that of Reference, but could also be in preliminary pilot studies where the appropriate study design for the pivotal BE study is established. For instance, characterization of exposure-response relationship and determination of the dose or dose duration which will subsequently be used in pivotal studies for the comparison of Test vs. Ref, are done within the pilot studies. This is the case for topical dermatological corticosteroids.

Topical dermatological corticosteroids play a pivotal role in the treatment of inflammatory skin diseases, such as atopic dermatitis, eczema, and psoriasis. The clinical effectiveness of topical corticosteroids in the treatment of these skin conditions is related to their vasoconstrictive, anti-inflammatory, immunosuppressive and anti-proliferative effects [369, 442]. Topical corticosteroids exert their pharmacological effect by reaching their site of action, the glucocorticoid receptors on fibroblasts which are involved in the immune and inflammatory response and reside in dermis [369]. The local vasoconstriction of the skin microvasculature and the consequent blood flow reduction by corticosteroids cause a blanching in the skin.

The intensity of skin blanching response for topical corticosteroids presumably relates to the amount of the drug that enters the skin and therefore has become a basis for BE assessment of two physically alike corticosteroid formulations. In July 1992, the US FDA's OGD issued an interim guidance for BE assessment of topical corticosteroids based on PD response for the first time. The interim guidance recommended characterization of skin blanching data (AUEC versus dose duration) in terms of a simple E_{max} model for each subject. Due to the difficulty experienced in fitting the E_{max} model to the individual subject data during the period 1992-1994, the OGD revised the guidance and removed the recommendation for modeling of individual subject data.

On June 2, 1995, the FDA published the Guidance document ''Guidance Topical Dermatologic Corticosteroids: in vivo Bioequivalence'', and recommended the use of population modeling method, either naïve pooled data or non-linear mixed effect modeling, for fitting the skin blanching data [155]. Since then, the FDA is the only regulatory agency with the Guidance document for the BE assessment of topical corticosteroids. Other

regulatory agencies, HC and EMA, follow the FDA's instructions. Currently, the BE assessment of topical corticosteroids is based on the comparison of skin blanching response of the test versus reference product at the dose duration corresponding to the population $DD_{50,}$ the dose duration at which the effect is half-maximal, of a simple E_{max} model.

In the FDA 1995 Guidance [155], a case study with P-PHARM® is presented as an example for data fitting with the non-linear mixed effect modeling method. P-PHARM®, developed by SIMED, is a software package, incorporating Expectation Maximization (EM) as the non-linear mixed effect modeling method. P-PHARM® was then incorporated into the Kinetica® suite of software (Innaphase).

Although the FDA Guidance provides the example of P-PHARM® with EM method, over the years a number of other non-linear mixed effect modeling algorithms (methods) and software packages have been developed. The non-linear mixed effect modeling methods, in general, fall into two main categories, EM-like methods (such as MLEM, MCPEM, SAEM) and FOCE-like methods.

Given the availability of different types of non-linear mixed effect modeling methods, each sponsor could choose a different one. When more than one method can be employed for fitting a dataset, while similar results will increase the confidence in estimates, conflicting results may undermine the reliability of the estimates. The literature data showed that when implementing different non-linear mixed effect modeling methods, different estimates were obtained [166-168, 424].

Obtaining reliable population DD_{50} estimates is crucial in BE assessment of topical corticosteroids as the extent of skin blanching of test formulation to its reference is compared at this dose duration. Inaccurate estimation of DD_{50}, therefore, can lead to erroneous BE conclusion. The FDA 1995 Guidance does not specify which type of non-linear mixed effect modeling method could or could not be used. Nor does it specify the necessary assumptions for distribution of the PD parameters that need to be set before such an analysis is conducted.

We hypothesize that due to the different estimation methods and statistical solvers implemented in different non-linear mixed effect algorithms, fitting the same dataset with different non-linear mixed effect algorithms can lead to different population estimates. The objective for the second part of this research project was, therefore, to investigate whether different non-linear mixed effect modeling algorithms, and different basic fitting assumptions

would lead to different population mean estimates of DD_{50}, and if they do, which non-linear mixed effect method/algorithm and fitting assumption should be prioritized over the other. To this purpose, AUEC versus dose duration data from 11 distinct skin blanching pilot studies were fitted using two non-linear mixed effect modeling methods: FOCE, as implemented in NONMEM®, and an EM algorithm (MLEM) implemented in ADAPT®5. While FOCE approximates the maximum likelihood estimates, the MLEM algorithm offers an exact estimation of the maximum likelihood estimates (**Section 5.4.2.4**). Therefore, different population estimates from FOCE and MLEM methods were expected. The following article presents the results of this study. The recommendations that we put forward in this research should be considered by the US FDA to update its Guidance, or by other regulatory agencies such as HC or EMA for approving topical corticosteroids.

The reader is advised to note that the DD_{50} is commonly referred to as ED_{50} in the FDA 1995 Guidance and literature. As a matter of consistency, DD_{50} in the following article is presented as ED_{50}.

3.2 Article #2

Revisiting FDA's 1995 Guidance on Bioequivalence Establishment of Topical Dermatologic Corticosteroids: New Research Based Recommendations

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3.2.1 Abstract

Purpose: As per the US FDA guidance issued on June 2, 1995, the establishment of bioequivalence for topical dermatologic corticosteroids is based on comparing the pharmacodynamic (PD) effects of Test and Reference products at the dose duration corresponding to the population ED_{50}, determined either by naïve pooled data or non-linear mixed effect modeling. The guidance was introduced using a study case example where the Expectation Maximization (EM) non-linear mixed effect algorithm, as implemented in P-PHARM®, was used. Although EM methods are relatively common, other methods such as the First-Order Conditional Estimation (FOCE) as implemented in the NONMEM® software are even more common. The objective of this study was to investigate the impact of using different parametric population modeling/analysis methods and distribution assumptions on population analysis results.

Methods: The dose duration-response data from 11 distinct skin blanching blinded pilot studies were fitted using FOCE (NONMEM®) and an EM algorithm (ADAPT®5 (MLEM)). Three different E_{max} models were tested for each method. Population PD estimates and associated CV%, and the agreement between model predicted values and observed data were compared between the two methods. The impact of assuming different distributions of PD parameters was also investigated.

Results: The simple E_{max} model, as proposed in the FDA guidance, appeared to best characterize the data compared to more complex alternatives. The MLEM method in general appeared to provide better results than FOCE; lower population PD estimates with less inter-individual variability, and no variance shrinkage issues. The results also favored ln-normal versus normal distribution assumptions.

Conclusions: The population ED_{50} estimates were influenced by both the type of population modeling methods and the distribution assumptions. We recommend updating the FDA guidance with more specific instructions related to the population approach to be used (EM-like versus FOCE-like methods) and to the normality assumptions that need to be set (ln-normal versus normal distribution).

3.2.2 **Introduction**

Bioequivalence (BE) between a Test and Reference (Ref) product is mainly demonstrated using pharmacokinetic (PK) endpoints, such as the area under the curve (AUC) and peak drug concentrations (Cmax), metrics that are related to the rate and extent of exposure of a moiety in the systemic circulation. For locally acting drug products that are not intended to be systemically absorbed such as those administered topically, bioequivalence may be demonstrated using alternative approaches. According to the US FDA 1995 guidance (1), an in vivo pharmacodynamic (PD) endpoint is currently an acceptable surrogate to use for BE assessment of topical corticosteroid drug products. The PD response following application of a topical corticosteroid to the skin is its ability to produce a vasoconstriction of the microvasculature of the skin, leading to skin blanching at the site of application. The skin blanching response is then measured visually and/or with a chromameter and is expressed in terms of the Area Under the Effect Curve (AUEC). The use of this PD endpoint for demonstrating BE for topical corticosteroids presumes that skin blanching is sufficiently correlated with the clinical effect, so that two formulations that differ clinically will also differ in terms of skin blanching (2-6).

According to the US FDA 1995 guidance, the BE assessment of topical corticosteroids involves the conduct of two separate studies, a pilot and a pivotal study. As a first step, a pilot study is performed solely with the reference listed drug product to establish the response vs. dose-duration relationship, from which PD parameters for use in a pivotal BE study can be determined. To this purpose a topical corticosteroid formulation is applied to the skin of human subjects for differing periods of time, i.e. dose durations. To obtain the PD response for each dose duration, an AUEC is calculated over a time course after drug removal, with time 0 hour representing the time at which the residual drug product was removed until 24 hours later ($AUEC_{(0\text{-}24)}$). The calculated PD responses ($AUEC_{(0\text{-}24)}$) are plotted as a function of dose duration to obtain the response vs. dose-duration relationship. The relationship is characterized in terms of an E_{max} model. From the relationship, population mean estimates of PD parameters (E_{max} and ED_{50}) and other discriminative time points such as *D1* and *D2* for use in the pivotal bioequivalence study are determined. E_{max} is the maximum PD response or, alternatively, defined as the maximum AUEC. ED_{50} is the dose duration required to produce 50% of this maximum PD response. *D1* and *D2* are dose durations at which approximately 33% and 67% of the maximal effect is produced and are determined as half and double the

ED_{50}, respectively. Therefore, the calculations of *D1* and *D2* are directly influenced by the estimated value of the ED_{50}.

Within a pivotal study, the BE of a multisource dermatologic corticosteroid is determined by comparing the skin blanching effect produced by the Test formulation to that of the Ref at the population ED_{50} estimate identified in the pilot study. This to ensure that the response attributable to this dose duration will fall within a sensitive log-linear region (20% to 80% of E_{max}) of the dose-response curve (7). To make sure that the study is sensitive, only the data from those subjects whose *D2*/*D1* ratios of PD responses meet a specified minimum value of 1.25 may be included in the BE assessment. A robust estimation of the ED_{50} from the pilot study is therefore crucial as it may not only affect the Test/Ref ratio of the PD response for BE assessment, but it may also impact on the overall sensitivity of the study in establishing BE.

In order to characterize the response vs. dose-duration relationship and to determine the population ED_{50} and E_{max} estimates, the US FDA 1995 guidance recommends to fit the PD response data using either naïve pooled data or a parametric non-linear mixed effect modeling method. But this latest method requires setting distribution assumptions, which are absent in the guidance. Naïve pooled data does not take into account inter-individual variability when estimating population parameters. As a result, population estimates based on the naïve pooled data method poorly correlate with the observed data, may not accurately represent the study population (8, 9), and should thus not be the method of choice. Non-linear mixed effect modeling is therefore the only option of first choice to be used for the determination of the population parameters of interest such as the ED_{50} and the E_{max}. However, there are gaps in the guidance concerning which non-linear mixed effect modeling method should or could be used. A case study was included in the guidance when it was issued, and the P-PHARM® software was used by the FDA to fit the skin blanching data to the E_{max} PD model. P-PHARM®, developed at the time by SIMED, was a software package incorporating the Expectation Maximization (EM) method for non-linear mixed effect modeling (10, 11). The EM method, pioneered by Alan Schumitzky and Walker (12), is an estimation method that is based on true likelihood estimation and is incorporated in a large variety of different software such as ADAPT-5® developed and supported by the Biomedical Simulations Resource (BMSR) at the University of Southern California, MONOLIX® by Lixsoft, Phoenix® WinNonlin® by Certara, L.P. (11, 13, 14). Despite this large availability of the EM method for

non-linear mixed effect, the NONMEM® software and its FOCE method, originally developed by Beal and Sheiner, is often the method that scientists think of first in terms of non-linear mixed effect and is based on likelihood approximation (15-17). We therefore tested two main methods in this study; the EM algorithm as implemented in the ADAPT-5® software from D'Argenio & Schumitzky (9, 18) and the FOCE algorithm as implemented in the NONMEM® software.

The availability of different population modeling methods is an advantage, as each has its own features and limitations. However, results from different population modeling methods can vary due to their different estimation approaches in data analysis (14, 19, 20). While some studies obtained different population mean estimates when using different population modeling methods (14, 17, 21, 22), some others found comparable results (11, 19, 23).

If employing different population modeling methods results in different population ED_{50} estimates, divergent conclusions on BE documentation between topical corticosteroids could be made. Therefore, inconsistency in estimated PD parameters decreases our confidence in BE assessment results for topical corticosteroids. Lack of reproducibility and conflicting results from tape stripping in BE assessment of topical tretinoin gel formulations in two laboratories is the very reason which led to withdrawal of the FDA 1998 Draft Guidance for bioavailability and BE assessment of topical dermatological drug products (24) in May 2002 (25-27). Hence, it is essential for regulatory agencies to update their guidance with instructions recommending more consistent approaches to be followed by pharmaceutical manufacturers.

Similar to other FDA draft Guidance documents, the 1995 Corticosteroids Guidance is open to various interpretations, and alternative approaches can be used to estimate the PD parameters as long as they comply with the requirements of the regulations. The US FDA Guidance (1) recommends either non-linear mixed effect modeling or naïve pooled data method for skin blanching data analysis to determine the population ED_{50} and E_{max}. However, it does not specify which type of non-linear mixed effect modeling method should or should not be used, nor does it specify the necessary assumptions for distribution of the PD parameters that need to be set before such an analysis is conducted. Interestingly, in a letter published by FDA in 1998 (28) in response to Demana et al. (29), the absence of any consideration for the nature of the distribution (normal or ln-normal) of population parameters

was mentioned among the reasons to discourage the use of naïve pooled data method. However, in the very guidance, no distribution profile was recommended to be assumed for PD parameters when using parametric non-linear mixed effect modeling method. Therefore, one can describe skin blanching data using different PD models, different fitting methods, and different distribution profile assumptions.

As the ED_{50} is an essential component in influencing the BE evaluation of topical corticosteroids, its robust estimation is crucial. In this study, we therefore investigated whether different population modeling methods, and different basic fitting assumptions would lead to different population mean estimates of ED_{50} and E_{max}. At the time of this research, no other study with real clinical data had been found in the literature investigating the influence of the abovementioned factors on ED_{50} estimates for BE assessment of topical corticosteroids. In this study, the objective was to compare population PD estimates obtained from two different non-linear mixed effect population modeling methods as well as different assumptions, and to conclude whether one method/assumption should be prioritized over the other. To ensure practicality and objectivity, we based our analysis on real-life blinded clinical data sets; they were therefore not specifically designed to demonstrate differences among methods. The recommendations that we are putting forward may provide an opportunity for the FDA to update its guidance as well as other regulatory agencies such as Health Canada and European Medicines Agency should they want to consider publishing guidances on the BE assessment of topical corticosteroids using this technique.

3.2.3 Methods

Data Collected

The data of each study included the PD response for each tested dose duration for one strength of an RLD cream formulation of a corticosteroid. Pharmacodynamic responses were measured in terms of AUEC (unit: scale*time) by means of a chromameter, which was then corrected for baseline and untreated control site for each dose-duration on ventral forearm. A total of 8-10 dose durations were used in each study and the tested dose durations ranged from a minimum of 3 min to a maximum of 360 min. The number of subjects ranged from 16 to 24. The skin blanching data were available from 11 studies. The data were sent blinded in terms of patients and RLD products from Cliantha/Hilltop to Learn and Confirm. It was not possible

for the scientists at Learn and Confirm to know what RLD products were tested, but it was mentioned that the same RLD was administered in two of these 11 studies (Studies 6 and 11).

Population Modeling/Fitting of the Response vs. Dose-Duration Data

By use of PD models we attempted to produce the best fit of $AUEC_{(0\text{-}24)}$ versus dose duration data to characterize the response vs. dose-duration relationship from which population mean estimates of E_{max} (scale*min) and ED_{50} (min) could be determined.

For each study the $AUEC_{(0\text{-}24)}$ versus dose duration data were fitted twice using two different population modeling methods, the FOCE method as implemented in NONMEM® version VII and the EM method as implemented in ADAPT-5® MLEM version 5.0.53. In each method, the same dataset was fitted separately to three different E_{max} models, the simple E_{max} model as per the FDA guidance, and two modifications to it, an E_{max} model with Hill factor, and an E_{max} model with a minimum Dose-Duration threshold. Goodness of fit measures were used for model discrimination. The best fitting model to the set of observations was selected and was consequently used to compare FOCE and MLEM. The methods were compared in terms of their agreement in population mean estimates of PD parameters and associated inter-individual variability (CV%), and the agreement between model predicted values and observed data. As parametric population modeling methods require certain distribution profile to be assumed, the impact of assuming ln-normal versus normal distribution of PD parameters on analysis results was also investigated.

When using MLEM, each population analysis was run until 1000 population iterations with sampling methods (including important sampling) set at 2000. Results from each MLEM analysis were considered to have started attaining convergence when all PK parameter values had converged graphically (for example, if all PK parameters appeared to have reached stable values starting at population iteration 600). Then the log likelihood estimates for the following 200 population iterations at convergence (in the current example between population iterations 600 and 800) were studied and were verified to not vary between the minimum and maximum estimates by more than 1% for these 200 consecutive iterations. The population iteration number at convergence was then chosen as the first population iteration within that set of 200 that resulted in the exact median convergence estimate (for example if the median value was 1290.20, then if the first population iteration that reached this exact value was 700, then convergence was set to be achieved at population iteration 700). Once the

population iteration at convergence was determined, the MLEM analysis was re-run until this exact population iteration in order to get the population parameter values and their associated individual estimates ("post-hocs").

When using FOCE, the analyses were permitted to converge automatically by NONMEM® and results were only used if optimization was concluded successfully with a minimum of 3 significant digits.

For both MLEM and FOCE, the values for the initial estimates ("priors") per study for E_{max} and ED_{50} were set as the average of the last three AUEC values of all subjects and as the first tested dose duration, respectively.

Structures of the PK-PD Models

As mentioned earlier, the AUEC represents the estimate of the extent of PD response. Therefore, the effect (E) was represented as AUEC in the PK-PD models.

Pharmacological effects being reductions in skin color, the baseline responses were higher than the responses seen following drug applications. Therefore, the AUEC versus dose duration curves have negative slopes, which are reflected by the minus sign in the E_{max} model equations. For all models and for all studies, AUECs are in fact baseline-adjusted control site-corrected $AUEC_{(0\text{-}24)}$, E_{max} is the maximum fitted value of AUEC, ED_{50} is the dose duration required to produce 50% of the fitted E_{max} value, and Time represents each tested dose duration.

Three different models were evaluated for their ability at best characterizing the observed AUEC data from all 11 studies. The simple E_{max} model, as recommended in the FDA guidance, was the base model to which two other E_{max} models were compared. The first one incorporated one additional parameter, a minimum effective dose duration threshold below which no effect could be seen. The second one was the commonly used "sigmoidal E_{max}" model and thereby also incorporated only one additional parameter versus the base model, the "Hill" coefficient. The formulas describing the three models are presented below:

Base model (Simple E_{max} model)

$$AUEC_{(\text{baseline-adjusted})} = 0 - \frac{\boldsymbol{Emax}\,.\,\boldsymbol{Time}}{\boldsymbol{ED_{50}} + \boldsymbol{Time}} \qquad (1)$$

Base model with a Minimum Effective Dose Duration Threshold ("MIN")

IF (TIME.LT.MIN) AND IF ((TIME-MIN).LT.0)
THEN Z= 0
ELSE Z=1

$$AUEC_{(baseline\text{-}adjusted)} = 0 - \left(\frac{\boldsymbol{Emax}.\ (\boldsymbol{Time-MIN})}{(\boldsymbol{ED_{50}-MIN})+(\boldsymbol{Time-MIN})}\right).Z \quad (2)$$

Sigmoidal E_{max} model

$$AUEC_{(baseline\text{-}adjusted)} = 0 - \frac{\boldsymbol{Emax}.\ \boldsymbol{Time^{HILL}}}{\boldsymbol{ED_{50}^{HILL}+Time^{HILL}}} \quad (3)$$

For each model, parameters fitted were associated with an inter-individual variability, and the residual variability was fitted using both a proportional and an additive error component. Due to convergence issues, the model with the minimum effective dose duration threshold could only be fitted in NONMEM® with an additive component error.

Statistical Analyses

This study investigated whether the PD parameters were better assumed as ln-normally or normally distributed. In the absence of a known distribution profile, non-parametric statistical tests at a 5% level of significance were used to compare the results (30). The Kruskal-Wallis ANOVA and the Wilcoxon Signed-rank tests were used to compare more than two independent groups and two paired groups, respectively (31, 32). To compare the frequencies of occurrence of a nominal variable (e.g. shrinkage) between two categories (in here, FOCE and MLEM methods), two-sided Fisher's exact test was used (33). SPSS® version 25 (IBM Corp. 2017) and PSI-Plot® version 8.8 (Poly Software International, Inc. 2012, Pearl River, NY) were used for statistical tests and additional generation of graphs.

Model Discrimination

According to the law of parsimony, the simplest model should be used preferentially over more complicated models if models fit the data similarly and the standard model discrimination criteria are similar between models (34, 35). The criterion determining the selection of the most appropriate model was the lowest observed value of the Akaike Information Criterion Test (9, 35) when using ADAPT®5 and the lowest observed value of the Minimum Value of the Objective Function (MOF) according to a chi-square distribution ($p<0.05$) (36) when using NONMEM®.

Influence of Different Distribution Assumptions

The impact of different distribution assumptions was investigated based on population mean estimates of PD parameters and their associated inter-individual variability in terms of coefficient of variation (CV%), and their distribution profile. This analysis was performed only with MLEM, as the distribution type must be chosen by the modeller prior to analysis. Therefore, each study dataset was presented to MLEM algorithm twice when using simple E_{max} model; once assuming normal and the other time ln-normal distribution of the PD parameters. This test was not conducted with NONMEM® with FOCE algorithm as the population mean estimate is a geometric mean.

Population PD estimates and associated CV% were compared when normal versus ln-normal distribution were assumed.

Distribution profiles of data were assessed both graphically and numerically for both assumptions. Histograms of PD parameters were generated for graphical assessment. As a numerical test for assessing normality, the Shapiro-Wilk test was used.

Comparison of NONMEM® FOCE and ADAPT®5 MLEM Methods

FOCE and MLEM methods were to be compared only on the most appropriate found structural model, and only when assuming that parameters were ln-normally distributed. Population mean estimates and their associated inter-individual variability in terms of coefficient of variation (CV%), as well as the agreement between model predicted values and observed data (the quality of fit) from the two methods were compared.

Residual variability and Goodness-Of-Fit (GOF) plots were used as the measures of the agreement between model predicted values and observed data. The scatterplots of "observed AUEC versus the corresponding post-hoc predicted values" (IPRED versus DV plot) and "weighted residuals versus population predicted AUEC values" (WRES versus PRED plot) were chosen as GOF plots.

Ratios of PD estimates obtained from MLEM versus FOCE and their associated 90% confidence intervals (CIs) were calculated for each study. The two methods were deemed to be similar for specific parameter estimation if their ratios and 90% CIs were completely within the range of 0.80 to 1.25 interval.

3.2.4 Results

Model Discrimination

The more elaborate models did not improve goodness of fit any further. Therefore, the simple E_{max} model was selected as the most appropriate model to characterize the data, out of the three different models tested. Results of the discrimination process are presented in Table 1.

Influence of Different Distribution Assumptions

Different distribution assumptions caused a statistically significant difference in population mean estimates of ED_{50} and E_{max} ($P<0.05$). Medians for population ED_{50} estimates were 96.7 and 126.0 min and for population E_{max} estimates were 43.10 and 49.0 scale*min, when normal and ln-normal distribution were assumed, respectively. Inter-individual variabilities around population mean estimates were significantly different when normal versus ln-normal distribution were assumed ($P<0.05$). Population mean estimates and associated CV% under normal and ln-normal distribution assumptions for each study are given in Table 2.

No specific trend could be observed for distribution profile of population E_{max} estimates; in some studies distribution of E_{max} estimates was either normal or ln-normal and in the others neither of them, regardless of initial assumption. For example in study 1, E_{max} estimates were ln-normally distributed while in Study 2 they were neither normally nor ln-normally distributed when ln-normal distribution was assumed.

When ln-normal distribution was assumed, the histogram of population ED_{50} estimates displayed a ln-normal distribution. Shapiro-Wilk test results of ln transformed population ED_{50} estimates also confirmed the assumed distribution (Figure 1, panel a). When normal distribution was assumed, however, neither normal nor ln-normal distribution profile characterized the obtained ED_{50} estimates (Figure 1, panel b).

Table 1 PD model discrimination.

Model	Ref. model	Description	No. of system parameters	MOF	Change in MOF	Selected Model
Base model		"Additive+ proportional" error model	6	1062.6		
Base model + MIN	Base model	"Proportional" error model	7	1063.7	+1.1 (<3.841, P<0.05)	Base model
Sigmoidal E_{max} model	Base model	"Additive+ proportional error model"	8	1070.9	+8.3 (<5.991, P<0.05)	Base model
PD model discrimination in FOCE based on Minimizing the Objective Function (MOF) according to a Chi Square distribution (P<0.05)						

Model	Ref. model	Description	No. of system parameters	AIC	Selected Model
Base model		"Additive+ proportional" error model	6	1395.8	
Base model + MIN	Base model	"Additive+ proportional" error model	8	1406.3	Base model
Sigmoidal E_{max} model	Base model	"Additive+ proportional" error model	8	1396.9	Base model
PD model discrimination in MLEM based on minimizing the Akaike's Information Criterion (AIC) test					

Table 2 Comparison of population mean estimates and associated inter-individual variability (CV%) of each study when different distribution profiles for PD parameters were assumed.

Study	Ln-normal distribution		Normal distribution	
	Geo Mean	CV%	Arith Mean	CV%
1	87.6	128	48.4	49.9
2	255	169	188	57.1
3	501	127	293	43.2
4	38	134	32.6	50
5	37.5	17	44.9	30.7
6	126	120	124	50.7
7	145	166	96.7	55
8	235	91	161	34
9	77	102	50.2	38.2
10	33.7	166	44.2	70.9
11	1220	160	611	49.4

Data represents population geometric (Geo) mean and arithmetic (Arith) mean estimates with associated CV% for ED_{50} (min).

Study	Ln-normal distribution		Normal distribution	
	Geo Mean	CV%	Arith Mean	CV%
1	35	19.3	30.2	48.8
2	48.5	36.6	39.2	49.4
3	82.8	26.7	55.1	40.8
4	56	33.3	50.1	37.8
5	16.4	76.1	22.1	62.6
6	58	34.4	51.9	29.7
7	57.1	6.31	43.1	38.7
8	47.8	16.3	42.6	48.3
9	53.3	8.64	43.5	28.9
10	49	31.7	46	32.8
11	30.6	11.7	22.9	51.4

Data represents population geometric (Geo) mean and arithmetic (Arith) mean estimates with associated CV% for E_{max} (scale*min).

As an additional evidence of distribution profile, the geometric mean/median ratios were compared with the arithmetic mean/median ratios of individual PD estimates. When ln-normal distribution was assumed, geometric means and medians of individual *ED50* estimates appeared to be more in agreement with each other than their arithmetic means and medians, implying ln-normal distribution of ED_{50} estimates being more likely. In contrast, comparing the ratios for individual E_{max} estimates was unavailing under either assumption (Figure 2).

Comparison of NONMEM® FOCE and ADAPT®5 MLEM Methods

Assuming ln-normal distribution of PD parameters, the base model was used to compare FOCE and MLEM methods, as it was selected as the best fitting model to the set of observations.

The NONMEM® FOCE and the ADAPT®5 MLEM methods were found to result in differences. They disagree more often than not between each other, especially in terms of ED_{50} and associated CV% rather than E_{max}. Figure 3 shows the dispersion of the ratios of "PD estimates from MLEM versus PD estimates from FOCE" within the range of 0.80-1.25 interval. The 90% CI for the ratios of population E_{max} estimates from all studies, except study 11, fell within the range of 0.80-1.25 which shows the general agreement between two population modeling methods in estimating population E_{max} estimates. However, the dispersion of the ED_{50} ratios showed that MLEM estimates in majority tend to be lower than FOCE estimates among which 90% CI for the ratios, in 5 out of 6 studies, completely fell off the lower bound of the range (p=NS, n=11). Population mean estimates and associated CV% for each study are given for FOCE and MLEM in Table 3.

PD parameter variances may be underestimated during the modeling process. When the post-hoc estimates are very close to the population values, the variance of post-hoc estimates distribution is shrinking towards zero and it becomes difficult to estimate the differences between subjects. This phenomenon is defined as η-shrinkage (shη) (36). When the data was fitted with FOCE, 6 out of 22 variance estimates (27%) were associated with shrinkage issue (marked as bolded in Table 3), while there were no instances of this issue when MLEM was used. The number of shrinkage issues with FOCE was significantly higher than with MLEM ($P<0.05$, Fisher's exact test). For the purposes of this study, a severely underestimated variance was associated with an inter-CV<1%.

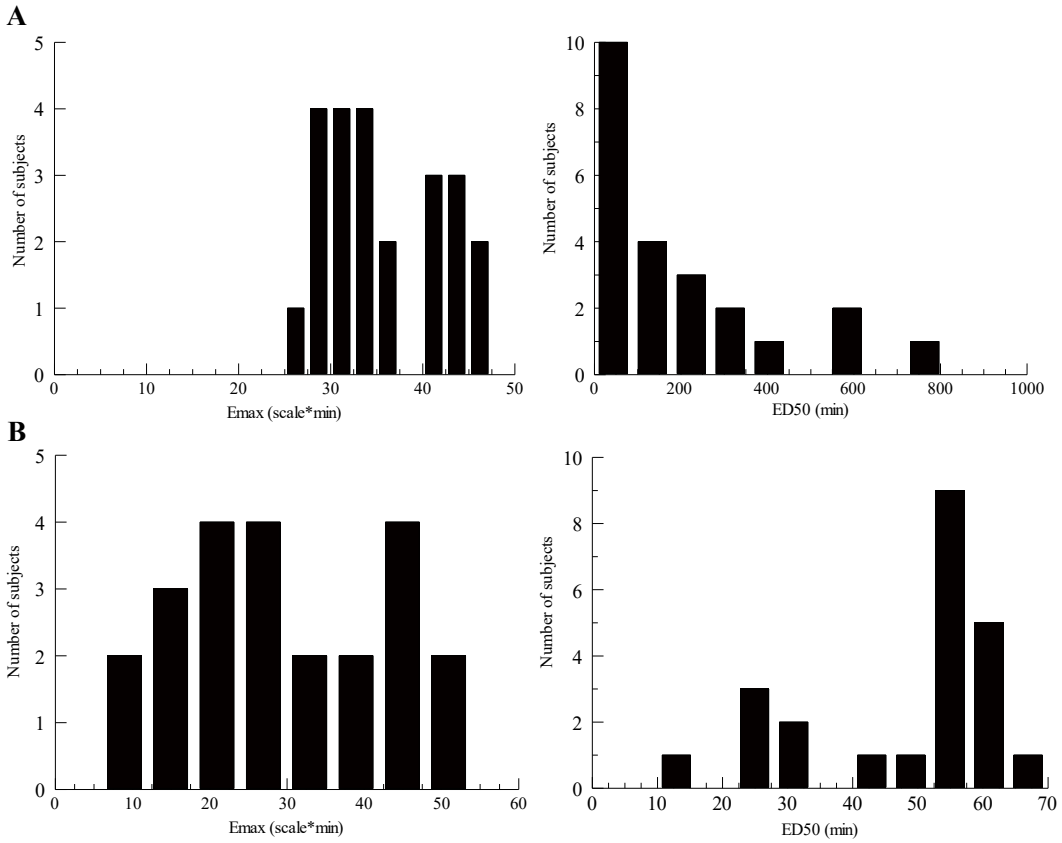

Figure 1. The histogram of population PD estimates for study 1 (n=23). A, when ln-normal distribution was assumed. B, when normal distribution was assumed.

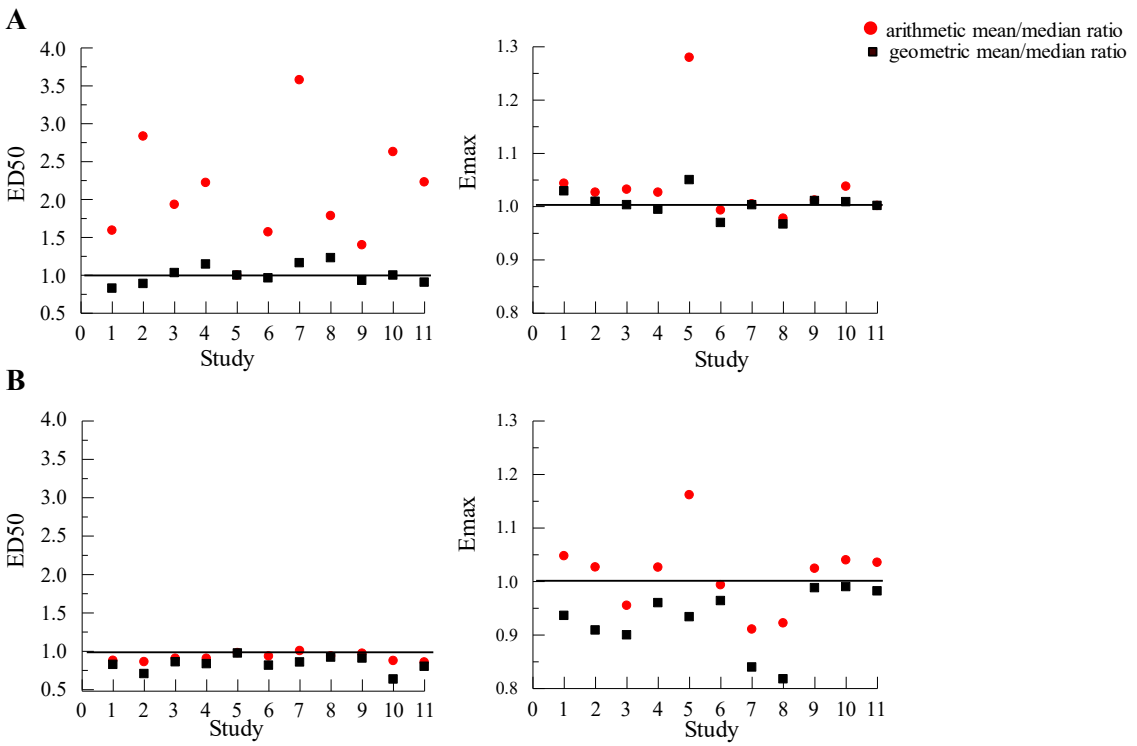

Figure 2. Ratios of arithmetic and geometric mean to the median of *post-hoc* PD estimates. A, when ln-normal distribution was assumed. B, when normal distribution was assumed. The perfect overlay of mean with median is denoted by the long horizontal line.

In study 6 (n=16) and study 11 (n=24) the same RLD formulation was used to characterize the response vs. dose-duration relationship. Therefore, the variability in results of population analysis of repeated applications of the same RLD were determined and compared between MLEM and FOCE. Although MLEM appeared to be more reproducible, the variabilities in population mean estimates were too large with both methods.

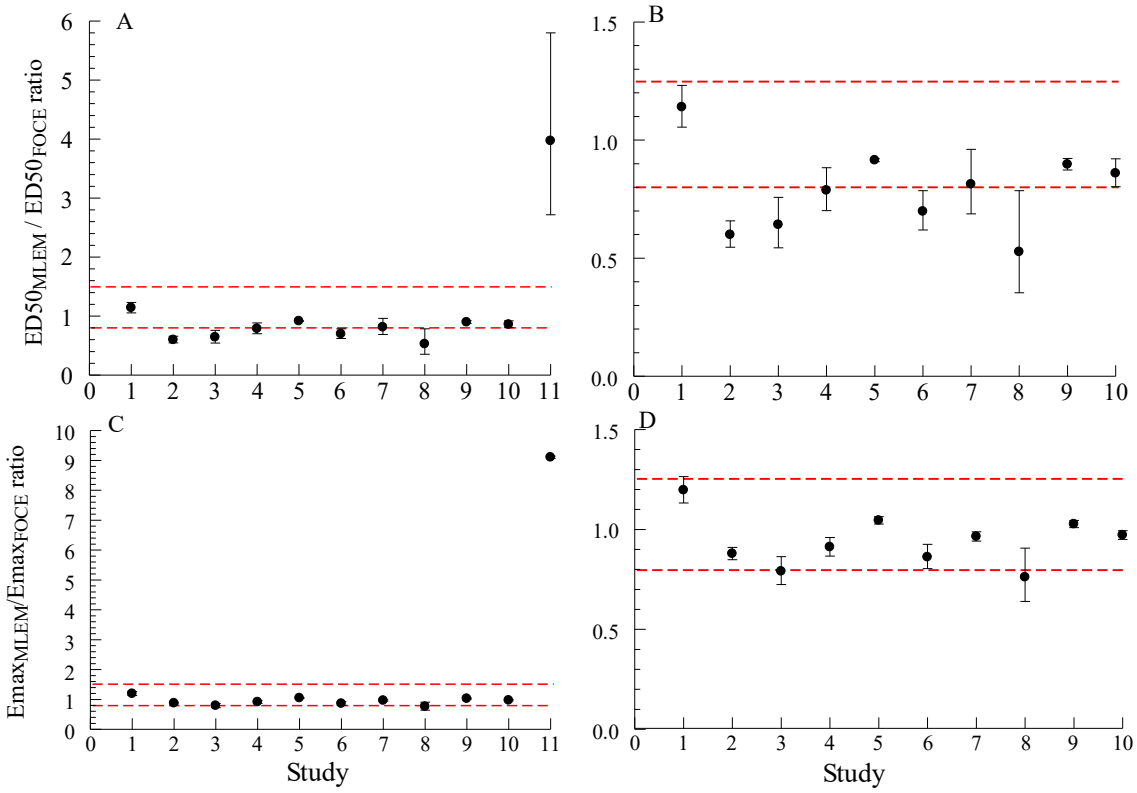

Figure 3. Dispersion of the ratios of "PD estimates from MLEM versus PD estimates from FOCE" within the acceptance range of 0.80-1.25. Each point represents the ratio in each study and black solid bar represent 90% confidence interval for each ratio. Due to the greater variability of PD estimates of study 11, the dispersion profiles were also shown when excluding study 11. A, ratio of ED_{50} in all studies. B, ratio of ED_{50} in all studies except study 11. C, ratio of E_{max} in all studies. D, ratio of E_{max} in all studies except study 11.

There was no statistically significant difference in residual variability of the fitted model whether FOCE or MLEM were used (P>0.05). When the dispersion of the ratios of residual variability of the model from FOCE versus MLEM was generated, the ratio fell within the range of 0.80-1.25 in 10 out of 11 studies (Figure 4), which implies that the residual variability was similar when different methods were used. In the meantime, GOF plots did not suggest any apparent differences between the two methods. In conclusion, comparative results of residual variability and GOF plots between FOCE and MLEM were indicative of the same overall quality of fit for both methods.

3.2.5 **Discussion**

Although the BE assessment of many topical formulations still relies on establishing similar clinical efficacy in a comparative clinical study using clinical endpoints in patients, topical corticosteroids are the only exception for which BE can be assessed solely based on an in vivo PD study endpoint (i.e., skin blanching) in healthy volunteers. The response vs. dose-duration relationship for topical corticosteroids is demonstrated by application of one strength of drug formulation for varying durations of time as this method has the least manipulation of experimental parameters (37).

Table 3 Comparison of population mean estimates and associated inter-individual variability (CV%) obtained from two NLME modeling methods for each study.

Study	**Base model**		**Base model + MIN**		**Sigmoidal E_{max} model**	
	Geo Mean	**CV%**	**Geo Mean**	**CV%**	**Geo Mean**	**CV%**
1	87.6	128	57.6	173	37	72
2	255	169	252.9	169	1950	22
3	501	127	192.1	121	122	63.1
4	38	134	34.3	150	43.4	148
5	37	17.6	62.6	83.4	35.4	16.7
6	126	120	52.6	53.2	71	90.5
7	145	166	124	177	102	156
8	235	91	195	77.7	95.3	51.5
9	77	102	74.5	116	53.6	80.7
10	34	166	31.9	177	663	5.91
11	1220	160	15.6	11.3	221	117

Data represents population Geometric (Geo) mean estimates and CV% of ED_{50} (min) with MLEM.

Study	**Base model**		**Base model + MIN**		**Sigmoidal E_{max} model**	
	Geo Mean	**CV%**	**Geo Mean**	**CV%**	**Geo Mean**	**CV%**
1	118	320	113	473	170	353
2	219	318	219	320	2850	103
3	373	103	346	63.0	153	95.6
4	35.4	137	34.3	174	37.5	143
5	34.9	**0.55**	35.3	**0.55**	15	751
6	102	130	99.6	132	65.8	101
7	140	247	118	439	146	247
8	132	**0.55**	393	866	10.8	2603
9	78.3	146	71	206	1.4	2.27E+14
10	35.2	288	32.8	373	59.2	463
11	8550	120	2150	250	524	74.3

Data represents population Geometric (Geo) mean estimates and CV% of ED_{50} (min) with FOCE. The bolded values represent shrinkage. NLME: Non-linear mixed effect

The US FDA 1995 Guidance recommends characterizing this PD response in terms of an E_{max} model to determine population ED_{50} estimate. Correct estimation of ED_{50} is of particular importance as its value will affect validity of the consecutive pivotal study by directly influencing the shorter dose duration calibrator ($D1$= 0.5* ED_{50}) and the longer dose duration calibrator ($D2$= 2* ED_{50}).

One of the biggest challenges associated with dose duration-response quantification for topical corticosteroids is the determination of the ED_{50} of the Ref product as it may not always be reproducible and of adequate reliability (37-40). Due to the importance of accurately estimating population ED_{50} in BE assessment of topical corticosteroids, this project aimed to investigate whether different population modeling methods and different basic fitting assumptions would lead to different population PD estimates, and whether a recommendation should be put forward for regulatory agencies to consider for updating and improving guidance documents on the BE assessment of topical corticosteroids.

Given the availability of different types of non-linear mixed effect modeling methods, each study analyst could choose a different method for characterization of AUEC versus dose duration for skin blanching data. It may be acceptable for scientists to use different population modeling techniques if they result in the same estimates. We have, however, seen that this is not the case, and two of the most widely used techniques (e.g., NONMEM® FOCE, and ADAPT®5 MLEM) result in significantly different ED_{50} estimates. Discrepancies may be indicative of some insufficiency in one or another method or some difficulty arising from a particular dataset, potency, the model, poor starting values, or other sources which require further investigation (17, 19, 21, 41, 42). Some difference in population mean estimates from MLEM and FOCE would be expected due to their different estimation approaches in data analysis, but the differences observed in this study were rather large as the ratios of the ED_{50} estimates were rarely within +/-20% of each other.

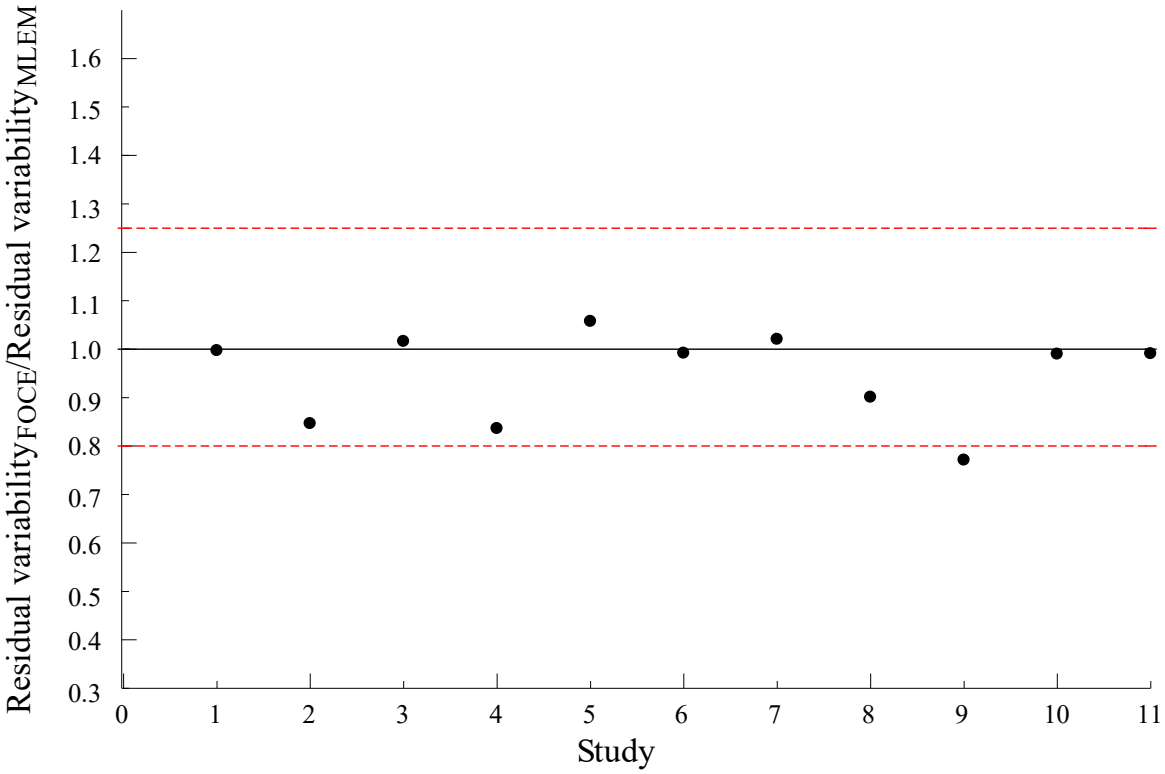

Figure 4. Dispersion of the ratios of residual variability of the model within the range of 0.80-1.25 when the FOCE versus MLEM method was used. Each point represents the residual variability ratio in each study and black solid bar represents equality of the residual variability in the FOCE and MLEM methods.

FOCE and similar estimation methods (FOCE-like methods) implement the estimation of maximum likelihood (ML) to solve the nonlinear problems. Therefore, the population mean estimates from FOCE-like methods are based on model approximation and not true ML estimators (20, 44). In addition, FOCE-like methods first fit the data by obtaining population mean estimates followed by a conditional second step with individual data estimates (post-hocs) in an iterative fashion. The fixed effects and random effects are fitted simultaneously with respect to population mean and variability estimates as well as the residual variability (11, 45). MLEM and similar estimation methods (EM-like methods), on the other hand, compute maximum likelihood with an iterative approach that involves 2 repetitive steps; an expectation step (E-step) and a maximization step (M-step). Since the linear approximation is replaced by importance sampling-based estimation method, the parameters obtained are true/exact ML estimates (11, 20, 44, 46, 47). In the E-step, parameter variables are estimated using the latest predicted parameter values and the observed data (Bayesian estimation of the

individual parameters). In the M-step, parameter values are estimated and updated to maximize the log-likelihood function in the E-step (estimation of population parameters). These new values are then reused for the subsequent iteration (46, 47). In the MLEM algorithm, ML is combined with an EM algorithm (10, 11, 48-50). EM-like methods, similar to FOCE-like methods, fit the data in an iterative fashion, but in a different order; they first compute the individual estimates (post-hocs) followed by the population estimates.

In this study, population PD analyses of topical RLD corticosteroid formulations were conducted for 11 different PD effect study data, using two different population modeling methods and different distribution profile assumptions. A satisfactory model was developed in both methods based on goodness of fit measures (MOF and AIC) and residual variability. In both methods, the simple E_{max} model described the skin blanching data better than two modified forms of it which were tested in this study. In general MLEM appeared to provide "better" results than FOCE did. MLEM provided lower population PD estimates (Figure 3) with less variability, and no issue of variance shrinkage (Table 3).

The simple E_{max} model (hyperbolic model) as suggested by the FDA 1995 guidance remains at this time preferable to more complex/modified models. This is in agreement with previously published results. Demana et al. investigated the suitability of two different PD E_{max} models to describe skin blanching data as the result of topical corticosteroid application; simple E_{max} model and sigmoidal E_{max} model. They concluded the chromameter data were best described by the simple E_{max} model (29). Later on, other studies also found that sigmoidal E_{max} model did not improve the model fit (51, 52).

Both FOCE and MLEM algorithms are designed to estimate the central tendency of population data using parametric methods (17). Therefore a certain type of distribution assumption is required to be made prior to performing the population analysis. Owing to the different shapes of them, the central tendency values of normal and ln-normal distributions vary; while in normal distribution the arithmetic mean and median overlay, in ln-normal distribution geometric mean and median overlay (53, 54). As a consequence, different distribution profiles may cause difference in central tendency values, in our case, population mean estimates of E_{max} and ED_{50}. The FDA 1995 Guidance does not recommend a particular type of distribution (normal versus ln-normal) to be assumed for running non-linear mixed effect population modeling. Based on our literature search, only two studies mentioned the type of distribution profile that they assumed within population analysis for fitting AUEC

versus dose duration data (51, 52). Both studies assumed normal and ln-normal distribution for E_{max} and ED_{50} parameters, respectively. However, neither mentioned the basis for this choice. In this study, the distribution profile of data was assessed both graphically and numerically. The initial distribution assumptions significantly affected the population E_{max} and ED_{50} estimates and associated CV%, as well as the distribution profile of population ED_{50} estimates. When ln-normal distribution was initially assumed, population ED_{50} estimates appeared to be ln-normally distributed. When normal distribution was assumed, however, neither normal nor ln-normal distribution profile could be achieved. Investigating the influence of the two assumptions on distribution profile of population E_{max} estimates was unpersuasive. Given the fact that ED_{50} serves as an essential component in influencing BE evaluation of topical corticosteroids, the results of analysis for ED_{50} estimates were prioritized in making a conclusion. The results, therefore, suggested that assuming ln-normal distribution of the PD parameters should be favored over normal distribution for skin blanching studies. Although not written in the guidance, lower ED_{50} estimates should be preferred to ensure that the comparison between a Test and Ref in pivotal study remains in the sensitive portion of the dose duration response curve. For this reason, the population modeling method which provided lower estimates was considered to be preferable in this study. Numerical comparison of the results of each study suggested that the estimates were different between the two methods. The dispersion of the ratios of PD estimates from MLEM versus PD estimates from FOCE (Figure 3) shows that ED50 estimates from MLEM in majority appeared to be lower than FOCE estimates. This is in agreement with previously published results. Staatz and Tett also found population mean estimates lower when they used EM-like versus FOCE-like method to fit the blood concentration–time data of orally administered tarcrolimus (17). Many other studies also reported different results from FOCE-like and EM-like methods (14, 21, 22), but none had used skin blanching data of topical corticosteroids.

The estimated inter-individual CVs around each PD parameter by different methods for the same dataset may be indicative of the uncertainty associated with the population estimate of the PD parameter (55). Given the example provided in the 1995 US FDA guidance, no data indicating the inter-individual variability associated with the population ED_{50} estimate appears to be required by the agency. As a consequence one could never know the inter-individual variability around the estimated ED_{50} and, therefore, on the *D1* and *D2* time points. In this study, we assumed that a better population modeling method would be

associated with detectable (e.g. No shrinking issues) but lesser inter-individual variability around the ED_{50} estimate. Results showed that inter-individual variabilities around population mean estimates generally appeared lower in MLEM than in FOCE and no variance shrinkage was observed with MLEM while it was an issue with FOCE. This finding was in agreement with the results previously found by Colucci et al (42). However, the CV% associated with ED_{50} estimates were significantly higher than CV% associated with E_{max} estimates for both methods (median of 137.34% and 128.0% versus 25.27% and 26.70% when FOCE and MLEM were used, respectively). Given that we often "hear" that sponsors experience difficulties at determining the ED_{50} values in pilot skin blanching studies, the greater variability in ED_{50} estimates seen was not surprising. Tsai et al also found a higher CV% for population ED_{50} estimates than for population E_{max} parameters (≈40-90% versus ≈10-27%) in a dose duration-response characterization study for clobetasol 17-propionate (52).

In this study, the same RLD formulation was used in study 6 and 11. Therefore population mean estimates obtained from the two studies were expected to be comparable. In contrast, a large difference was observed between the population mean estimates for both non-linear mixed effect methods, implying neither FOCE nor MLEM were of adequate reproducibility for this dataset. In two surveys conducted by FDA, one of which was on 88 ANDAs with vasoconstrictor BE studies submitted from January 1992 to April 2015 (56), high variability and lack of reproducibility and consistency in PD response/skin blanching data were reported alike as the difficulties with topical corticosteroids experienced by sponsors (39, 43, 51).

Wide variation in residual variabilities of ≈30% to 250% was observed in our results with both methods; median residual variabilities with the E_{max} model were 61.89 and 67.20 when FOCE and MLEM were used, respectively, implying high uncertainty left after the data were fitted and therefore overall low degree of model fit to the E_{max} model. The literature search showed that the data of skin blanching study is inherently associated with high degree of variability. High variability in AUEC data have been found by many investigators (28, 30, 38, 57, 58). In a study performed by Smith et al, there were extensively large standard deviations about the mean values of AUEC at each time point with no differentiation between the means. Approximately 20-50% variability were found in skin blanching data (30). As such, an intra-individual variability of 60%-139% was found in a study performed by Singh et al (51) which was inversely related to dose duration and to the potency of the dermatologic

corticosteroid product. In a more recent study conducted by Lehman and Franz (58), variability ranging from 78-126% were reported in the skin blanching data which were fitted to the E_{max} model. The authors, however, did not specify which fitting method was used. The variability in the mass of formulation applied to the skin, different application sites along the forearm, chromameter probe manipulations, ambient temperature, relative humidity, posture, one application site for each dose duration, and adjusting the chromameter readings for the baseline are some sources contributing to the high variability in skin blanching data (29, 51, 59-61).

In conclusion, this study demonstrated that using different population modeling methods and different assumptions regarding distribution of PD parameters affected the population estimation of the PD parameters and their associated variability. Regardless of the population modeling method used, more complex versions of the simple E_{max} model did not appear to be necessary to describe skin blanching data better. The study results suggested that EM-like methods may provide better population ED_{50} estimates of skin blanching data. It also suggested that ln-normal distribution should be assumed for the distribution of the ED_{50} parameter.

As population ED_{50} estimates play a critical role in the BE assessment of topical corticosteroid products, any difference in estimated PD parameters could influence the outcome of BE evaluation for these products. Due to the availability of several methods for performing population modeling and their parametric approach in data analysis, updating the US FDA 1995 Guidance with more specific instructions related to the population approach and normality assumptions, would favor a more consistent approach to be followed by pharmaceutical manufacturers, and would increase the confidence in BE assessment results of these products.

3.2.6 References

1. US FDA Guidance for Industry: Topical Dermatologic Corticosteroids: In Vivo Bioequivalence, 02/June/1995. Last accessed 05/Aug/2016, at: http://www.fda.gov/ohrms/dockets/dockets/04p0206/04p-0206-ref0001-08-FDA-Guidance-for-Industry-06-1995-vol3.pdf.

2. Hauck WW. Bioequivalence studies of topical preparations: statistical considerations. International journal of dermatology. 1992;31 Suppl 1:29-33.

3. Stoughton RB. The vasoconstrictor assay in bioequivalence testing: practical concerns and recent developments. International journal of dermatology. 1992;31 Suppl 1:26-8.

4. Cornell RC, Stoughton RB. Correlation of the vasoconstriction assay and clinical activity in psoriasis. Archives of dermatology. 1985;121(1):63-7.

5. Gibson JR, Kirsch JM, Darley CR, Harvey SG, Burke CA, Hanson ME. An assessment of the relationship between vasoconstrictor assay findings, clinical efficacy and skin thinning effects of a variety of undiluted and diluted corticosteroid preparations. The British journal of dermatology. 1984;111 Suppl 27:204-12.

6. Pershing LK, Silver BS, Krueger GG, Shah VP, Skelley JP. Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching bioassay. Pharm Res. 1992;9(1):45-51.

7. Samara E, Granneman R. Role of population pharmacokinetics in drug development. A pharmaceutical industry perspective. Clin Pharmacokinet. 1997;32(4):294-312.

8. Bonate PL. Recommended reading in population pharmacokinetic pharmacodynamics. The AAPS Journal. 2005;7(2):E363-E73.

9. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. III. Monoexponential model: Routine clinical pharmacokinetic data. Journal of Pharmacokinetics and Biopharmaceutics. 1983;11(3):303-19.

10. Aarons L. Software for Population Pharmacokinetics and Pharmacodynamics. Clinical Pharmacokinetics. 1999;36(4):255-64.

11. Mentre F, Gomeni R. A two-step iterative algorithm for estimation in nonlinear mixed-effect models with an evaluation in population pharmacokinetics. Journal of biopharmaceutical statistics. 1995;5(2):141-58.

12. Walker S. An EM algorithm for nonlinear random effects models. Biometrics 1996; 52(3):934-944.

13. Ng CM. Novel hybrid GPU-CPU implementation of parallelized Monte Carlo parametric expectation maximization estimation method for population pharmacokinetic data analysis. Aaps j. 2013;15(4):1212-21.

14. Plan EL, Maloney A, Mentre F, Karlsson MO, Bertrand J. Performance comparison of various maximum likelihood nonlinear mixed-effects estimation methods for dose-response models. Aaps j. 2012;14(3):420-32.

15. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetics parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data. J Pharmacokinet Biopharm. 1980;8(6):553-71.

16. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. II. Biexponential model and experimental pharmacokinetic data. J Pharmacokinet Biopharm. 1981;9(5):635-51.

17. Staatz CE, Tett SE. Comparison of two population pharmacokinetic programs, NONMEM and P-PHARM, for tacrolimus. Eur J Clin Pharmacol. 2002;58(9):597-605.

18. Schumitzky A. EM Algorithms and two stage methods in pharmacokinetic population analysis. In: Advanced methods of pharmacokinetic and pharmacodynamic system analyses. Vol. II. D'Argenio, DZ editor. Plenum Press; New York:1995. p 145-160.

19. Roe DJ. Comparison of population pharmacokinetic modeling methods using simulated data: results from the Population Modeling Workgroup. Statistics in medicine. 1997;16(11):1241-57; discussion 57-62.

20. Bauer RJ, Guzy S, Ng C. A survey of population analysis methods and software for complex pharmacokinetic and pharmacodynamic models with examples. Aaps j. 2007;9(1):E60-83.

21. Bennett JE, Wakefield JC. A comparison of a Bayesian population method with two methods as implemented in commercially available software. J Pharmacokinet Biopharm. 1996;24(4):403-32.

22. Girard P, Mentré F. A comparison of estimation methods in nonlinear mixed effects models using a blind analysis. In: Population Approach Group in Europe P, editor. Population Approach Group in Europe, PAGE; Pamplona, Spain. Pamplona, Spain: Population Approach Group in Europe, PAGE; 2005.

23. McLachlan AJ, Tett SE. Pharmacokinetics of fluconazole in people with HIV infection: a population analysis. British journal of clinical pharmacology. 1996;41(4):291-8.

24. US-FDA Guidance for Industry: Topical Dermatologic Drug Product NDAs and ANDAs-In Vivo Bioavailability, Bioequivalence, In Vitro Release, and Associated Studies, 1998. Bethesda (MD): Center for Drug Evaluation and Research, FDA.

25. D.P. Conner, Differences in DPK Methods, http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_01_conner/index.htm, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, MD, November 29, 2001.

26. Franz, TJ. Study #1, Avita Gel 0.025% vs Retin-A Gel 0.025%, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, MD, November 29, 2001.

27. Pershing, LK. Bioequivalence assessment of three 0.025% tretinoin gel products: Dermatopharmacokinetic vs. clinical trial methods, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, MD, November 29, 2001.

28. Singh GJ, Fleischer N, Lesko L, Williams R. Evaluation of the proposed FDA pilot dose-response methodology for topical corticosteroid bioequivalence testing. Pharm Res. 1998;15(1):4-7.
29. Demana PH, Smith EW, Walker RB, Haigh JM, Kanfer I. Evaluation of the Proposed FDA Pilot Dose-Response Methodology for Topical Corticosteroid Bioequivalence Testing. Pharmaceutical Research. 1997;14(3):303-8.
30. Smith EW, Haigh JM, Walker RB. Analysis of chromameter results obtained from corticosteroid-induced skin blanching. I: Manipulation of data. Pharm Res. 1998;15(2):280-5.
31. Lynn KP. Assessment of Topical Corticosteroid-Induced Skin Blanching Response Using the Visual Mckenzie-Stoughton and Colorimetric Methods. Drug information journal. 1995;29(3):923-34.
32. Holford NH, Sheiner LB. Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. Clin Pharmacokinet. 1981;6(6):429-53.
33. Fisher RA. On the interpretation of chi square from contingency tables, and the calculation of P. Journal of the Royal Statistical Society. 1922;85(1):87-94.
34. Wade JR, Beal SL, Sambol NC. Interaction between structural, statistical, and covariate models in population pharmacokinetic analysis. J Pharmacokinet Biopharm. 1994;22(2):165-77.
35. Yamaoka K, Nakagawa T, Uno T. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. Journal of Pharmacokinetics and Biopharmaceutics. 1978;6(2):165-75.
36. Savic RM, Karlsson MO. Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. AAPS J. 2009;11(3):558-69.
37. Pershing LK, Lambert L, Wright ED, Shah VP, Williams RL. Topical 0.050% betamethasone dipropionate. Pharmacokinetic and pharmacodynamic dose-response studies in humans. Archives of dermatology. 1994;130(6):740-7.
38. Walker RB, Haigh JM, Smith EW. Application of the Minolta Chromameter to the Assessment of Corticosteroid-lnduced Skin Blanching. In: Schwindt DA, Maibach HI, editors. Cutaneous Biometrics. Boston, MA: Springer US; 2000. p. 295-305.
39. Keith G. Bioequivalence of topical corticosteroids: Design and data analysis challenges with the vasoconstrictor assay. 6th World Congress on Bioavailability & Bioequivalence: BA/BE Studies Summit; August 17-19, 2015; Chicago, USA: Journal of Bioequivalence & Bioavailability; 2015. p. 31.
40. Gibson JR, Kirsch J, Darley CR, Burke CA. An attempt to evaluate the relative clinical potencies of various diluted and undiluted proprietary corticosteroid preparations. The British journal of dermatology. 1983;109 Suppl 25:114-6.

41. Aarons L, Balant LP, Mentre F, Morselli PL, Rowland M, Steimer JL, et al. Population approaches in drug development. Report on an expert meeting to discuss population pharmacokinetic/pharmacodynamic software. Eur J Clin Pharmacol. 1994;46(5):389-91.

42. Colucci P, Grenier J, Yue CS, Turgeon J, Ducharme MP. Performance of Different Population Pharmacokinetic Algorithms. Therapeutic Drug Monitoring. 2011;33(5):583-91.

43. Ren K, Braddy A, Wang R, Caramenico H, Conner D. FDA Perspective on Current Challenges with the Use of Pharmacodynamic Endpoint Evaluation of Bioequivalence of Topical Dermatologic Corticosteroids. AAPS; November 10-13, 2013; San Antonio2013.

44. Davidian M, Giltinan DM. Nonlinear models for repeated measures data. New York: Chapman & Hall; 1995.

45. Seng Yue C and Ducharme MP: empirical models, mechanistic models, statistical moments, and noncompartmental analysis. In Shargel L, Yu Andrew BC (eds). Applied Biopharmaceutics and Pharmacokinetics. 7th ed. The McGraw-Hill Inc. 2016. .

46. Gomeni R, Pineau G, Mentre F. Population kinetics and conditional assessment of the optimal dosage regimen using the P-PHARM software package. Anticancer research. 1994;14(6a):2321-6.

47. Kinowski J-M, Bressolle F, Rodier M, Augey V, Fabre D, Richard JL, et al. A Limited Sampling Model with Bayesian Estimation to Determine Inulin Pharmacokinetics Using the Population Data Modelling Program P-PHARM. Clinical Drug Investigation. 1995;9(5):260-9.

48. Wang X, Schumitzky A, D'Argenio DZ. Nonlinear Random Effects Mixture Models: Maximum Likelihood Estimation via the EM Algorithm. Computational statistics & data analysis. 2007;51(12):6614-23.

49. Wang J, Weiss M, D'Argenio DZ. A note on population analysis of dissolution-absorption models using the inverse Gaussian function. Journal of clinical pharmacology. 2008;48(6):719-25.

50. Wang X, Schumitzky A, D'Argenio DZ. Population Pharmacokinetic/Pharmacodyanamic Mixture Models via Maximum a Posteriori Estimation. Computational statistics & data analysis. 2009;53(12):3907-15.

51. Singh GJ, Adams WP, Lesko LJ, Shah VP, Molzon JA, Williams RL, et al. Development of in vivo bioequivalence methodology for dermatologic corticosteroids based on pharmacodynamic modeling. Clinical pharmacology and therapeutics. 1999;66(4):346-57.

52. Tsai J-C, Cheng C-L, Tsai Y-F, Sheu H-M, Chou C-H. Evaluation of in vivo bioequivalence methodology for topical clobetasol 17-propionate based on pharmacodynamic modeling using Chinese skin. Journal of pharmaceutical sciences. 2004;93(1):207-17.

53. Heath DF. Normal or Log-Normal - Appropriate Distributions. Nature. 1967;213(5081):1159.

54. Weippl G, Pantlits.M. Log-Normal or Normal Distribution as A Basic Requirement for Knowledge of Normal Values1970.

55. Graaf PHvd. Introduction to Population Pharmacokinetic/Pharmacodynamic Analysis With Nonlinear Mixed Effects Models. CPT: Pharmacometrics & Systems Pharmacology. 2014;3(12):e153.
56. Kim J, Wang Y, Ren K, Wang R, Braddy AC, Tampal N, et al. Evaluation of Quality of Submission of Vasoconstrictor Assay Bioequivalence Studies for Topical Dermatologic Corticosteroid Products in Abbreviated New Drug Applications (ANDAs). AAPS; October 25–29; Orange County Convention Center, Orlando, Florida.2015.
57. Pershing LK, Bakhtian S, Poncelet CE, Corlett JL, Shah VP. Comparison of skin stripping, in vitro release, and skin blanching response methods to measure dose response and similarity of triamcinolone acetonide cream strengths from two manufactured sources. Journal of pharmaceutical sciences. 2002;91(5):1312-23.
58. Lehman PA, Franz TJ. Assessing Topical Bioavailability and Bioequivalence: A Comparison of the In vitro Permeation Test and the Vasoconstrictor Assay. Pharmaceutical Research. 2014;31(12):3529-37.
59. Kirsch J, Gibson JR, Darley CR, Barth J, Burke CA. Forearm site variation with the corticosteroid vasoconstrictor assay. The British journal of dermatology. 1982;106(4):495.
60. Ale SI, Laugier JP, Maibach HI. Spacial variability of basal skin chromametry on the ventral forearm of healthy volunteers. Archives of dermatological research. 1996;288(12):774-7.
61. Sommer A, Veraart J, Neumann M, Kessels A. Evaluation of the vasoconstrictive effects of topical steroids by laser-Doppler-perfusion-imaging. Acta dermato-venereologica. 1998;78(1):15-8.

Chapter 4

4 Novel Approach for the Bioequivalence Assessment of Topical Cream Formulations: Model-Based Analysis of Tape Stripping Data Correctly Concludes BE and BIE

4.1 Preface

One of the other fields in the context of BE yet to be improved is the BE assessment of topical dermatological products. Similar to all other locally acting drugs, bioequivalence of these products is a long standing challenge in generic drug development. Plasma concentration profiles of these products are often not appropriate surrogates of their pharmacological activity. Therefore, with the exception of topical corticosteroids, the only acceptable approach by almost all regulatory agencies for BE demonstration of a topical dermatological drug product to an innovator product is comparative clinical trials. Nevertheless, this approach is the least accurate, sensitive, and reproducible of the general approaches for demonstrating bioequivalence. For this reason, many attempts have been directed to establish a BE approach based on PK principles at the local site of action, the skin itself. The study of pharmacokinetics of topical drug products in the skin has emerged as a method called Dermatopharmacokinetic (DPK). The most common procedure in DPK approach for extracting the skin data is sequential removal of skin layers by application of adhesive tapes on the surface of the skin, called tape stripping.

Dermatopharmacokinetic approach was first introduced by the US FDA's OGD as a universal method for demonstrating BE of all topical drug products in June 1998 [443] but then was withdrawn in May 2002 due to the conflicting BE assessment results for the same formulations, in two different laboratories [382, 384, 385]. Although the apparent conflict in BE assessment results was subsequently determined to be attributable to differences in the design and methodologies followed by the two research groups and not lack of reproducibility of the DPK approach [385], the FDA has since been critically reevaluating DPK, with a view to improving sensitivity, reproducibility, and reducing complexity of the approach.

Since 2002, many efforts have been directed to the refinement of tape stripping study design [388-390, 444-454]. Although promising, all attempted approaches to date are associated with certain limitations and complexity. Aside from their tedious and labor-intensive study protocols, they were not successful in offering the PK metrics reflective of the rate and extent of exposure in the skin. Moreover, the estimation of PK parameters in all tested approaches required numerous measurements, several assumptions, and sophisticated

calculations. Numerous measurements, per se, contribute to the high variability of skin stripping data. None of the approaches was successful yet to adopt tape stripping back as a reliable method for regulatory purposes. Currently, Japan and South Africa are the only jurisdictions that may accept DPK approach for BE demonstration of topical drug products [198].

The third part of this thesis project aimed to develop a novel DPK approach by (a) refining the study design and (b) proposing a novel approach in generating the DPK profile and analysis of the skin data collected from the tape stripping procedure.

Refinement of study design ((a) as referred to, above) comprised of simplified procedure by conducting tape stripping only at one dose duration during the uptake phase which corresponded to DD_{50}. As for the novelty in data analysis ((b) as referred to, above), for the first time in the literature, population CPT modeling was implemented in the analysis of tape stripping data and the PK parameters reflective of rate (K_{in}) and extent (F_S) of exposure or input into the skin were estimated directly from fitting the data, circumventing several assumptions and sophisticated calculation in previous methods. The feasibility of this approach in both bioequivalence and bioinequivalence assessment was tested using three different topical formulations of acyclovir, the RLD and generic products of acyclovir, and a 70% lower in strength bioinequivalent formulation.

The following article demonstrates how our novel DPK approach was successful in concluding BE and distinguishing BIE. The advantages of this approach over previous ones are also discussed in this article.

4.2 Article #3

Novel Approach for the Bioequivalence Assessment of Topical Cream Formulations: Model-Based Analysis of Tape Stripping Data Correctly Concludes BE and BIE

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4.2.1 Abstract

Purpose: The purpose of this study was (a) to suggest a novel dermatopharmacokinetic (DPK) approach from which pharmacokinetic parameters relevant to the bioequivalence (BE) assessment of a topical formulation can be deduced while circumventing the need for numerous measurements and assumptions, and (b) to investigate whether this approach enables the correct conclusion of BE and bioinequivalence (BIE).

Methods: Bioequivalent and bioinequivalent formulations of acyclovir were compared versus a reference product (Zovirax®). Tape Stripping was conducted at only one dose duration during the uptake phase to generate drug content in *stratum corneum* versus time profiles, each time point corresponding to one stripped layer. Nonlinear mixed effect modeling (ADAPT®5) (MLEM algorithm) was used to fit the DPK data and to estimate the rate (K_{in}) and extent (F_S) of drug absorption/input into the skin. Results were evaluated using the average BE approach.

Results: Estimated exposure metrics were within the usual BE limits for the bioequivalent formulation (F_S: 102.4 [90% CI: 97.5–107.7]; K_{in}: 94.2 [90% CI: 83.7–106.0]), but outside those limits for the bioinequivalent formulation (F_S: 43.4 [90% CI: 27.9–67.6]; K_{in}: 54.5 [90% CI: 36.6–81.1]).

Conclusions: The proposed novel DPK approach was shown to be successful, robust and applicable to assess BE and BIE correctly between topical formulations.

Keywords: Bioequivalence and Bioinequivalence, Population PK Modeling, Tape Stripping, Topical Dermatological Formulations, Regulatory Sciences

4.2.2 **Abbreviations**

ACV	Acyclovir
API	Active pharmaceutical ingredient
AUC	Area Under Curve
BA	Bioavailability
BE	Bioequivalence/Bioequivalent
BIE	Bioinequivalence/Bioinequivalent
C_{max}	Maximum concentration
CPT	Compartment
CV	Coefficient of variation
DD	Dose Duration
DPK	Dermatopharmacokinetics
ED_{50}	The dose duration at which 50% of E_{max} is obtained
E_{max}	Maximum effect
F_S	Extent of absorption/input into the skin (%)
FDA	Food and Drug Administration
GMR	Geometric Mean Ratio
GOF	Goodness-of-Fit
HV	Healthy volunteers
IOV	Inter-Occasion Variability
IPRED	Individual-level predictions
K_{diff}	Diffusion rate constant
K_{in}	First-order absorption/input rate constant into the skin
K_{out}	Diffusion rate constant out from the last compartment
Max	Maximum
Min	Minimum
ML	Maximum Likelihood
MLEM	Maximum Likelihood Expectation Maximization
MOF	Minimum Value of the Objective Function
PK	Pharmacokinetics
Pop	Population
PRED	Population-level predictions
Q_{max}	Maximum amount
Ref	Reference
RLD	Reference Listed Drug
RP	Reference Product

SC	*Stratum Corneum*
STS	Standard 2-Stage
TOST	Two One-Sided Tests
TS	Tape Stripping
VPC	Visual Predictive Check
WRES	Weighted residuals

4.2.3 **Introduction**

Assessment and comparisons of drug product performances are usually made using pharmacokinetic (PK) measures that reflect the rate and extent of drug exposure in a clinically relevant and accessible biological matrix (e.g., blood, plasma, or serum). Regulatory agencies typically request PK metrics for the assessment of bioequivalence (BE) of drug products that are absorbed systemically, as similar PK profiles indicate that a similar amount has reached the sites of action, and a similar safety and efficacy could be expected between the bioequivalent products (1). Topical dermatological drug products, however, are designed for local action and drug effect occurs prior to systemic exposure. Hence, systemic concentrations may not correlate with drug efficacy (2, 3). Instead, drug quantity in the skin is a more clinically relevant measure for the assessment of topical BE based on a PK approach. Nevertheless, PK-based BE assessment for topical drug products is more challenging compared to drug products intended for the systemic circulation (4, 5) since the measurement of drug concentrations in blood for such products is considered inappropriate.

Currently, the most common approach recommended by regulatory agencies for BE assessment of topical dermatological drug products, except for corticosteroids, is acquiring clinical endpoints in patients. In the case of topical dermatological corticosteroids, regulatory agencies recommend BE studies with pharmacodynamic endpoints where skin blanching at the application site is compared between products (5, 6).

The use of comparative clinical endpoints in patients is considered the least sensitive and reproducible approach among all other approaches to demonstrate BE (7). Such studies are more complicated and costlier to run, as they generally require a large number of patients as opposed to the use of healthy volunteers in studies with PK endpoints. Even though studies

with clinical endpoints typically have a large sample size, they are often insensitive to formulation differences. Regulatory agencies and researchers have acknowledged the need to find faster, less expensive, and more reproducible and sensitive approaches to reliably demonstrate BE for topical dermatological products (5, 6, 8).

As previously mentioned, the skin is the target organ of topically administered dermatological drug products and presents the most clinically relevant matrix for drug measurements for the demonstration of BE of topical products (5). Efforts to develop a method with measurable PK endpoints for BE assessment of topical dermatological formulations at the site of action (*i.e.*, the skin) have given rise to the emergence of dermatopharmacokinetics (DPK). Among these efforts, the studies conducted by Rougier et al. (9-12) are of value. The principal assumption of DPK is that regardless of how far through the skin layers the drug needs to penetrate, it first needs to pass through the *stratum corneum* (SC) before reaching deeper skin layers. Hence, differences in the rate and extent of the active ingredient exposure at site(s) of action deeper in the epidermis or dermis are assumed to arise from differences in drug quantity vs. time profiles in the SC (3, 6, 13, 14). As a consequence, the amount of drug recovered from the SC may theoretically be related to the amount reaching the target site (15). This assumption is analogous to the one made for traditional PK-based BE assessments for orally administered drugs. Concentrations of a drug in blood/urine are related to and will influence the amount that reaches the target tissue(s). Therefore, two oral formulations are judged to have equivalent safety and efficacy profiles if they have equivalent plasma concentration vs. time profiles. Similarly, two formulations that have comparable drug amount vs. time profiles in the SC can be considered to have equivalent safety and efficacy as they should have comparable profiles at the site of action(s) (6, 13). The most commonly used non-invasive methods of DPK for the assessment of BA and BE of topically applied dermatological drug products in SC is tape stripping (TS) (14).

DPK was introduced in a Draft FDA Guidance in June 1998 as a universal method to document BA/BE for all topical formulations (16). However, the Draft Guidance was withdrawn 4 years later in May 2002, in part due to the contradictory BE assessment results found by two different expert laboratories (17). The TS method was used to compare the commercially available Reference Listed Drug (RLD) of tretinoin gel (Retin-A®); and non-

bioequivalent tretinoin gel (Avita®) (18). The apparent conflict of these results was initially attributed to the lack of reproducibility of the TS method. It was proposed much later that these contradictory findings were the result of protocol and methodology differences between the two studies (17). However, the credibility of the DPK approach had already suffered to the extent that continuing research in the field became largely restrained. Currently, Japan appears to be the only jurisdiction that accepts *in vivo* DPK studies (8).

Following its 2002 withdrawal, many concerns have been raised regarding the FDA's 1998 TS Draft Guidance (2, 3, 14, 19-25). Several accounts of methods were performed to refine and improve the TS procedure (2, 3, 14, 21, 22, 24-27). However, these DPK approaches involve numerous calculations and assumptions related to the estimation of parameters characterizing the "absorption" into the skin, which may not directly reflect the rate and extent of "absorption" (for transdermal formulations) or input into the skin (for topical formulations)[2]. Some of these DPK approaches also involve the estimation of parameters characterizing elimination/clearance from the SC which do not offer an advantage for BE purposes. To date, none of the proposed TS methods have been adopted by regulatory agencies.

We herein propose a simpler DPK approach for a more direct estimation of the rate and extent of "absorption" or input into the skin for BA/BE assessment. A simpler TS procedure, involving application at a determined discriminative time point of uptake phase, was adopted by testing the data described in a publication by Nallagundla *et al.* (28). Our methods were thus tested by comparing a generic formulation of acyclovir (ACV) that had been shown to be BE to the Reference Product (RP) (Study 1), and a lower potency formulation of ACV that was bioinequivalent (BIE) to the RP (Study 2). The DPK approach consisted of a pilot Dose Duration (DD) study where the ED_{50} at the discriminative part of the E_{max} model was estimated (28), followed by the collection of TS samples at ED_{50} over a specified duration of time, thus yielding drug amount vs. time profiles. Using a novel population PK approach, the

[2] Studied ACV creams are locally effective topical formulations; therefore, input into the skin should be preferred over absorption which is rather reflective of systemic exposure. For the purpose of simplicity, however, absorption and input into the skin may interchangeably be used throughout this text.

rate (K_{in}) and extent (F_S) of absorption/input into the skin of each formulation were calculated and compared between test and reference products to investigate whether our proposed strategy could correctly conclude BE and distinguish BIE.

4.2.4 Materials and Methods

The clinical TS procedure and details (Formulations, Experimental Procedure and Data Acquisition) have been published earlier by Nallagundla et al. (28).

The TS was conducted only at one Dose Duration (DD) during the uptake phase. The procedure was successfully used in previously published studies to assess the BE of topical clotrimazole (29) and clobetasol propionate formulations (30). Similarly, the procedure was adopted in the Japanese Guideline for Bioequivalence Studies for Topical Dermatological-Applied Generic Products (31). Further descriptions and applications of this method have been published in book chapters (32, 33).

4.2.4.1 Formulations

Zovirax® cream (5% ACV, GlaxoSmithKline (Pty) Ltd, South Africa) was used as RP and an approved generic acyclovir cream (5% ACV, Adco®, Adcock-Ingram (Pty) Ltd, South Africa) was the test product shown to be BE. The BIE formulation was a lower potency 1.5% acyclovir cream (consisting of the same composition as Adco®).

4.2.4.2 Experimental Procedure and Data Acquisition

Twenty human volunteers in Study 1 and 10 in Study 2 were included. All subjects had healthy skin and no history of dermatological disease. Study 1 (n=20) compared concurrent applications of Zovirax® (Reference) and 5% Adco® (Test, BE product), while Study 2 (n=10) compared that of Zovirax® (Reference) and a 1.5% ACV cream formulation (Test, BIE formulation). Each drug product was applied twice, one on each arm, for a total of 4 application sites per subject.

Approximately 20 mg of each cream formulation was applied, providing a total dose of 1000 mcg for the 5% ACV formulations and 300 mcg for the 1.5% ACV formulation. All treatments were applied over a DD of 8 min. The decision to choose an 8-minute exposure

was presented in a previously referenced paper (28) based on the E_{max} model which provides estimates of E_{max} and ED_{50}. The DD of 8 min is equal to the ED_{50} determined from the DD-response relationship and is the time required for the absorption of 50% of the applied dose. A total of 15 successive strips were collected from each application site. However, the first strip was discarded as the drug amount on the first strip was assumed to represent unabsorbed drug left after cleaning off the excess cream.

Each stripping procedure lasted approximately 30 seconds (except for the first strip as it was discarded). One time point was attributed for each stripped layer. Therefore, for each drug application, a total of 14 time points were collected, starting from 8 minutes to 14.5 minutes. As each formulation was applied twice (once on each arm), a total of 28 tape stripping samples were collected per formulation, per subject. Therefore, the dataset of Study 1 included a total of 1120 observations (28 tape stripping samples x 2 formulations x 20 subjects) and Study 2 included a total of 560 observations (28 tape stripping samples x 2 formulations x 10 subjects).

4.2.4.3 Population PK Modeling

Construction of the PK Model

The structure of the PK model was developed using only data from the RP from Study 1. Once the final model for the RP was selected, the model was modified to allow for the estimation of PK parameters for the Test when fitting of Test and RP simultaneously. Data fitting using the population approach was conducted separately for each study. Ln-normal distribution of PK parameters was assumed in the process of model development.

All tested PK models consisted of 14 compartments (CPTs) as one CPT was attributed to each stripped layer. Each model was comprised of two types of PK parameters: formulation-related PK parameters and PK parameters independent of formulation factors. Formulation-related PK parameters are those that reflect the local BA of drug product in terms of the rate and extent of input into the skin. Therefore, they are reflective of drug product performance and are the pertinent PK parameters for BE assessment. On the other hand, PK parameters independent of formulation factors were related to characteristics of the Active

Pharmaceutical Ingredient (API) (i.e., ACV). Hence, these PK parameters are the same for the test and reference products.

The formulation-related PK parameters included in each PK model were: the first-order absorption/input rate constant (K_{in}) which represented the rate of "absorption", and % absorbed/delivered amount of applied ACV dose from the cream into the skin (F_S) which represented the extent of "absorption".

Due to the duplicate application of each formulation in each subject and the likelihood of having some variability in the amount of formulation applied to the skin between these two dosing occasions, an additional F parameter was then introduced into the model, accounting for inter-occasion variability (IOV). Therefore, the % absorbed/delivered amount of applied dose into the skin, at each dosing occasion, was estimated separately. The IOV was subsequently calculated from F estimates in terms of geometric CV%.

The PK parameters independent of formulation factors were the diffusion/transfer rate constants between two consequent layers of the SC. These parameters were introduced to describe the diffusion of API from one layer to the next one and were not used in BE assessment. The diffusion rate constants (K_{diff}) were estimated assuming a first-order process. The simplest model was built by assigning one diffusion rate constant (K_{diff1}) to all 14 CPTs, while the most complicated model included a diffusion rate constant attributed to each CPT (K_{diffn}). Other structural PK models were nested by different pooling of diffusion rate constants between the CPTs. The diffusion rate constant describing drug output from the last CPT was defined as K_{out}.

Each PK parameter was associated with an inter-individual variability. Additive and proportional error components were used to characterize the unexplained (residual) variability.

Fitting of the PK Data

Population PK analyses were performed using the software ADAPT®5 version 5.0.53 with the Maximum Likelihood Expectation Maximization (MLEM) algorithm. The first step in fitting the data was to obtain results using a standard two stage (STS) with maximum likelihood (ML) algorithm. The median of the estimated PK parameters from ML were then used as priors (initial estimates) for the MLEM nonlinear mixed effect modeling approach.

Each Population analysis was run until 1000 population iterations with importance sampling set at 2000. Results from each MLEM analysis were considered to have started attaining convergence when all PK parameter values had converged graphically (i.e., when all PK parameters appeared to have reached stable values). The minimum value of the objective function (MOF) was chosen as the lowest found -2LogLikelihood over the range of converged population iterations. The population iteration number at convergence was then chosen as the first population iteration (with a minimum of 200 stable population iterations) that resulted in the exact median convergence estimate. Once the population iteration at convergence was determined, the MLEM analysis was re-run until this exact population iteration in order to get the population parameter values and their associated individual estimates ("*post-hocs*").

Model Evaluation

The official criterion determining the selection of the most appropriate model was the minimum value of the objective function (MOF) according to a chi-square distribution ($P<0.01$). If the difference between the MOF values for two tested models was greater than the critical value based on a chi-square test with a p-value of 0.01, the more complicated model was chosen (34). As an example, a model with two additional parameters (degree of freedom = 2) would require a decrease in the MOF of at least 9.21 to achieve statistical significance, and be selected as the superior model. Residual variability was also monitored as a supplementary measure of quality of fit.

The quality of the fit of the final model was assessed using the following diagnostic Goodness-of-Fit (GOF) plots: observations vs. population-level predictions (DV vs. PRED), observations vs. individual-level predictions (DV vs. IPRED), weighted residuals vs. population-level predictions (WRES vs. PRED), and weighted residuals vs. time (WRES vs. time).

Population means and post-hoc estimates of PK parameters were compared to investigate whether the model predictions in population and individual levels complied with each other and whether model predictions of both levels could be used. Visual Predictive Check (VPC) procedure was conducted for internal validation of the final model. Using the final model, and the population PK parameters and variability estimated from this model,

ACV amount vs. time profiles for 1000 subjects were simulated. The 5th and 95th percentile of the simulated predictions were plotted to check if 90% of the observations were included within this interval. A VPC was conducted for each dosing occasion.

4.2.4.4 Bioequivalence Evaluation

In agreement with the Two One-Sided Tests (TOST) procedure for BE assessment (35), the Geometric Mean Ratio (GMR) (Test/Ref) and the 90% CIs for F_S and K_{in} PK parameters, based on ln-transformed post-hoc estimates, were calculated for each study. Following the traditional FDA criteria, formulations were to be considered (a) bioequivalent if the GMR (Test/Ref) and the 90% CIs for both the rate and extent of "absorption" into the skin were contained completely within the 80.00 to 125.00% interval, (b) bioinequivalent if the GMR (Test/Ref) and the 90% CIs for both the rate and extent of "absorption" into the skin were outside the 80.00 to 125.00% interval, and (c) inconclusive if neither the BE nor BIE criterion was met.

Since two F_S parameters for each formulation were introduced into the model (i.e., one for each dosing occasion), the GMR (Test/Ref) and the 90% CI for F_S were calculated using a statistical procedure for BE assessment of 4-way fully replicated crossover studies (36). Post-hoc estimates of $F_{S(Test)}$ and $F_{S(Ref)}$ in each dosing occasion were ln-transformed and the arithmetic means were calculated for $LnF_{S(Test)}$ and $LnF_{S(Ref)}$ for each subject. The difference between the average $LnF_{S(Test)}$ and $LnF_{S(Ref)}$ was calculated for each subject; these values were then averaged (arithmetic mean) across all subjects. The GMR was then determined by taking the antilogarithm of the average ($LnF_{S(Test)}$-$LnF_{S(Ref)}$).

4.2.5 Results

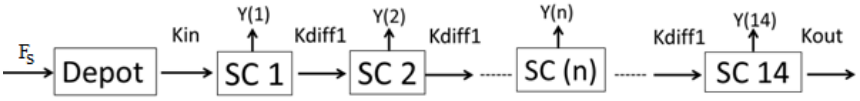
4.2.5.1 Population PK Modeling

Construction of the PK Model

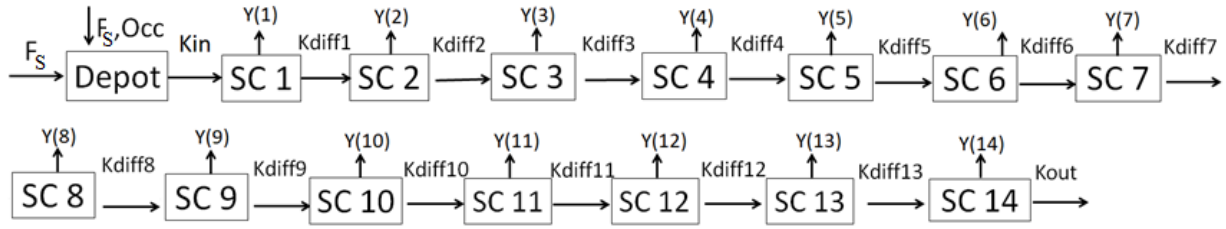
Eight structural PK models were tested, starting from the simplest to the most complicated one. The initial model included one F_S for both dosing occasions for each formulation, and the final model included two F_S (i.e., one for each dosing occasion for each

formulation). The schematic diagrams of the compartmental structure of different PK models are presented in **Fig.1**.

Model 1 (the simplest)



Model 8 (the most complicated)



Final PK model

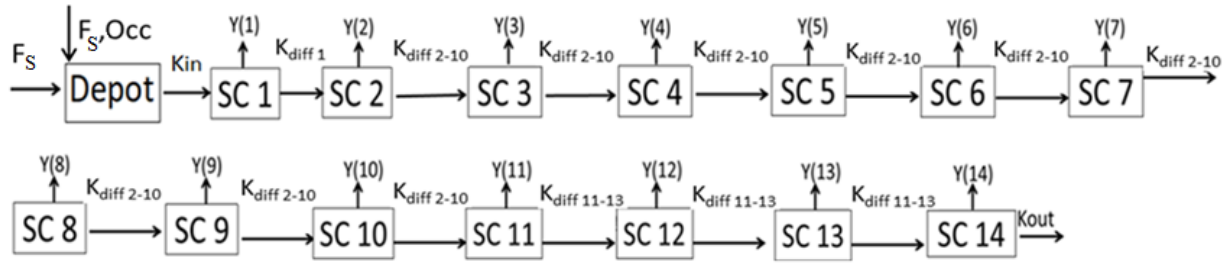


Figure 1. Structural representation of the simplest and most complicated models, as well as the final PK model. K_{in}, first-order absorption/input rate constant (min^{-1}); $K_{diff(n)}$, K_{out}, first-order transfer/diffusion rate constants from one CPT to the next one (min^{-1}); FS, % absorbed/delivered amount of applied dose in dosing occasion 1; $F_{S,Occ}$ (also known as F_{S2}), % absorbed/delivered amount of applied dose in dosing occasion 2; Y(n), predicted amount (µg) of ACV in each stripped layer (CPT).

Final PK Model

Based on the results of fitting the reference DPK data using ADAPT®5, Model 3 gave a better fit than all other tested models, and was considered as the final PK model. The PK model discrimination process is summarized in **Table I**. Model 3 was used for the simultaneous fitting of Test and RP data for the purpose of BE assessment (data fitted per study).

Estimated parameters in the final PK model (Model 3) included the percentages (F_{S1} in dosing occasion 1 and F_{S2} in dosing occasion 2) of the delivered ACV dose into the skin by a first-order process (K_{in}), and four first-order diffusion rate constants (K_{diff1}, $K_{diff(2-10)}$, $K_{diff(11-13)}$ and K_{out}). Introducing an additional F parameter into the model resulted in a decrease of 142.877 units in the MOF value, which indicated a statistically significant improvement in quality of fit of the model (P<0.01). The MOF for models with additional diffusion rate constants (model 4 – model 8) increased, implying on deterioration of the quality of fit (**Table 1**).

Table 1 PK model discrimination

Model	Ref Model	No. of parameters (Theta, ETA, EPS)	MOF at iteration No.	Change in MOF (P<0.01)	Residual variability
1		10 (4 , 4, 2)	-111.904 at 966		22%
2	1	14 (6, 6, 2)	-189.777 at 840	-77.873 (> -13.277)	22.60%
3[a]	**2**	**16** (7, 7, 2)	**-332.654 at 891**	-142.877 (> -9.21)	20.60%
4	3	18 (8, 8, 2)	-229.387 at 964	+103.267 (> -9.21)	21.70%
5	3	20 (9, 9, 2)	-239.672 at 989	+92.982 (> -13.277)	21.40%
6	3	22 (10, 10, 2)	-232.138 at 989	+100.516 (> -16.812)	22.60%
7	3	24 (11, 11, 2)	-240.977 at 989	+91.677 (> -20.09)	21.40%
8	3	34 (16, 16, 2)	-255.527 at 997	+77.127 (> -34.805)	22%

[a] Selected model is shown in bold

The final PK model was mathematically described by the following series of differential equations, where: K_{in} is the first-order input rate constant for RP; K_{inT} is first-order input rate constant for Test; % delivered amount of applied dose of ACV from first and second dosing occasions were represented as F_{S1} and F_{S2} for the RP, respectively, and F_{S1T} and F_{S2T} for the Test, respectively; K_{diff1}, $K_{diff(2-10)}$, $K_{diff(11-13)}$ and K_{out} are first-order diffusion rate constants.

14-CPT Model

Z1=1

Z2=1

Z3=1

Z4=1

IF(T.GT.8) Z1=0

```
IF(T.GT.5008) Z2=0
IF(T.GT.10008) Z3=0
IF(T.GT.15008) Z4=0

XP(1)=+R(1)*F1-Kin*Z1*X(1)
XP(2)=+Kin*Z1*X(1)+Kin*Z2*X(16)-Kdiff1*X(2)+KinT*Z3*X(17)+KinT*Z4*X(18)
XP(3)=+Kdiff1*X(2)-Kdiff(2-10)*X(3)
XP(4)=+Kdiff(2-10)*X(3)-Kdiff(2-10)*X(4)
XP(5)=+Kdiff(2-10)*X(4)-Kdiff(2-10)*X(5)
XP(6)=+Kdiff(2-10)*X(5)-Kdiff(2-10)*X(6)
XP(7)=+Kdiff(2-10)*X(6)- Kdiff(2-10)*X(7)
XP(8)=+Kdiff(2-10)*X(7)-Kdiff(2-10)*X(8)
XP(9)=+Kdiff(2-10)*X(8)-Kdiff(2-10)*X(9)
XP(10)=+Kdiff(2-10)*X(9)-Kdiff(2-10)*X(10)
XP(11)=+Kdiff(2-10)*X(10)-Kdiff(2-10)*X(11)
XP(12)=+Kdiff(2-10)*X(11)-Kdiff(11-13)*X(12)
XP(13)=+Kdiff(11-13)*X(12)-Kdiff(11-13)*X(13)
XP(14)=+Kdiff(11-13)*X(13)-Kdiff(11-13)*X(14)
XP(15)=+Kdiff(11-13)*X(14)-Kout*X(15)
XP(16)=+R(2)*F2-Kin*Z2*X(16)
XP(17)=+R(3)*F1T-KinT*Z3*X(17)
XP(18)=+R(4)*F2T-KinT*Z4*X(18)
```

Z1 to Z4 are the flags which play a role as on/off switch for absorption from formulation into the skin. Before removal of formulation at the 8th min, the absorption from the formulation into the skin could start at any time. However, at the 8th min, the formulation was removed and therefore no more absorption could occur after 8 min. Introducing Z flags into the model switches on and off the absorption before and after 8th min, respectively. Z1 and Z2 correspond to the first and second dosing occasions for RP, respectively, and Z3 and Z4 correspond to the first and second dosing occasions for Test, respectively. Absorption/input rates ($\mu g.min^{-1}$) into the skin from the first and second dosing occasions were represented as

R1 and R2 for the RP, respectively, and R3 and R4 for the Test, respectively. X(n) is the estimated amount of ACV in each CPT.

Fitting of Test and Ref

PK parameters and their inter-individual variability (CV%) are presented in **Table 2** for the fitting of the generic product and RP, and in **Table 3** for the fitting of the BIE formulation and RP. When fitting the DPK data of generic product vs. RP, the IOV of 9.22% and 7.00% were found for $F_{S(Ref)}$ and $F_{S(Generic)}$, respectively. When fitting the DPK data of BIE formulation vs. RP, the IOVs of 10.99% and 20.37% were found for $F_{S(Ref)}$ and $F_{S(BIE)}$, respectively.

Table 2 PK parameter estimates and their inter-individual variability (geometric CV%) following 20 mg cream application of 5% ACV (1000 µg dose) Generic [a] and Ref [b] products for 8 min (based on the study by Nallagundla et al. (28), 50% of the bioavailable dose was absorbed after 8 min)

Parameter	Geometric Mean (CV%)	Median (Min-Max)
$F_{S1(Ref)}$	0.461 (11.86%)	0.488 (0.333-0.499)
$F_{S2(Ref)}$	0.452 (20.46%)	0.481 (0.198-0.498)
$F_{S1(Generic)}$	0.465 (9.16%)	0.489 (0.369-0.500)
$F_{S2(Generic)}$	0.470 (5.96%)	0.480 (0.384-0.498)
$K_{in(Ref)}$ (min^{-1})	0.0030 (20.33%)	0.0028 (0.0022-0.0052)
$K_{in(Generic)}$ (min^{-1})	0.0028 (15.41%)	0.0028 (0.0020-0.0036)
K_{diff1} (min^{-1})	0.420 (10.82%)	0.424 (0.315-0.511)
$K_{diff(2-10)}$ (min^{-1})	0.553 (7.41%)	0.559 (0.455-0.657)
$K_{diff(11-13)}$ (min^{-1})	0.511 (46.27%)	0.535 (0.113-0.995)
K_{out} (min^{-1})	0.910 (136.10%)	0.955 (0.04- 4.695)

[a] Adco® (5% ACV)

[b] Zovirax® (5% ACV)

Table 3 PK parameter estimates and their inter-individual variability (geometric CV%) following 20 mg application of 1.5% ACV BIE formulation [a] (300 µg dose) and 5% ACV Ref product [b] (1000 µg dose) for 8 min (based on the study by Nallagundla et al. (28), 50% of the bioavailable dose was absorbed after 8 min)

Parameter	Geometric Mean (CV%)	Median (Min-Max)
$F_{S1(Ref)}$	0.419 (13.68%)	0.441 (0.327 – 0.483)
$F_{S2(Ref)}$	0.366 (11.28%)	0.363 (0.294 – 0.417)
$F_{S1(BIE)}$	0.175 (92.08%)	0.260 (0.055 – 0.456)
$F_{S2(BIE)}$	0.166 (53.14%)	0.199 (0.081 – 0.348)
$K_{in(Ref)}$ (min^{-1})	0.0026 (19.27%)	0.0028 (0.0020 – 0.0034)
$K_{in(BIE)}$ (min^{-1})	0.0014 (51.63%)	0.0014 (0.0006 – 0.0028)
K_{diff1} (min^{-1})	0.370 (17.90%)	0.362 (0.284 – 0.554)
$K_{diff(2-10)}$ (min^{-1})	0.613 (4.28%)	0.614 (0.568 – 0.644)
$K_{diff(11-13)}$(min^{-1})	0.530 (9.79%)	0.535 (0.466 – 0.627)
K_{out} (min^{-1})	0.397 (25.79%)	0.386 (0.264 – 0.626)

[a] 1.5% ACV

[b] Zovirax® (5% ACV)

Model Evaluation

The diagnostic plots of the final model are presented in **Fig. 2** As demonstrated by the GOF plots, the model adequately describes observed amounts of AVC in all stripped layers. The individual- and population-level predicted ACV amounts were in reasonable agreement with the observed amounts recovered from stripped layers. In addition, no obvious biased patterns were observed for the plots of WRES vs. PRED and WRES vs. time. Residual variabilities of all tested models ranged from 20% to 22%.

Geometric mean values of observed and fitted amounts of ACV at each time point were compared between the RP and test formulations. **Fig. 3** shows that the observed and fitted ACV amount vs. time profile from generic and Ref products overlaid, while the profile from RLD and BIE formulation and Ref product did not. The ACV amount vs. time profile

also shows a remarkable agreement between the model-predicted and observed ACV amounts for each formulation.

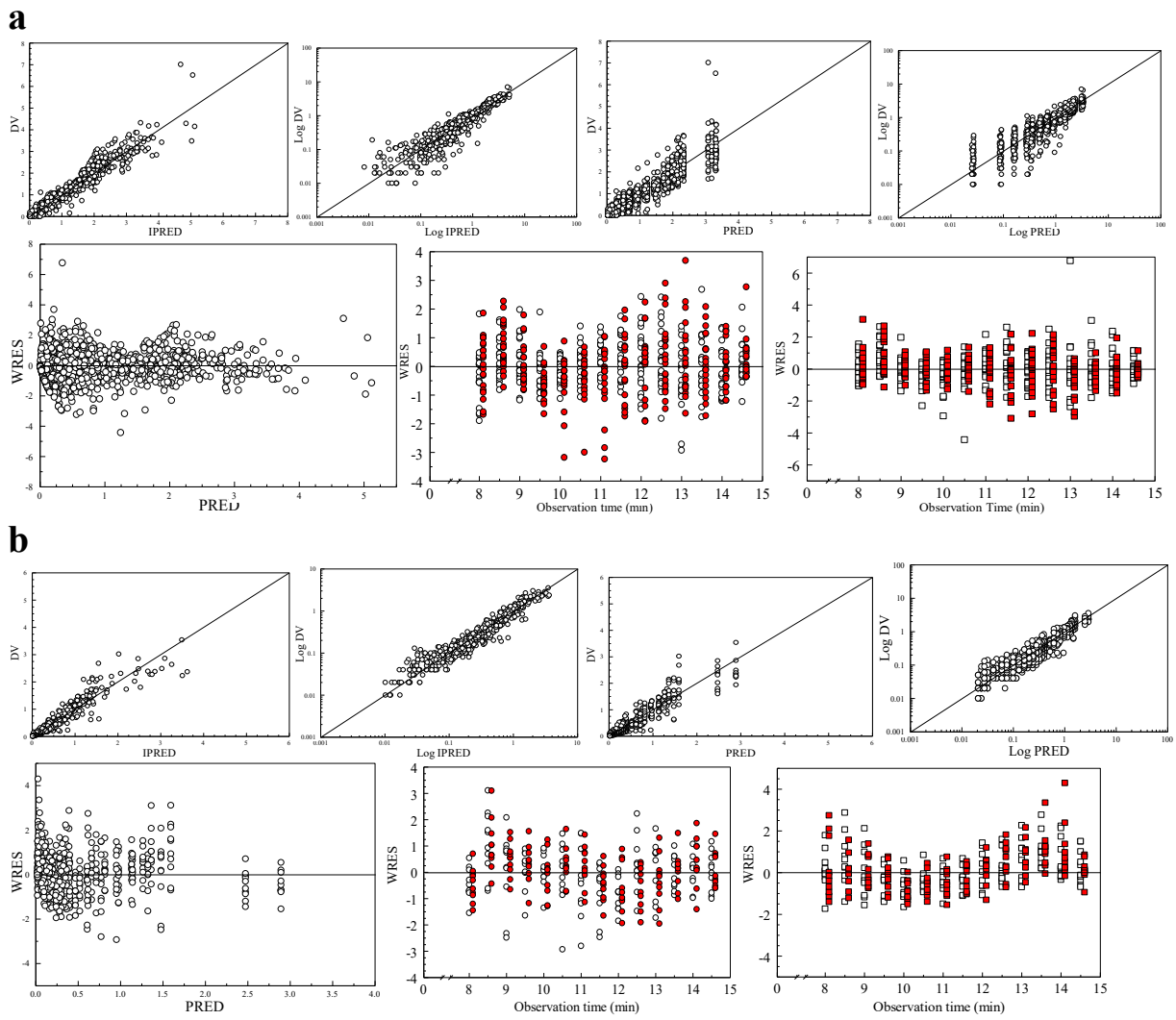


Figure 2. Goodness-of-fit plots for the final population PK model (a) when fitting Generic vs. Ref Product, (b) when fitting BIE formulation vs Ref Product (b). From left to right for each panel: observations (DV) vs. individual-level model predictions (IPRED), observations vs. individual-level model predictions on a log scale, observations vs. population-level model predictions (PRED), observations vs. population-level model predictions on a log scale, weighted residuals (WRES) vs. population-level model predictions, weighted residuals vs. time for RLD, weighted residuals vs. time for Test. Legend: Solid line = the line of identity, Blank and red filled symbols in WRES vs. time plots are representative of dosing occasion 1 and 2, respectively.

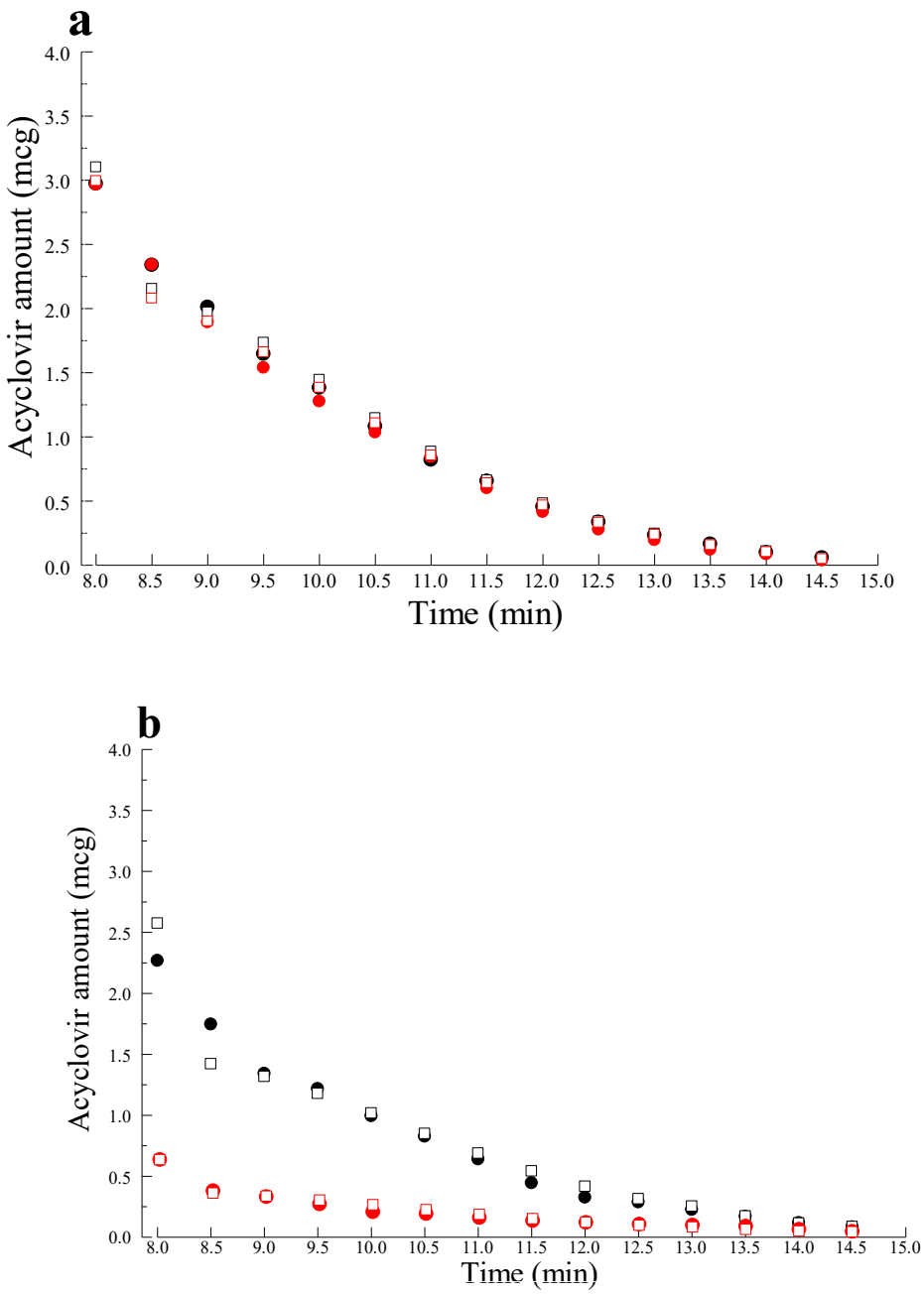


Figure 3. Individual fitted (squares) and observed (circles) amount vs. time profiles of ACV in stratum corneum after 8 min topical administration of (a) Ref product (black) vs. generic product (red), and (b) Ref product (black) vs. BIE formulation (red) in healthy human subjects; geometric mean of fitted and observed data at each time point are plotted.

VPCs generated for the internal validation showed that the simulated ACV amount vs. time curves conformed to the observed values in each dosing occasion (**Fig. 4**), suggesting that the final model was stable and the PK parameters were well estimated.

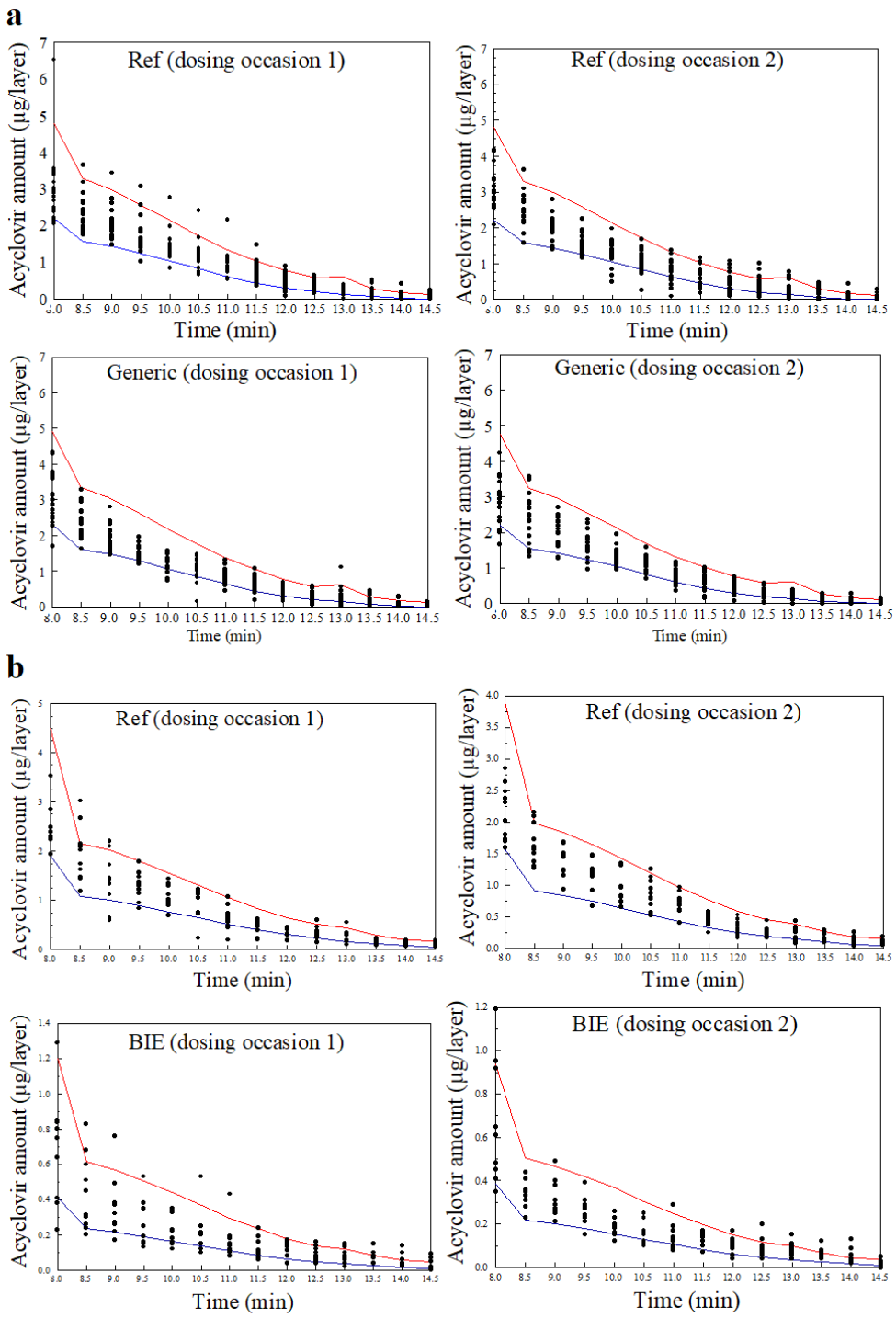


Figure 4. Visual predictive checks of the PK model for ACV recovered amount after topical administration of cream formulations (a) when fitting Generic vs. Ref product, (b) when fitting BIE formulation vs. Ref product (b), used for model validation. Black dots represent the observed recovered amount in each layer; the red and blue lines represent the upper and lower bound of 90% prediction interval of the model, respectively.

4.2.5.2 Bioequivalence Evaluation

The population and post-hoc estimates of formulation-related PK parameters and their associated inter-individual variability (CV%) and IOV (when relevant) from the final model are presented in **Table 4**.

Table 4 Estimated PK parameters for BE Assessment (based on the study by Nallagundla et al. (28), 50% of the bioavailable dose was absorbed after 8 min)

PK Parameter	Population Estimates	Post-hoc Estimates	IOV (Geo CV%) [c]
	Geo. mean (CV%) [a]	Geo mean (CV%) [a] Arith. mean (CV%) [b]	
When fitting generic product vs. RP [d]			
$F_{S(Ref)}$	48.77% (4.65%)	45.61% (16.5%) 46.11 % (12.60%)	9.22%
$F_{S(Generic)}$	48.70% (4.05%)	46.73% (7.64%) 46.86% (7.18%)	7.00%
$K_{in(Ref)}$ (min^{-1})	0.0030 (0.030%)	0.0030 (20.33%) 0.0030 (22.74%)	-
$K_{in(Generic)}$ (min^{-1})	0.0028 (0.022%)	0.0028 (15.41%) 0.0028 (14.64%)	-
When fitting BIE formulation vs. RP [d]			
$F_{S(Ref)}$	40. 39% (13.56%)	39.11% (14.04%) 39.47% (13.76%)	10.99%
$F_{S(BIE)}$	20.21% (370.43%)	16.99% (71.13%) 20.19% (54.84%)	20.37%
$K_{in(Ref)}$ (min^{-1})	0.0026 (0.02%)	0.0026 (19.28%) 0.0026 (18.81%)	-
$K_{in(BIE)}$ (min^{-1})	0.0014 (0.04%)	0.0014 (51.63%) 0.0016 (46. 89%)	-

[a] Geometric mean (Geometric CV%)
[b] Arithmetic mean (Arithmetic CV%)
[c] Inter-occasion variability is given as geometric CV%
[d] Reference Product

When fitting the generic product and RP (Study 1), the GMR (Generic Test/Ref) for both F_S and K_{in} and their 90% CIs were within the equivalence interval of 80.00 to 125.00%. Therefore, BE was successfully concluded between the generic and Ref products. When fitting BIE formulation and RP (Study 2), the GMR (BIE Test/Ref) and the 90% CIs for both the rate and extent of delivered dose into the skin fell outside the 80.00-125.00% interval. Therefore,

BIE was concluded. A summary of the BE assessment results for each study is provided in **Table 5**.

Table 5 Summary of the Comparative Local Bioavailability from Population Analysis Predictions When (a) Comparing Generic vs. Ref Product, and (b) Comparing BIE Formulation vs. Ref Product

(a)

PK Parameter	GMR (%) [a]	90% CI (%) [b]
F_S (%)	102.4	97.5 – 107.7
$K_{in}(min^{-1})$	94.2	83.7 – 106.0

(b)

PK Parameter	GMR (%)	90% CI (%)
F_S (%)	43.4	27.9 – 67.6
$K_{in}(min^{-1})$	54.5	36.6 – 81.1

[a] Geometric mean ratio (%)
[b] 90% Confidence interval

4.2.6 **Discussion**

This work proposes a novel DPK approach using a simplified TS procedure for the demonstration of BE between two topical dermatological drug products which involves a refinement of the TS procedure and a model-based approach for the analysis of the DPK data. Refinement of the TS procedure consisted of (1) the reduced number of DDs which simplified the experimental procedure in comparison to other DPK approaches, and (2) conducting TS at the DD equal to ED_{50} during the uptake phase which rendered the procedure to be more discriminative of formulation differences. The model-based approach for the analysis of the DPK data reduced the variability of the data, but more importantly, enabled the direct estimation of rate and extent of "absorption" or "delivery" into the skin, while parameters used in other DPK approaches (2, 3, 14, 24, 26, 37) are not direct measures of rate and extent of absorption" or "delivery" into the skin. A summary of the DPK approaches which have been investigated to-date is given in **Table 6**. Their comparisons to our novel approach are summarized in **Table 7** and discussed in detail below.

Table 6 A Summary of different DPK approaches and PK metrics for topical BA/BE assessment which were used in different studies

Number of DD	Number of stripped sites	DPK profile	BA/BE measures	Reference
4 1	8 (4 in uptake and 4 in clearance) 2 (1 in uptake and 1 in clearance)	Q vs. time [a]	Q_{max} and AUC	N'Dri-Stempfer et al. (25)
4	8 (4 in uptake and 4 in clearance)	Q vs. time	Q_{max} and AUC	Pershing et al. (18)
1	2 (1 in uptake and 1 in clearance)	C vs. depth [b]	Q_{total} [e], Flux, K_{Cl} [f]	Cordery et al. (23)
1	2 (1 in uptake and 1 in clearance)	- [c]	Q_{total} [e] in uptake Q_{total} in clearance	N'Dri-Stempfer et al. (24)
1	2 (1 in input and 1 in clearance)	Q vs. time [d]	Q_{total} in uptake Q_{total} in clearance	Pedon de Araujo et al. (45)
1	2 (1 in input and 1 in clearance)	-	-	Bunge et al. (27)
1	1 in uptake	C vs. depth	K and D/L^2	Alberti et al. (3)
1	1 in uptake	C vs. depth	K and D/L^2	Alberti et al.(21)
1	1 in uptake	C vs. depth	K and D/L^2	Pirot et al. (22)
1	1 in uptake	C vs. depth	K and D/L^2	Alberti et al. (50)
1	1 in uptake or 1 in clearance	C vs. depth	K and D/L^2	Reddy et al. (44)
1	1 in uptake	Q vs. depth	AUC	Parfitt et al. (29)
1	1 in uptake	C vs. depth	K and D/L^2	Herkenne et al. (14)
1	1 in uptake	C vs. depth	K and D/L^2	Herkenne et al. (51)
1	1 in uptake	C vs. depth	K and D/L^2	Wiedersberg et al. (52)
1	1 in uptake	C vs. depth	K and D/L^2	Herkenne et al. (53)
1	1 in uptake	C vs. depth	K and D/L^2	Herkenne et al. (54)

[a] SC drug amount vs. time
[b] SC drug concentration vs. relative depth into the SC
[c] information was not specified/accessible
[d] In this study SC drug amount vs. time profile was generated by an approach similar to *FDA proposed method*, not with *Two-Time method*.
[e] Total drug amount recovered from all stripped layers
[f] First-order clearance rate constant

The TS procedure consists of successive applications and removal of adhesive tapes to the surface of the skin. Each adhesive tape removes a microscopic layer of the outermost skin layer (i.e., the SC). Measuring the drug content in the removed skin layers enables the measurement of drug in the SC as a function of time, and then allows for the generation of a SC drug content vs. time profile, called the DPK profile (2, 24).

As per the 1998 FDA guidance (38), in order to generate a DPK profile, the amount of drug in the SC should be determined in no less than eight sites: 4 sites in the uptake phase corresponding to 4 different drug exposure periods of time (called DD), and 4 sites in the clearance phase corresponding to different post-drug removal time points. Having 8 sites for each of the test and reference product, and collecting approximately 10-15 strips at each DD yields a minimum of 160-240 samples per subject to be analyzed, rendering a DPK evaluation burdensome in terms of the amount of work involved. Moreover, the guidance recommended generating the DPK profile in terms of SC drug quantity vs. time (18, 39) from which classical PK measures of maximum drug quantity per unit area ($ng.cm^{-2}$) in the SC (Q_{max}), time to reach this maximum (T_{max}), and the area under the curve (AUC) for the BA/BE assessment can be deduced, in a similar fashion to PK measurements for an orally administered drug. As such, BE is recommended to be assessed by comparing Q_{max} (also referred to as "C_{max}" in the literature) and AUC between Test and Ref. However, Q_{max} (or C_{max}) and AUC in the SC are not analogous to the peak plasma drug concentration (C_{max}) and AUC, respectively, obtained after oral administration of systemically absorbed drugs. After oral administration, C_{max} and AUC are calculated from plasma drug concentrations, which represent the measured amount of active moiety that reaches the systemic circulation per unit of volume of distribution. Two simultaneous processes govern plasma concentrations: ongoing absorption of the remainder of administered dose from the gastrointestinal tract into the small intestinal enterocytes, and the clearance from the bloodstream (40). As such, both C_{max} and AUC are affected by the kinetics of drug absorption, distribution, metabolism and elimination. This is not the case for topical drug delivery into the skin. The maximum amount quantified in the skin (Q_{max}) is not measured per unit of volume. Therefore, unlike C_{max}, Q_{max} is not representative of concentration, but rather an amount. In addition, the mechanisms of uptake into and elimination from the SC are almost always controlled by penetration across the skin, often through the SC itself (41). The amount of drug in the SC represents the measured amount that reaches deeper layers of the SC post-removal of the excess formulation (18, 24), where (1) clearance does not occur, except the clearance that is initiated artificially by removing the product from the skin surface rather than through the depletion of the applied dose (26), and (2) a volume of distribution does not exist (42). The absence of clearance and a volume of

distribution at the sampling site in topical administration render AUC in SC to be different than the systemic AUC obtained from plasma drug concentration vs. time profiles.

A challenge of the TS method is that stripping does not remove the same amount of SC across sequentially stripped layers from the same skin location (43). The integrity of the SC is maintained by corneosomes that form specialized inter-corneocyte linkages. Corneosomes increase from the surface of the skin towards the granular layer. Due to the greater cohesive properties of corneosomes in deeper layers versus their looser packing in the surface, permeability barrier function of the skin and therefore diffusivity of the drug substance in different layers can vary. As a result, the amount of drug stripped from each layer will be measured in different volumes of the matrix. Moreover, different tapes and different experimentalists will inevitably strip the SC in a variable fashion, making inter- and intra-laboratory comparisons difficult, if not impossible. Even when the type of tape is standardized, and an attempt is made to exert an equal pressure on each tape applied to the skin, the SC amount removed still depends on, for example, the speed with which the tape is subsequently removed from the skin and exactly where on the forearm the TS is performed (2, 3). As standardization appeared to be very difficult to achieve, new approaches emerged to identify a method by which the amount of stripped SC can be quantified, thereby allowing the uptake of drug into this tissue to be normalized (2, 28-33).

Some of these efforts led to the *Relative-Depth method* where the uptake of drug into the SC was normalized by expressing drug concentration in the SC (an amount per unit volume or weight of SC) as a function of normalized SC depth which, in turn, requires quantification of the mass of SC removed (2, 3, 14). In this method, the DPK profile is reported as a concentration (drug amount per volume of SC [mol L^{-1}]) vs. normalized depth of the SC removed (x/L), where x and L represent the thickness of SC removed and the full thickness of the unstripped SC, respectively (2, 3, 22). This is different from the *FDA Proposed method* where the DPK profile is represented by the total amount in SC ($ng.cm^{-2}$) as a function of time (h) (18, 22, 38). The *Relative-Depth method* approach has the advantage of measuring drug quantity only at one DD in the uptake phase instead of a minimum of 8; however, it necessitates the measuring of the mass of the SC removed in each layer (ng) and incorporation of assumptions to derive the total thickness of the SC (cm) from the removed

mass (19, 20). Bioequivalence based on the *Relative-Depth method* is assessed by comparing two parameters between Ref and Test: K, which is the SC-vehicle partition coefficient of the drug, and D/L^2, which is the diffusivity (D) of the drug across the SC of thickness L. The ratio D/L^2 represents first-order rate constant for drug transport or diffusion across the SC, or the rate of "absorption", while K represents the extent of "absorption" of the drug across the SC. These two parameters have been used to compare topical drug BA from different formulations and assess bioequivalence (or not) between different drug formulations (3, 14, 22). Nevertheless, due to the numerous assumptions related to the estimation of these parameters, and their rather complicated calculations, we would argue that they may not directly reflect the rate and extent of "absorption" or input into the skin. Another limitation of the *Relative-Depth method* is that it doesn't take into consideration that drug continues to move across the SC layer throughout the tape stripping procedure. Unless tape stripping is rapidly conducted, the SC drug concentration measured in each TS may be different from the concentration at that location when the exposure ends. This can affect the accuracy in the estimation of K and D/L^2 (44).

Of the most significant efforts that simplified the TS procedure were the studies performed by Bunge et al. (27) and N'Dri-Stempfer et al. (24), which introduced the *Two-Time method* (26, 37), although a relatively arbitrary DD was used. In this method, several modifications to the withdrawn FDA 1998 Draft Guidance TS procedure were made: the number of DDs was reduced to only one with duplicate application of each formulation for that DD, and TS was conducted once in the uptake phase and once in the clearance phase (23-25, 27).

In the *Two-Time method*, the total amount of drug per unit area in the SC (Q_{total}) is determined by summation of drug amount recovered from all stripped samples and normalizing it by the sample area (ng cm^{-2}). The normalization of drug quantity by sample area in the *Two-Time method* is less complicated than using depth of the stripped and the total SC in the *Relative-Depth method*. Reducing the number of dose durations and using less complicated normalizations, resulted in a simplification of the TS procedure while duplicating the application of each formulation reduced the variability of the DPK data and improved reproducibility of this method (23, 26). Although the *Two-Time method* was relatively a

simpler DPK approach in comparison to the FDA *Proposed* and the *Relative-Depth methods*, it did not provide a DPK profile from which the rate of input into the SC could be derived. Therefore, BE can be assessed only based on the extent of input into the skin, comparing the total drug amount in the uptake ($Q_{total(uptake)}$) and clearance phase ($Q_{total(clearance)}$) of Test versus Ref, instead of AUC (24, 37, 45).

In the study performed by Parfitt et al. (28-33), the AUC from a plot of the SC drug amount vs. normalized depth was calculated using the trapezoidal rule to compare the BA between test and reference products. Nevertheless, no PK parameter representative of the rate of "absorption" or input into the skin was deduced with this method.

In summary, to date different PK parameters such as AUC, C_{max} and/or Q_{max} and thermodynamic parameters such as K and D/L^2 have been used for the purpose of assessing topical BA and BE (3, 14, 18, 22, 29). However, there is no consensus which PK parameters should be used as a universal PK metric in the DPK procedure for the BA or BE assessment of dermatological drug products. Moreover, even if a consensus is achieved, the PK parameters may not necessarily represent the same PK measure – since multiple ways of calculating the same PK parameter have been utilized. For instance, the AUC calculated from SC drug concentration vs. depth profile used Fick's second law of diffusion for the *Relative-Depth method* (3, 14, 22, 46), while in the *FDA Proposed method* the AUC was calculated from SC drug amount vs. time profile using the trapezoidal rule (18). Likewise, Q_{max} in SC is not the same as C_{max} but it is confusingly referred to as C_{max} in the FDA 1998 Draft Guidance (16) and by Pershing et al. (18).

In this project, we developed a novel approach of generating a DPK profile from which the PK parameters reflecting the rate and extent of absorption/input into the SC could directly be derived. The derived PK parameters in our approach are analogous to those used in PK-based BE studies for systemically available drugs. Therefore, with our proposed refined strategy, we addressed some of the limitations and complexities that exist in the *FDA Proposed method* and the other DPK approaches.

In our DPK approach, in accordance with the procedure described by Nallagundla et al. (28-33), each formulation was applied twice based on the assumption that it may decrease

variability in the DPK data. Duplicate application of each formulation was previously found to reduce the magnitude of the experimental error considerably (26), thereby reducing the variability in the DPK data and improving reproducibility (23, 24, 27). Numerous measurements of drug amount were avoided by performing TS only at one DD. A total of 56 samples per subject were collected for analysis. This number would have been increased to 224 samples per subject with the *FDA Proposed method* (14 TS x 8 sites for Test and 14 TS x 8 sites for Ref) and to 112 samples per subject with the *Two-Time method* (28 TS for Test and 28 TS for Ref in each of uptake and clearance phases). In our proposed approach, the DD was selected within the uptake phase. The rationale behind using one DD within the uptake phase is due to the ultimate purpose of the study, the BE assessment which is the test of formulation performance. Performance of topical dermatological formulations is one step before "absorption" into the skin which comprises the release of the drug substance from formulation and its partition into the SC. All other steps following partition of drug substance into the SC are related to the behavior of drug substance alone, out of formulation, and are not directly related to formulation performance. Therefore, elimination/clearance from the SC was not characterized as it would not offer an advantage for BE purposes.

At the end of the single exposure period (DD) of 8 minutes, sequential stripped layers were harvested, with each TS procedure lasting 30 seconds, and one time point was attributed to each stripped layer. This enabled us to generate a DPK profile that consisted of the *SC drug quantity* (μg) as a function of *time*. It is of importance to clarify that neither the *SC drug quantity* nor the *time* concept in our approach is similar to those in other approaches. *SC drug quantity* in this research is simply the amount of drug recovered per strip with a unit of mass (μg or ng), instead of SC drug concentration (ng cm^{-3}) as per *Relative-Depth method* which necessitates gravimetric SC quantification and TEWL measurements per stripped layer. Accordingly, expressing the *SC drug quantity* as a function of *time*, instead of depth, circumvents the need to quantify the amount of SC removed and TEWL measurements per stripped layer, as well as numerous assumptions for converting the removed SC mass to the SC thickness/depth for BE assessment. This also eliminates a part of variability arising from experimental errors that could occur in these measurements. Therefore, an advantage of our

method in comparison to the *Relative-Depth method* is that our approach simplifies the DPK procedure and reduces the variability in the DPK data in terms of drug quantity measurement.

Likewise, the *time* concept in our approach is different from the *time* concept specified in the *FDA Proposed method*. In the *FDA Proposed method*, each time point represented one DD, and to develop a DPK profile, one needed a minimum of 4 DDs to characterize the input phase and another 4 DDs to characterize the clearance of drug from the SC, yielding to a total of 8 DDs. However, in this novel approach, TS was performed at the end of only one period of exposure (only at one DD) and the *time* in DPK profile represents the time points at which the SC was harvested. Therefore, an advantage of our methodology in comparison to the *FDA Proposed method* is that our approach circumvents performing TS at many DDs and simplifies the DPK procedure.

Population PK modeling was used to fit the DPK data. The use of population PK modeling enabled us to deduce the PK parameters that directly reflect the rate and extent of "absorption" or input into the skin. It also circumvented numerous assumptions in the estimation of the parameters for topical BA/BE assessment and their sophisticated calculations which have previously been used by other methods. With population PK modeling, the estimates of the rate and extent of "absorption" are expected to be more precise and associated with less inter-individual variability, despite the highly variable nature of DPK data. However, the advantages and disadvantages of our method in comparison to others would have been better understood if the same formulations were used in our study and previous ones.

The PK models were described by introducing only the identifiable PK parameters. In our model, K_{in} stands for the first-order input rate constant which, more precisely, consists of the rate constants of the release process of the drug substance from the dosage form and its partitioning into the SC. Diffusion of the drug substance through the SC layers was characterized by considering that the diffusivity may vary in different layers due to the varying amounts of corneosomes throughout the SC (43). To this purpose, in the model development process, different poolings of the layers were tested with the presence of one common diffusion rate constant for all layers in the simplest model (Model 1) and the presence of one diffusion rate constant for each layer in the most complicated one (Model 8) (**Fig. 1**).

Based on the model discrimination process (**Table 1**), attributing one diffusion rate constant to each stripped layer did not significantly improve the quality of fit. This may be due to the fact that TS could approximately remove a total of 0.5-1 μm of the SC (2) and within this depth, cohesive properties of skin tissues do not change significantly. A model with 4 transfer rate constants for 14 stripped layers (Model 3) was found to describe the stripping data most successfully.

The input rate constant (K_{in}) and the diffusion rate constant (K_{diff}) PK parameters were estimated assuming a first-order process, because the SC is a Fickian membrane (43). Therefore, the flux (*J*) or the rate of diffusion of a drug through the SC can be described most simply by Fick's first law of passive diffusion. Given Fick's law of diffusion, the delivered amount of API per unit time to each layer depends on the amount in the previous layer which is explained by the first-order absorption process.

As topical dermatological products are not designed to deliver the drug into the systemic circulation, the permeation process is expected to stop at diffusion through the layers, before ending into the cutaneous circulation. Therefore, we did not introduce clearance and volume of distribution PK parameters as they would be unidentifiable. Instead, K_{out} was the last diffusion rate constant that represented the diffusion of the drug substance from the last stripped layer to the deeper one(s).

In our analysis, the PK parameters of importance for BE assessment were the rate and extent of absorption/input into the skin which were introduced into the model as first-order absorption/input rate constant (K_{in}) and % absorbed/delivered amount of applied ACV dose from the cream into the skin (F_S). Due to the likelihood of variability in the amount of formulation applied to the skin in two dosing occasions, two F_S parameters were introduced into the model, accounting for inter-occasion variability (IOV). An IOV of approximately 10% was found for the extent of ACV input from RP into the skin ($F_{S(Ref)}$) in both Study 1 and Study 2. The IOVs estimated for $F_{S(Generic)}$ and $F_{S(BIE)}$ were ≈ 7% and ≈ 20%, respectively. Inter-occasion variability is important to be considered in the model development especially when individual PK parameters are of interest, such as when using population PK modeling for the purpose of BE assessment. Introducing the IOV into the model enabled us to differentiate a part of the variability arising from the likely differences in the applied mass of

the drug product at two dosing occasions from other sources of variability (residual variability). This differentiation cannot be attained by non-model-based approaches which have been tried to date.

Bioequivalence between the generic and Ref products was concluded based on the Two One-Sided Tests (TOST) procedure (35). The 90% CIs for the GMR (Test/Ref) were entirely contained within the equivalence interval of 80.00-125.00% for both PK parameters: GMR =102.4 [90% CI: 97.5–107.7] for F_S, GMR = 94.2 [90% CI: 83.7–106.0] for K_{in}). This suggests that the population PK modeling was successful in concluding BE when the Test (generic product) and RP were in fact bioequivalent.

To date, several studies have used nonlinear mixed effect modeling to test BE and all concluded the applicability of population PK modeling to BE demonstration (47-49); however, no study has been conducted to investigate the feasibility of population PK modeling in BIE assessment. We used model-based estimates to test the BIE between two formulations. Bioinequivalence can only be concluded when the GMR (Test/Ref) and the 90% CI for the ratio fall completely off the equivalence interval without touching the margins. If we define the BE region/interval as the range of $[80.00\%, 125.00\%]$, then the BIE regions should be considered as the union of two regions: $(0, 80.00\%) \cup (125.00\%, +\infty)$. As such, BIE can be concluded when the GMR (Test/Ref) and 90% CI are completely in either of the two BIE regions. Similar to BE, BIE was concluded based on the TOST procedure. The GMR (Test/Ref) and 90% CIs fell completely out of the 80.00-125.00% range for F_S (GMR=43.4 [90% CI: 27.9–67.6]). Similarly, GMR (Test/Ref) and 90% CIs fell out of the range for K_{in} (GMR=54.5 [90% CI: 36.6–81.1]). To conclude BIE, GMR (Test/Ref) and 90% CI for both PK parameters ideally should fall completely out of the 80.00-125.00% range. For K_{in} in this study, the upper limit of the CI exceeded the margin of 80%, but this can be attributed to the limited sample size of the study (n=10). It is very likely that in a new study with a larger sample size, the width of the 90% CI would decrease; hence, it would fall completely outside the 80.00-125.00% interval. This suggests that the population PK modeling was also successful in distinguishing BIE when the test formulation and RP were in fact not bioequivalent.

To our knowledge, population PK modeling of DPK data analysis for the purpose of topical BE/BIE assessment has not been described in the literature. Our proposed DPK approach with the use of population PK modeling is shown to be successful at concluding BE and distinguishing BIE correctly, for topical formulations.

4.2.7 **Conclusion**

From the analysis of ACV tape stripping data in this study, we conclude that the population PK analysis of drug amount in SC vs. time profile supports the ability of a protocol using one DD within the uptake phase to assess BE and distinguish BIE reliably with far fewer analyses. The proposed approach offers the significant advantage that (a) drug is measured at a single discriminatory DD with duplicate application by implementing the TS procedure published previously (28-30), and that (b) the measures of topical BA (the rate and extent of absorption/input into the skin) can directly be derived without an excessive list of assumptions and numerous measurements. We thus propose the model-base analysis of DPK data which can be obtained from the clinical TS procedure published earlier (28-30) as a means to implement TS into regulatory frameworks for the purpose of topical BE assessment as an alternative to clinical studies. For BIE assessment, further studies are warranted to investigate the feasibility and the discriminatory power of population PK modeling in detecting smaller differences in formulation strength between Test and Ref.

Table 7 Comparison of different DPK approaches and incorporated tape stripping data analyses

DPK/TS approach	*FDA Proposed method* [a]	Modified/Improved approaches		Our proposed approach
		Relative-Depth method	*Two-Time method*	
Procedure	**1.** Quantification of total amount of drug (e.g., ng) at 8 sites (4 sites to characterize input into the SC, 4 sites to characterize output from the SC): - During the uptake phase TS is performed at 4 sites, each site is attributed to one DD. - During the clearance phase TS is performed at 4 sites, each site is attributed to a different post-removal period for a single DD at steady state. **2.** The formulation is applied only once at each DD.	**1.** Quantification of drug concentration (e.g., $ng.cm^{-3}$) at 1 DD during the uptake phase: - The DD is not necessarily equal to ED_{50}. - Drug amount on each strip is measured and is considered as one observation.	**1.** Quantification of total amount of drug per unit area (e.g. $ng.cm^{-2}$) at 1 DD: - The DD is not necessarily equal to ED_{50}. - TS is conducted once during the uptake phase, which is immediately after product removal, and the other time after a period of clearance. - Drug amount on each strip is measured, but the amounts on all tapes are combined to obtain Q_{total}. **2.** Duplicate application of drug at the tested DD	**1.** Quantification of drug amount (e.g., ng) at 1 DD during the uptake phase: - The DD is equal to ED_{50} - Drug amount on each strip is measured and is considered as one observation. **2.** Duplicate application of the drug at the tested DD
DPK Profile	- SC drug content per unit area (e.g., $ng.cm^{-2}$) vs. time - Each time point, in the uptake phase, corresponds to 1 DD; and in the clearance phase corresponds to 1 post-removal period for a single	-SC drug concentration (e.g., $ng.cm^{-3}$) vs. normalized depth (x/L) - Each time point corresponds to one of the strips, all of which were taken at 1 DD.	No PK profile is produced.	- SC drug content (e.g., ng) vs. time - Each time point corresponds to one of the strips, all of which were taken at 1 DD.

	DD at steady state.			
PK parameters for BA/BE assessment	Q_{max}, AUC Q_{max} and AUC are obtained from DPK profile; - Q_{max} is the maximum observed drug amount during the uptake phase - AUC is calculated for the time interval from zero to the longest clearance time using the trapezoidal rule.	K, D/L^2, AUC K and D/L^2 are obtained from DPK profile; - AUC is obtained from integration of the equation of Fick's second law of diffusion across the SC thickness (i.e., from x/L= 0 to x/L=1). - AUC represents the overall uptake of drug into the skin.	$Q_{total(uptake)}$, $Q_{total(clearance)}$	F_S, K_{in} F_S and K_{in} are obtained from DPK profile; - Both are directly estimated by fitting the DPK profile using population PK modeling.
Advantages	Only recovered amount of drug (e.g., ng) from stripped layers needs to be quantified.	**1.** Drug quantification at 1 DD and 1 site (in the uptake phase) instead of a minimum of 8 sites. **2.** Standardized/normalized data with better reproducibility.	**1.** Drug quantification at 1 DD and 2 sites (1 in the uptake, another in the clearance phase) instead of a minimum of 8 sites. **2.** More reproducible results and less variability in PK estimates for BA/BE assessment.	**1.** Drug quantification at 1 DD and 2 sites (duplicate application for 1 DD in the uptake phase) instead of a minimum of 8 **2.** Neither the amount of SC removed on each strip, nor the full thickness of SC needs to be quantified/deduced. Therefore; **-** No excessive list of assumption needs to be made. **2.** More reproducible results and less variability in PK estimates for BA/BE

				assessment. **3.** Better discrimination of formulation differences is expected due to the conduction of TS at the DD equal to ED_{50} of the DD-response relationship.
Disadvantages	**1.** The procedure is burdensome; - A minimum of 8 sites are required for demonstration of the DPK profile. - Numerous measurements are required (15-20 tape strippings at each site per subject, thus, a total of ≈120 analyses per formulation per subject). **2.** the method is not standardized; high variability in the data and lack of reproducibility **3.** C_{max} (more precisely, Q_{max}) and AUC in SC are not comparable/analogous to the C_{max} and AUC in systemic circulation, in a sense to be directly reflective of the rate and extent of "absorption".	**1.** Removed amount of SC on each strip must be quantified (in weight units), in addition to that of permeated drug. **2.** This amount must then be converted to removed thickness (depth) on each strip **3.** Full thickness of SC (L) must be measured. **4.** Weight to depth conversion and L calculation require many assumptions to be made: - $\rho \sim 1$ g cm^{-3} [b] - K ~ 0.06 [c] - $\Delta C \sim 55$ M ≈1 g·cm^{-3} [d] - Homogeneous barrier function of SC to water diffusion - Lack of a universal method in deduction of the full thickness of the SC (Linear vs. Non-Linear Model	**1.** The amount of drug in samples collected during the clearance phase is likely to be below the limit of quantification especially for slowly diffused drugs, even if the tapes are combined and extracted in groups to achieve high quantities for analysis. This can introduce additional variability into the DPK data. **2.** The method cannot be used for BA assessment, given that only the extent of exposure ($Q_{total(uptake)}$ [e], $Q_{total(clearance)}$ [f]) is determined and compared between the formulations. The rate of input into the skin cannot be calculated. **3.** DPK profile is not generated; therefore, it cannot be indicative of topical formulation performance (steps encompassing drug substance release from	Further studies are warranted to distinguish the power of the method in distinguishing the non-bioequivalence between the formulations which fail to be either BE or BIE.

		approaches) **5.** Estimation of PK parameters for BE assessment is sophisticated and they are not directly reflective of rate and extent of absorption for BE purposes. **6.** SC drug concentration measured in each TS may be different from the concentration at that location when the exposure ended, which would affect estimated values for K and D/L^2, unless the TS procedure is fast.	formulation, and its partition into the SC).	
Recommended applications	BE assessment (old approach)	It may be more useful for formulation development than BE assessment	BE assessment	BA/BE assessment

[a] Recommended approach in Withdrawn FDA 1998 Draft Guidance
[b] ρ: Density of SC
[c] K: SC-viable tissue partition coefficient of water
[d] ΔC: the water concentration difference across the membrane (tissue concentration minus that in the atmosphere above the skin surface
[e] Total amount of drug in the SC during the uptake phase
[f] Total amount of drug in the SC during the clearance phase

4.2.8 References

1. Chen ML, Lesko L, Williams RL. Measures of exposure versus measures of rate and extent of absorption. Clin Pharmacokinet. 2001;40(8):565-72.
2. Herkenne C, Alberti I, Naik A, Kalia YN, Mathy F-X, Préat V, et al. In Vivo Methods for the Assessment of Topical Drug Bioavailability. Pharmaceutical research. 2007;25(1):87.
3. Alberti I, Kalia YN, Naik A, Guy RH. Assessment and Prediction of the Cutaneous Bioavailability of Topical Terbinafine, In Vivo, in Man. Pharmaceutical research. 2001;18(10):1472-5.
4. Borsadia S, Ghanem AH, Seta Y, Higuchi WI, Flynn GL, Behl CR, et al. Factors to be considered in the evaluation of bioavailability and bioequivalence of topical formulations. Skin pharmacology : the official journal of the Skin Pharmacology Society. 1992;5(3):129-45.
5. Yacobi A, Shah VP, Bashaw ED, Benfeldt E, Davit B, Ganes D, et al. Current challenges in bioequivalence, quality, and novel assessment technologies for topical products. Pharmaceutical research. 2014;31(4):837-46.
6. Shah VP, Flynn GL, Yacobi A, Maibach HI, Bon C, Fleischer NM, et al. Bioequivalence of topical dermatological dosage forms-methods of evaluation of bioequivalence. Pharmaceutical research. 1998;15(2):167-71.
7. United States Food and Drug Administration, Title 21 Code of Federal Regulations (CFR) Part 320, Section 24. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=320.24. Accessed 08 Feb 2018.
8. Braddy AC, Davit BM, Stier EM, Conner DP. Survey of international regulatory bioequivalence recommendations for approval of generic topical dermatological drug products. The AAPS journal. 2015;17(1):121-33.
9. Rougier A, Dupuis D, Lotte C, Roguet R, Wester RC, Maibach HI. Regional variation in percutaneous absorption in man: measurement by the stripping method. Archives of dermatological research. 1986;278(6):465-9.
10. Rougier A, Dupuis D, Lotte C, Roguet R, Schaefer H. In vivo correlation between stratum corneum reservoir function and percutaneous absorption. The Journal of investigative dermatology. 1983;81(3):275-8.
11. Rougier A, Rallis M, Krien P, Lotte C. In vivo percutaneous absorption: a key role for stratum corneum/vehicle partitioning. Archives of dermatological research. 1990;282(8):498-505.
12. Rougier A, Lotte C, Maibach HI. In vivo percutaneous penetration of some organic compounds related to anatomic site in humans: predictive assessment by the stripping method. Journal of pharmaceutical sciences. 1987;76(6):451-4.

13. Raney SG, Franz TJ, Lehman PA, Lionberger R, Chen M-L. Pharmacokinetics-Based Approaches for Bioequivalence Evaluation of Topical Dermatological Drug Products. Clinical Pharmacokinetics. 2015;54(11):1095-106.
14. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Dermatopharmacokinetic prediction of topical drug bioavailability in vivo. The Journal of investigative dermatology. 2007;127(4):887-94.
15. Pershing LK, Bakhtian S, Poncelet CE, Corlett JL, Shah VP. Comparison of skin stripping, in vitro release, and skin blanching response methods to measure dose response and similarity of triamcinolone acetonide cream strengths from two manufactured sources. Journal of pharmaceutical sciences. 2002;91(5):1312-23.
16. US-FDA Guidance for Industry: Topical dermatologic drug product NDAs and ANDAs-in vivo bioavailability, bioequivalence, in vitro release, and associated studies, 1998. Bethesda (MD): Center for Drug Evaluation and Research, FDA.
17. Franz TJ. Study #1, Avita gel 0.025 % vs Retin-A gel 0.025 %. Transcribed presentation to the Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, MD, November 29, 2001. Transcript of presentation.pp.47–61.
http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3804t2_01_Morning_Session.pdf. Accessed 08 Feb 2018.
18. Pershing LK, Nelson JL, Corlett JL, Shrivastava SP, Hare DB, Shah VP. Assessment of dermatopharmacokinetic approach in the bioequivalence determination of topical tretinoin gel products. Journal of the American Academy of Dermatology. 2003;48(5):740-51.
19. Kalia YN, Alberti I, Sekkat N, Curdy C, Naik A, Guy RH. Normalization of stratum corneum barrier function and transepidermal water loss in vivo. Pharmaceutical research. 2000;17(9):1148-50.
20. Pirot F, Berardesca E, Kalia YN, Singh M, Maibach HI, Guy RH. Stratum corneum thickness and apparent water diffusivity: facile and noninvasive quantitation in vivo. Pharmaceutical research. 1998;15(3):492-4.
21. Alberti I, Kalia YN, Naik A, Bonny J, Guy RH. Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. International journal of pharmaceutics. 2001;219(1-2):11-9.
22. Pirot F, Kalia YN, Stinchcomb AL, Keating G, Bunge A, Guy RH. Characterization of the permeability barrier of human skin in vivo. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(4):1562-7.

23. Cordery SF, Pensado A, Chiu WS, Shehab MZ, Bunge AL, Delgado-Charro MB, et al. Topical bioavailability of diclofenac from locally-acting, dermatological formulations. International journal of pharmaceutics. 2017;529(1-2):55-64.
24. N'Dri-Stempfer B, Navidi WC, Guy RH, Bunge AL. Improved Bioequivalence Assessment of Topical Dermatological Drug Products Using Dermatopharmacokinetics. Pharmaceutical research. 2008;26(2):316.
25. N'Dri-Stempfer B, Navidi WC, Guy RH, Bunge AL. Optimizing metrics for the assessment of bioequivalence between topical drug products. Pharmaceutical research. 2009;25(7):1621-30.
26. Navidi W, Hutchinson A, N'Dri-Stempfer B, Bunge A. Determining bioequivalence of topical dermatological drug products by tape-stripping. Journal of pharmacokinetics and pharmacodynamics. 2008;35(3):337.
27. Bunge A, N'Dri-Stempfer B, Navidi W, R G. Final report to food and drug administration (FDA): therapeutic equivalence of topical products, Colorado School of Mines, Golden, Colorado.; 2007.
28. Nallagundla S, Patnala S, Kanfer I. Application of an Optimized Tape Stripping Method for the Bioequivalence Assessment of Topical Acyclovir Creams. AAPS PharmSciTech. 2018;19(4):1567-73.
29. Parfitt NR, Skinner MF, Bon C, Kanfer I. Bioequivalence of topical clotrimazole formulations: an improved tape stripping method. Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques. 2011;14(3):347-57.
30. Au WL, Skinner M, Kanfer I. Comparison of tape stripping with the human skin blanching assay for the bioequivalence assessment of topical clobetasol propionate formulations. Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques. 2010;13(1):11-20.
31. Riku J, Muranushi N. Japan. In: Kanfer I, editor. Bioequivalence Requirements in Various Global Jurisdictions. Cham: Springer International Publishing; 2017. p. 127-58.
32. Kanfer I. Methods for the Assessment of Bioequivalence of Topical Dosage Forms: Correlations, Optimization Strategies, and Innovative Approaches. In: Shah VP, Maibach HI, Jenner J, editors. Topical Drug Bioavailability, Bioequivalence, and Penetration. New York, NY: Springer New York; 2014. p. 113-51.
33. Kanfer I, Tettey-Amlalo RNO, Au WL, Hughes-Formella B. Assessment of Topical Dosage Forms Intended for Local or Regional Activity. In: Shargel L, Kanfer I, editors. Generic drug product development specialty dosage forms. New York: Informa Healthcare USA; 2010. p. 54-103.

34. D'Argenio DZ, Schumitzky A, Wang X. ADAPT 5 User's Guide: Pharmacokinetic/Pharmacodynamic Systems Analysis Software. Los Angeles, CA: Biomedical Simulations Resource; 2009.

35. Schuirmann DJ. A comparison of the Two One-Sided Tests Procedure and the Power Approach for assessing the equivalence of average bioavailability. Journal of Pharmacokinetics and Biopharmaceutics. 1987;15(6):657-80.

36. FDA Draft Guidance on Warfarin Sodium. Guidelines, bioequivalence recommendation for specific products. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm201283.pdf. Accessed 22 May 2018.

37. Russell LM, Guy RH. Measurement and prediction of the rate and extent of drug delivery into and through the skin. Expert Opinion on Drug Delivery. 2009;6(4):355-69.

38. Olsen EA. A double-blind controlled comparison of generic and trade-name topical steroids using the vasoconstriction assay. Archives of Dermatology. 1991;127(2):197-201.

39. Pinkus H. Examination of the epidermis by the strip method of removing horny layers. I. Observations on thickness of the horny layer, and on mitotic activity after stripping. The Journal of investigative dermatology. 1951;16(6):383-6.

40. Duan JZ. Pharmacokinetics of Oral Absorption. In: Shargel L, Yu ABC, editors. Applied Biopharmaceutics & Pharmacokinetics; Pharmacokinetics, 7e. New York, NY: McGraw-Hill Education; 2016.

41. Scheuplein RJ, Blank IH. Permeability of the skin. Physiological reviews. 1971;51(4):702-47.

42. Elias PM. Epidermal lipids, membranes, and keratinization. International journal of dermatology. 1981;20(1):1-19.

43. Kalia YN, Pirot F, Guy RH. Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo. Biophysical journal. 1996;71(5):2692-700.

44. Reddy MB, Stinchcomb AL, Guy RH, Bunge AL. Determining dermal absorption parameters in vivo from tape strip data. Pharmaceutical research. 2002;19(3):292-8.

45. de Araujo TP, Fittipaldi IM, Bedor DCG, Duarte ML, Cordery SF, Guy RH, et al. Topical bio(in)equivalence of metronidazole formulations in vivo. International journal of pharmaceutics. 2018;541(1-2):167-72.

46. Crank J. Diffusion in a plane sheet. The mathematics of diffusion: Oxford University Press, London; 1975. p. 44–68.

47. Tsai JC, Cheng CL, Tsai YF, Sheu HM, Chou CH. Evaluation of in vivo bioequivalence methodology for topical clobetasol 17-propionate based on pharmacodynamic modeling using Chinese skin. Journal of pharmaceutical sciences. 2004;93(1):207-17.
48. Fradette C, Lavigne J, Waters D, Ducharme MP. The utility of the population approach applied to bioequivalence in patients: comparison of 2 formulations of cyclosporine. Therapeutic drug monitoring. 2005;27(5):592-600.
49. Panhard X, Mentre F. Evaluation by simulation of tests based on non-linear mixed-effects models in pharmacokinetic interaction and bioequivalence cross-over trials. Statistics in medicine. 2005;24(10):1509-24.
50. Alberti I, Kalia YN, Naik A, Bonny J-D, Guy RH. In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum. Journal of Controlled Release. 2001;71(3):319-27.
51. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Pig Ear Skin ex Vivo as a Model for in Vivo Dermatopharmacokinetic Studies in Man. Pharmaceutical research. 2006;23(8):1850-6.
52. Wiedersberg S, Naik A, Leopold CS, Guy RH. Pharmacodynamics and dermatopharmacokinetics of betamethasone 17-valerate: assessment of topical bioavailability. The British journal of dermatology. 2009;160(3):676-86.
53. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Effect of propylene glycol on ibuprofen absorption into human skin in vivo. Journal of pharmaceutical sciences. 2008;97(1):185-97.
54. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Ibuprofen transport into and through skin from topical formulations: in vitro-in vivo comparison. The Journal of investigative dermatology. 2007;127(1):135-42.

Chapter 5 - GENERAL DISCUSSION

The three research projects presented within the context of this thesis present some of the present-day challenges in the practice of bioequivalence for generic drugs at different levels. While the first article raises question on reliability of PK-endpoint BE study results when the studies are conducted in populations other than the target one, the second article puts forward some recommendations to the FDA 1995 Guidance document for topical corticosteroids [155], and lastly, the third article offers a novel and alternative approach for BE assessment of topical dermatological drug products by implementing population compartmental modeling in skin's data analysis.

Article #1

Extrapolation of BE study results has always been a controversial issue. Although regulatory agencies support the applicability of BE results from one population to another, scientists and clinicians have not yet been convinced. Therefore some studies to date have been conducted to address the raised question marks, mostly for the extrapolation from HVs to the patient population. As described in Article #1, the results from some animal models and clinical studies suggest that two formulations that are found to be bioequivalent under certain physiological conditions will not necessarily demonstrate BE under altered physiological conditions [434-440]. This is at odds with the current regulatory perspective.

PK-endpoint BE studies are conventionally conducted in HVs, although the targeted population for a developed drug is always patients. The use of HVs is assumed to minimize both inter- and intra-subject variability, and therefore conducting BE studies in HVs would be more discriminative of formulation differences. It is well recognized that PK profile and BA of a drug may differ between HVs and particular types of patients owing to different (patho)physiological conditions. Many factors, such as, but not limited to, diseased conditions, genetic polymorphism, and the difference in the level of expression and activity of CYP metabolizing enzymes and transporters can alter physiological conditions. The PK profile and BA of a drug, in turn, may differ under different (patho)physiological conditions. However, their influence on the relative BA of the test and reference products has conventionally been considered negligible for a given subject. It relies on conventional understanding of the sole

objective of BE, that is, to measure and compare formulation performance between these products.

For an oral dosage form, formulation performance is one step before the absorption and involves disintegration of the drug product, subsequent release of the drug substance, and dissolution of the drug substance in an aqueous environment eventually leading to BA of drug substance. These pre-absorptive steps are determined by the formulation factors such as incorporated excipients, release profile, manufacturing technology, etc. All other steps at the time of absorption and afterward are subject-related processes and are not directly related to formulation performance [141, 142].

By convention, it is expected that any difference observed between BA from two drug products would be due to the difference between *in vivo* formulation performances of these products. Indeed, that is the only stage where the formulation factors, i.e. excipients, can influence the disintegration of formulation, release of the drug substance from formulation and its dissolution, and therefore, regulate the fraction of drug to be absorbed and eventually drug BA. Accordingly, when the excipients and formulation factors are not the same between two formulations, such as in the case of generic and RLD products, the excipient can influence the absorption, and therefore, the BA of drug substance from formulations, differently. However, once the API is absorbed, it will continue the journey alone, independent of the excipients and other formulation factors. As a result, after the absorption, only physiological factors should influence drug BA.

Based on this conventional assumption, the BE studies can be conducted in different populations, mostly for economic reasons. Because, even if the physiological factors are different between these populations, it would be the case for the API from both the generic and RLD products. As a result, the BA of the API should alter similarly for both the generic and RLD products in a given population. Given that no formulation factor is expected at this stage, i.e. post-absorption, the relative BA of generic versus RLD product is expected to remain the same between these two populations.

One way to ensure that no formulation factor exists post-absorption is to study the impact of excipients on drug BA and BE outcomes. As described in **Section 3.8**, despite the

conventional understanding, excipients can influence drug BA and consequently BE outcomes significantly.

The influence of excipients on BE outcomes depends on many factors such as the type and concentrations of the excipients relevant to the pharmaceutical formulation, different combinations of excipients in formulation, and PK and physicochemical characteristics of incorporated API [231, 337-339]. As such, in agreement with the commentary published by authors affiliated with Health Canada, Spanish Agency for Medicines and Health Care Products, and Federal Institute for Drugs and Medical Devices of Germany [455], we believe that the impact of excipient on drug BA and BE outcome cannot be predictable without conducting a clinical PK study, and for each drug product separately.

We therefore speculate that the unpredictable impact of excipients on drug BA and BE outcomes, such as their impact on BA of highly permeable, highly soluble drugs, cannot be explained solely due to their role in regulating drug release and dissolution, differently said, their role in controlling the formulation performance.

The literature data shows that excipient can influence drug BA, not only by influencing drug release and its dissolution but also by their inhibitory or inductive effect on transporters and CYP enzymes expressed in enterocytes and hepatocytes. Examples for the study of inhibitory and inductive effect of excipients on transporters and CYP enzymes were presented previously in **Sections 1.6.1.1** and **1.6.1.2**. That means formulation factors can influence BA and BE outcomes not only before the absorption but all the way until the drug substance becomes available in the systemic circulation.

Given the influence of excipients on CYP enzymes and transporters, any interaction between excipients and CYP enzymes or excipients and transporters and their impact on drug BA could be observed only when there are CYP enzymes and transporters. As such, when there is a high level of expression or activity of transporters and enzymes, the interaction between excipients, CYP enzymes and/or transporters will influence drug BA more significantly. Differences in the ethnic distribution of various genotypes of genes encoding for CYP metabolizing enzymes and transporters have widely been shown in the literature [296, 304, 333]. More specifically, higher level of expression and prevalence of functional alleles of

transporters and CYP enzymes in North Americans and/or Caucasians than in Indians and/or Asians has been reported [296, 300, 303-305, 456].

Numerous studies have reported on different PK and BA of drugs in different ethnicities [323-335]. As described in **Section 3.7.3**, dosing recommendations for specific races or ethnicities have also been made in the label of certain drug products [308, 320-322]. Nevertheless, no study to date has acknowledged the risk of obtaining different BE study results in different ethnic or geographical populations.

Given that the majority of the PK BE for North American or European generic submissions have been performed in geographical or ethnic populations other than the intended ones, the first article of this thesis investigated whether there is any concern in extrapolating the BE study results from one ethnic population to another. To this purpose, ideally speaking, the BE study results of the same generic products versus their reference should be compared in two different populations. However, this was not possible because generic firms only conduct their pivotal BE studies once in one population. Therefore, we instead compared the FE results for a given RLD product between two different populations, based on the premise that, for two products to be bioequivalent in two different populations, they would require to be bioequivalent under both fasted and fed conditions in both populations, and that requires having a similar FE in both populations.

In order to calculate the FE in each population, we extracted the BA measures of RLD products ($AUC_{0\text{-}t}$ and C_{max}) from fasted and fed BE studies in generic submissions from regulatory resources publicly available by HC and the US FDA taking into consideration whether the BE study was carried out in Indian or Caucasian subjects. Because we combined the data from both HC and the US FDA resources, we did not calculate FE using $AUC_{0\text{-}\infty}$. The reason is the different regulatory requirements on the extrapolated portion of the $AUC_{0\text{-}\infty}$. As described in **Section 3.3.1.1**, only 12% of the $AUC_{0\text{-}\infty}$ can be extrapolated as per the US FDA [121], while it can be up to 20% as per HC [130]. Additionally, the last quantifiable concentration, C_t, in the calculation of $AUC_{0\text{-}\infty}$ has to be directly obtained from the observed concentration-time profile as per the US FDA [121], while HC recommends using C_t from a fitted concentration-time profile [130]. As a result, the values of $AUC_{0\text{-}\infty}$ could be slightly

different in generic submissions to HC and the US FDA. In order to maintain consistency in the incorporated data in our study, we relied only on the C_{max} and $AUC_{0\text{-}t}$.

Furthermore, we avoided including any data from randomly performed FE studies for research purposes, in order to ensure that all fasted and fed PK results are coming from the same standard conditions for regulatory purposes, such as the same subject inclusion criteria, the same meal composition, the same share of calorie from protein (150 kcal), carbohydrate (250 kcal), and fat (500-600 kcal), the same volume of the meal, the same volume of water for drug administration, the same fasting periods before and after meal intake, etc.

As for the statistical aspect of our study, we concluded summary FE in each population by prioritizing fixed-effect model meta-analysis, rather than random-effects model. That said, both approaches yielded the same conclusion eventually. In general, when results of multiple studies are combined for the purpose of meta-analysis, there are two levels variance, within-studies and between-studies. The within-studies variance is the sampling variance (random error) while the between-studies is the real variability, also known as tau-squared (τ^2).

The fixed-effect model assumes that all studies in the meta-analysis are drawn from a common population and all factors which could influence the effect size (here, the FE in each ANDS study) are the same in all the study populations; therefore, the effect size varies from one study to the next only because of the random error inherent in each study. Hence, under the fixed-effect model, we assign weights to all studies based entirely on the amount of information captured by that study. A large study would be given the big share of the weight, and a small study could be largely ignored [457, 458]. By contrast, the random-effects model assumes that the studies were drawn from different populations and the factors influencing the effect size in one study differ from those in another study. Therefore, the effect size will vary from one study to the next for two reasons, the random error inherent in each study and the true variation in effect size from one study to the next (between-studies variance). Given that each study is offering different information (effect-size), one wants to be sure that the information from all studies is included in the estimate of the summary effect. Therefore, as compared with the fixed-effect model, the weights assigned under random-effects are more balanced [458, 459].

In our analysis, we prioritized fixed-effect model meta-analysis for two reasons, the study conduct and the statistical perspectives. From a study conduct perspective, the FE in each study was calculated based on the data extracted from an ANDS. Therefore, all studies were expected to share the same study design in order to meet the regulatory requirements (e.g., the same RLD in the same ethnic population, the same volume of water for dose administration, the same meal with the same food volume and viscosity, etc.). As a result, all variables with a potential impact on effect size were assumed to be the same across the studies. This complies with the principles of fixed-effect model. From a statistical perspective, the number of studies in each study population was limited (≤5), which renders the estimate of between-studies variance (τ^2) to be imprecise and the random-effects model inappropriate.

In this study, we calculated and compared FE between the North American and Indian populations for nine drugs including amiodarone, carbamazepine, diltiazem, verapamil, esomeprazole, omeprazole, lansoprazole, pantoprazole, and rabeprazole, and the results suggested possible differences in FEs between the two populations.

This research is the first of its kind that investigated whether there is any concern in performing PK BE studies in populations other than target ones for which the comparator drug product is intended to be marketed and the results of our study suggest that more caution should be taken for extrapolating BE study results from one population to another. However, this study has limitations and challenges. Due to the limited access to the data of generic submissions, we were able to study only nine API (ten drug products) in this research and the study drugs were not all substrates to transporters. Among our study drugs, only amiodarone and diltiazem were substrates to P-gp transporters in addition to CYP enzymes. One of our arguments that undermines the traditional practice of BE was the expression of transporters at intestinal level, and therefore the occurrence of absorption in two directions rather than one direction towards systemic circulation. As such, our goal in this research was to preferably study drugs that are substrate to transporters. Tacrolimus [323, 324, 460, 461], sirolimus [85, 428, 462], cyclosporine [66, 265, 463, 464], saquinavir [238, 239, 284], indinavir [240, 241], fexofenadine [262, 263], atorvastatin [465], pravastatin [309, 310], and rosuvastatin [334, 335] are the drugs for which the predominant role of transporters in regulating their BA as well as

large FEs have widely been demonstrated, and were the targeted study drugs for the purpose of this project.

Furthermore, the racial or ethnic identity of subjects in this research was documented based on the information indicated in the product monographs from ANDS and NDA studies. In general, the subjects recruited for ANDS studies are either Caucasians or Indians/Asians which is explicitly indicated in the product monographs. As for the NDAs, the clinical PK and bioavailability studies in support of an NDA approval are known to be performed in the targeted population where the drug is intended to be marketed; in this case, in North America with majority of Caucasian subjects, but also sporadic representation of US racial or ethnic minorities such as Indians or Asian, Hispanics, Blacks, African Americans, Afro-Caribbeans, etc. Therefore, the FEs which were extracted from NDAs in our research could not be purely reflective of FEs in the Caucasian or North American populations.

In summary, further research is warranted to confirm whether there is any concern in extrapolating the BE results from one ethnic population to another. Drug substances with low BA are often those which undergo extensive first-pass metabolism. Therefore, the disposition of these drugs is expected to be more susceptible to ethnic differences in the expression and functionality of transporters and CYP enzymes.

In addition to drug substrates, the formulation type should also be strategically selected for future studies. Unlike the conventional IR formulations, MR products are specially designed in that the formulation and manufacturing variables control the release of the drug substance from the dosage form. Differently said, MR products specifically incorporate the excipients that would control the disintegration of the product, drug release, and its dissolution according to the surrounding physiological conditions such as fed versus fasted conditions, GI acidity, pancreatic and biliary secretions, gastric motility, etc. Food effect studies of different formulations of theophylline, a highly soluble and highly permeable drug, are well-known examples demonstrating the role of formulation factors in regulating formulation performance and drug BA. While a minimal food effect is documented for IR theophylline formulations [181, 466], different FE results were reported for its MR formulations. While early studies suggested no significant FE on the extent of exposure from controlled-release formulations (such as Uni-Dur® extended-release tablets, Theo-Dur® sustained-release tablet) [467-470],

some others documented either an increase (such as Theograd® controlled-release tablets, Theo-24® ultraslow-releasing hard gelatine capsules, Uniphyl® controlled-release tablets) [185-188] or a decrease in theophylline absorption from sustained release formulations in concomitant intake of high fat, high calorie foods [471, 472].

We recommend that drugs which are substrates to transporters and those with low BA as good candidates for future studies. As for formulation types, MR formulations are expected to be more responsive to physiological alterations, therefore, different performance of these products are expected across the ethnic populations with different physiological conditions.

Article #2

One of the other challenges in the context of BE is the characterization of response versus dose duration relationship, briefly called dose duration-response relationship, for topical dermatological corticosteroids before their pivotal BE studies. The main objective of the characterization of dose duration-response relationship is to determine the population DD_{50}, as well as *D1* and *D2*, of the RLD in question to be used in the subsequent pivotal BE study. In pivotal BE study, the generic product is compared with the RLD at dose duration approximately equal to RLD's DD_{50} as determined in the pilot study and only for the subjects whose *D2*/*D1* ratios of PD responses meet a specified minimum value of 1.25. Therefore, the manner in which the pilot study is performed and analyzed is critical because the protocol for the pivotal study, and hence BE study results, entirely rely on the results of the pilot study.

In an attempt to standardize the technique and methodology for BE assessment of topical corticosteroids, the US FDA has released a Guidance document [155]. As emphasized in the paper published in 1998 by the FDA's Office of Pharmaceutical Science [473], for precise and accurate assessment of BE for these products, the data analysis must be performed in the manner described in the Guidance. Any deviation from the proposed analysis may provide different results and influence the outcome of BE evaluation. The agency recommends two approaches for data analysis to determine population DD_{50}: non-linear mixed effect modeling, or naïve-pooled data approach. Nevertheless, due to the limitations of naïve-pooled data approach, determination of population PD parameters should preferably be based on the non-linear mixed effect modeling approach. The FDA Guidance provides a case study example with P-PHARM® software package, which incorporates EM algorithm as the non-linear mixed effect modeling method. However, due to the availability of a wide range of non-linear mixed effect modeling methods and software packages at present, different sponsors could employ a different method of choice, with NONMEM® software incorporating FOCE method being the most commonly used one.

Although the FDA underlines the importance of strict adherence to the instruction of the Guidance for obtaining precise and accurate study results, it leaves room for ambiguity by not specifying which type of non-linear mixed effect modeling method should or should not be used, and which initial fitting assumptions to be made. It is true that all non-linear mixed

effect modeling methods provide population estimates by accounting for both inter- and intra-individual variability, but the integrated mathematical and statistical models in different non-linear mixed effect algorithms and their estimation approaches are different. Therefore, employing different non-linear mixed effect algorithms, as well as different fitting assumptions, in the data analysis of skin blanching data makes it highly probable that different results will be obtained if the same study were to be performed by different investigators.

The second article of this thesis investigated the impact of using two different non-linear mixed effect modeling methods, FOCE and MLEM, and different distribution assumptions on population mean estimates of DD_{50}, commonly referred to as ED_{50}. To this purpose, the dose duration-response data from 11 distinct skin blanching blinded pilot studies were fitted using the FOCE algorithm incorporated in NONMEM® and an EM algorithm MLEM as incorporated in ADAPT®5. As expected, the population estimates of the two algorithms were different. The impact of population modeling on ED_{50} estimates has also been previously acknowledged by the FDA [474].

It is difficult to judge which estimates are true or "better", but given the estimation methods of the two algorithms, MLEM estimates are considered to be more accurate because the estimates of FOCE-like methods are based on model approximation while those of EM-like methods are true/exact ML estimates [420, 423, 424]. The findings of our study showed that MLEM estimates had lower of ED_{50} values compared to FOCE, suggesting that the EM estimates are probably located in the sensitive portion of the dose duration-response curve. In addition, the MLEM algorithm never had any issues with variance shrinkage while it was the case with FOCE. Therefore, we concluded that the use of EM-like algorithms should be favored over FOCE-like algorithms.

Unsurprisingly, the runtime until convergence with MLEM was longer than FOCE and that arises from the estimation process in MLEM. FOCE-like methods fit the data at two steps: population mean estimates are obtained at first step and individual estimates at second step. However, EM-like methods fit the data in two repetitive steps: an expectation step (E-step) where individual parameters are first estimated and a maximization step (M-step) where parameter values are updated to population estimates. Subsequently, E- and M-steps are repeated many times until convergence. The longer runtime of EM-like methods may,

therefore, appear as a disadvantage of these methods, but in facts it is the negligible cost for providing more coherent individual and population predictions. A summary of the key findings from comparison of the FOCE-like and EM-like methods as well as the advantages and disadvantages associated with use of each method are presented in **Table 1** in **Appendix 1**.

Another gap detected in the FDA 1995 Guidance [155], was the lack of recommendation for the type of distribution profile to be assumed for PD parameters. Both EM and FOCE-like methods are parametric estimation methods that find the central tendency of population data. Given the different shapes of normal and ln-normal distributions, the value of central tendency is expected to differ between these two distributions. The results of this research were confirmatory to our hypothesis and suggested that ln-normal distribution of the PD parameters should be favored over normal distribution for skin blanching studies.

Although this study addressed two major questions of ours regarding which type of non-linear mixed effect method and distribution profile to be chosen, further studies are encouraged to address other outstanding questions for better characterization of dose duration-response relationship of topical corticosteroids.

Skin blanching data inherently is associated with high variability. In BE study of topical corticosteroids, skin blanching should be quantified by use of a chromameter. The US FDA 1995 Guidance [155] recommends measurement of baseline values for each designated treated and untreated skin site. The readings of both treated and untreated sites should then be adjusted for the baseline values. Eventually, for generating dose duration-response relationship, the baseline-adjusted untreated sites readings must be subtracted from baseline-adjusted treated sites readings in order to obtain baseline-adjusted control-site corrected skin blanching response at each dose duration: (Treated site – Baseline) – (Untreated site – Baseline).

In accordance with the FDA Guidance, we used baseline-adjusted control-site corrected skin blanching data in this research. Nevertheless, it may be unnecessary to adjust the chromameter readings for baseline values [473, 475-477], because the net arithmetic effect

of this manipulation is the subtraction of the unadjusted untreated control site value from the unadjusted treated site value:

= (Treated site – Baseline) – (Untreated Control site – Baseline)

= Treated site – Baseline – Untreated Control site + Baseline

= Treated site - Untreated Control site

More importantly, adjusting the chromameter readings for the baseline could contribute to the variability of the data. Our datasets (**Appendix 1**, **Figure 1**), similar to the sample data presented in the FDA Guidance contain both positive and negative AUEC values while reading a positive value after corticosteroid application should not be possible. Adjusting the skin blanching measurements to the baseline values increases the variability in data, leading to positive and negative AUEC values in the dataset. Positive and negative AUEC values after baseline adjustment were also observed in other studies [343]. Such data would make modeling more problematic and the population mean estimates less reliable. On the other hand, in a study where the chromameter measurements of treated sites were only corrected for untreated control sites, all measurements were negative [477]. Therefore, further studies are warranted for characterization of dose duration-response relationship of topical corticosteroids using the data unadjusted for baseline values. Nevertheless, caution should be taken to use an appropriate E_{max} model when the data is not adjusted for the baseline. If the data are not corrected for baseline, the skin color measurements in the absence of drug are not equal to zero [114, 473]. Analysis of such data using the simple E_{max} model is not appropriate. Because based on this model the value of the baseline response ($AUEC_0$) in the absence of drug is assumed equal to zero:

$$\text{AUEC} = -\frac{AUEC_{max} \,.\, DD}{DD_{50} + DD}$$

$$= \text{AUEC} = 0 - \frac{AUEC_{max} \,.\, DD}{DD_{50} + DD}$$

An appropriate E_{max} model for unadjusted data for baseline values should incorporate the values of baseline response ($AUEC_0$), as below:

$$\text{AUEC} = \text{AUEC}_0 - \frac{AUEC_{max} \,.\, DD}{DD_{50} + DD}$$

where the AUEC is [Observed $\text{AUEC}_{(\text{Treated site})}$ − Observed $\text{AUEC}_{(\text{Untreated Control site})}$], and the AUEC_0 should be estimated as an additional parameter from fitting the data [473, 478]. Future studies can demonstrate whether the data unadjusted for baseline should be favored or not over the baseline-adjusted one for characterization of dose duration-response relationship of topical corticosteroids.

Article #3

The last part of this thesis attempted to investigate an alternative approach based on PK principles for bioequivalence assessment of topical dermatological products. Due to the local effect of topical formulations in the skin, we broaden the concept of pharmacokinetic-based studies to include methods that sample drug amounts at or near the site of action, that is, the skin itself. Sequential removal of the outermost layer of the skin and subsequent quantification of drug amounts in removed layers, known as tape stripping, is one of the most promising methods for characterizing the PK of drugs in the skin. Nevertheless, since the withdrawal of the FDA 1998 Guidance [443] in 2002, this method is not accepted for regulatory purposes, except in Japan and South Africa.

Many attempts to date have been directed to refine tape stripping methods, but almost all are associated with certain limitations and complexities. The efforts with the purpose of simplification of the procedure have partly been successful. For instance, while the FDA Guidance required tape stripping to be performed at four dose durations and eight sites, the more recent methods have suggested performing tape stripping at less number of dose durations and application sites for BE purposes. In this regard, as per the *Two-Time* method, tape stripping is performed at one dose duration and two sites, one during the uptake and another during the clearance phase; and as per the *Relative-Depth* method, tape stripping is performed at one dose duration during the uptake phase. Additionally some of the tested methods, e.g., *Two-Time* method, by proposing duplicate application of each formulation were successful in reducing the variability in the DPK data [363, 391].

However, all complexities and limitations are not addressed yet. For instance, as per the *Relative-Depth* method, although tape stripping is performed only at one dose duration, the amount of SC removed on each strip must be measured and the weight of removed SC must be converted to SC depth, which in turn requires certain assumptions. In comparison with the *Relative-Depth* method, the *Two-Time* method may seem a simpler alternative, but this method has its own limitations, mainly related to data analysis aspect. In this method, a PK profile cannot be generated and a PK metric reflective of rate of input into the skin cannot be estimated.

Briefly speaking, although some of the tested methods to date seem to simplify the tape stripping procedure, the major limitation is still outstanding and that is, the relevance of the PK metrics proposed by these tested methods is arguable for BA and BE assessments.

In traditional *FDA-proposed* method, Q_{max} and AUC parameters are derived from drug quantity versus time profile and, similarly to C_{max} and AUC for systemic effective oral dosage forms, are considered as the rate and extent of exposure or input into the skin. Nevertheless, the Q_{max} and AUC in the skin cannot be analogous to the C_{max} and AUC in systemic circulation as the kinetics of a drug in the skin is different from that of a drug in the oral delivery pathway.

In the *Relative-Depth* method, the diffusivity (D) of the drug across the SC of thickness L (D/L^2) and the SC-vehicle partition coefficient (K) are considered as representative of rate and extent of exposure or input into the skin and used as PK metrics for BE assessment. Nevertheless, as described in **Section 4.2.1.2.2**, due to the numerous assumptions related to the estimation of these parameters and their complicated calculations, we would argue that they may not directly reflect the rate and extent of input into the skin.

In the *Two-Time* method, as mentioned above, no rate of input into the skin can be estimated and the only metrics upon which the Test is compared to Ref is the cumulative amount of drug recovered from all stripped layers (Q_{tot}).

The third article of this thesis aimed to address these limitations and complexities by introducing a novel DPK approach. Our proposed approach includes refinements in the tape stripping procedure as well as in data analysis. Refinement of the TS procedure consisted of conducting tape stripping only at one dose duration equal to DD_{50} during the uptake phase which renders the procedure to be more discriminative of formulation differences. None of the previous methods performed tape stripping specifically at DD_{50}. Unlike previous methods (i.e., the *FDA-proposed* method and the *Two-Time* method) no data were collected during the clearance phase as the ultimate goal of a BE study is to measure and compare the drug product performance between Test and Ref formulations. Therefore, only PK parameters that are linked to the formulation performance will be relevant for BE assessment and those are the ones that can be estimated during the "absorption" or input of drugs to the biophase, in this

case, the skin. Once the drug substance reaches the skin layers, no more formulation factor is involved. Therefore, characterizing clearance or collecting data during the clearance phase would unnecessarily have increased the workload of the procedure. Furthermore, the DPK profile in our approach was generated by plotting the amount of drug extracted from stripped tapes versus time. Different than the *FDA-Proposed* method, the time points in the DPK profile in our method do not represent different studied dose durations. Instead, each time point corresponds to each of the tape strips, all of which were taken at the same dose duration, DD_{50}. Unlike the *Relative-Depth* method, quantification of the amount of the stripped SC was not required for the characterization of DPK profile. In summary, the refinements in our tape stripping procedure not only reduced the number of measurements (either by reducing the number of samples for drug amount quantification, or by eliminating SC amount quantification, and TEWL measurements) but also led to a data with lower variability and more discriminative of formulation differences.

Refinement in data analysis constitutes the principal difference between our method and others, and that is the implementation of population CPT modeling in fitting the DPK data which enabled direct estimation of rate and extent of exposure or input into the skin besides providing other common advantages of CPT analysis such as decreasing the uncertainty or variability associated with tape stripping data. To our knowledge, this is the first attempt in the literature that builds a population CPT model for tape stripping data.

The rate and extent of exposure in the skin were the only PK parameters that are linked to the formulation performance and can be different between two formulations, rendering them be relevant for BE assessment. All other parameters that are drug-specific (those describing the drug's disposition) would be identical between formulations. Simultaneous fitting of data from both Test and Ref allowed to account for the differences in rate and extent of exposure between two formulations as all other PK parameters were drug-specific, and therefore identical between two formulations.

In the developed PK model, the rate and extent of exposure into the skin were estimated as K_{in} and F_S, where K_{in} is the first-order input rate constant, and F_S is the % absorbed/delivered amount of applied dose from formulation into the skin. Drug-specific

parameters were those describing the diffusion of the drug between different layers of the SC and were introduced as diffusion rate constants (K_{diff}).

The PK parameters linked to the formulation performance, K_{in} and F_S, were then compared between the Test and Ref formulations in a manner analogous to the comparison of the C_{max} and AUC metrics with ln-transformed Test/Ref ratios and 90% CIs. Our PK model was successful in concluding BE when the generic topical cream formulation was compared versus the counterpart RLD, and in concluding BIE when a 70% lower in strength formulation was compared versus the RLD.

Our proposed approach should be considered for the generic development of other locally acting topical dermatological products. Nevertheless, it cannot still form the cornerstone for BE assessment of topical formulations as there are still aspects to be further investigated.

The use of K_{in} and F as potential endpoints for assessing BE is advantageous over all other parameters that have previously been described (K, D/L^2, AUC, Q_{max}, Q_{tot}). Nevertheless, caution should be taken when using K_{in}. For better comprehension of the underlying reason, it is useful to briefly recall the limitations of model-based PK parameters for systemic effective oral dosage forms.

The model-based PK parameters analogous to K_{in} and F_S, for systemic effective oral dosage forms are K_a and F. Unlike C_{max} which is a function of various processes, K_a for systemic effective oral dosage forms, is purely representative of the absorption rate. In fact, K_a only considers the shape of the curve, but not the height or position of the systemic concentration-time PK profile. Therefore, with a minuscule or negligible change in the PK profile, which may not be clinically relevant, K_a can change significantly. As a result, the changes in absorption rate constant frequently cannot be associated with clinical relevance. Additionally, given the susceptibility of K_a to small differences in release profiles of formulations, it is inherently associated with high variability which makes concluding equivalence even more difficult, even if the two products are in fact equivalent [136, 138, 431].

Therefore, in BE assessment between two formulations using model-based approaches, PK parameters associated with the PK model (K_a and F) in conjunction with model-fitted or model-predicted C_{max} and AUC, the default NCPT approach metrics, should be considered and be subjected to the same statistical comparisons [138].

As mentioned above, the parameter K_{in} for topical dermatological dosage forms, is analogous to K_a for oral dosage forms. Similar to K_a, K_{in} could be over-discriminating for bioequivalence comparisons. However, unlike for oral dosage forms, the C_{max} and AUC cannot be used in conjunction with K_{in} and F_S for BE assessment of topical drug products. Because, as described earlier, these parameters are not clinically relevant for BA and BE assessment of topical drug products. This is one of the fundamental reasons that we proposed this novel approach: to suggest a DPK approach with different PK metrics from those recommended by the FDA 1998 Guidance [443].

In this research project, despite the too-discriminatory nature of K_{in}, the point estimates (Test/Ref) and 90% CIs were entirely contained within the 80.00-125.00% equivalence interval. However, this needs to be studied for other types of topical formulations, and/or with other APIs. Due to the over-discriminatory nature of K_{in}, alternative metrics derived from other methods may be needed to be considered in conjunction with model-based BE assessment of topical formulations. For instance, an *in vitro* release test (IVRT) may be useful in capturing differences in composition of formulations, dosage form strengths, particle size of API, viscosity, and other formulation-related factors [13, 479]. The estimated K_{in} in our method consists of the rate constants of the release process of the drug substance from formulation and its partitioning into the SC. Considering IVRT results besides model-based PK parameters may be useful in differentiating the rate of drug release from formulation and input into the skin and may be used for BE evaluation between two topical formulations.

Additionally, in future studies, the amount of drug remaining in formulation can be quantified after the targeted exposure periods of time (dose duration) and then be extracted from the total dose that had initially been applied on the skin. The difference in terms of the percentage can then be compared with the PK parameter, F_S, which is the % absorbed/delivered amount of applied dose from formulation into the skin. This can be confirmatory that the model results were appropriate.

The discriminatory power of our approach in detecting formulation differences was tested by comparing a formulation 70% lower in strength versus the RLD. Due to the large difference in the strength between two formulations, BIE was expected to be concluded. Further to the conduct of TOST procedure, the 90% CIs for both K_{in} and F_S fell outside the 80.00-125.00% interval; however, the upper limit of the CI for K_{in} exceeded the margin of 80% (GMR=54.5 [90% CI: 36.6–81.1]). Considering the too-discriminatory nature of K_{in}, 90% CI was not expected to exceed the margin of equivalence interval. This was attributed to the limited sample size of the study (n=10). We postulated that with a larger sample size, the width of the 90% CI would decrease; hence, it would fall completely outside the 80.00-125.00% interval. Further studies are warranted to investigate the discriminatory power of our approach in detecting formulation differences, especially for formulations having smaller differences in strength.

In this study, we tested the discriminatory power of our approach only by comparing formulations with different strengths. Future studies are warranted to investigate whether the proposed approach could also be successful in discriminating formulation performances between drug products with the same strength but different microstructures such as particle size, viscosity, manufacturing process, release profile, etc.

Furthermore, tested formulations incorporated acyclovir as the API in this study. Acyclovir is a nucleoside analog antiviral drug that has been approved for the treatment of Herpes Simplex virus infection [480]. In order to produce its antiviral activity, acyclovir has to reach *stratum basale,* the target site of Herpes Simplex virus infections [481-483]. *Stratum basale* is the deepest layer of epidermis, separated from dermis by basement membrane (basal lamina) [481-483]. Future studies are encouraged to investigate the applicability of our approach for BE assessment of topical formulations incorporating APIs with deeper site of action in the skin layers, i.e., dermis.

In summary, the DPK approach that we proposed is in its infancy, and further studies are warranted to investigate its feasibility in BE assessment of different types of topical formulations in terms of formulation factors as well as incorporated APIs with different target site of action in skin layers.

Chapter 6 - CONCLUSION

Generic drug products play a vital role in today's healthcare system. More than four out of every five prescriptions dispensed in North America are generic versions of drug products. In the past decade, generic drugs have generated more than a trillion dollars in savings to the health care system. The major milestone in generic product development is the conduct of pivotal bioequivalence studies. Owing to scientifically sound bioequivalence standards, the development and approval of generic products have been accelerated and range of products for which generic versions are available has been expanded while maintaining high standards for quality, safety, and efficacy. Nevertheless, there are controversies and scientific challenges associated with certain fields in the context of bioequivalence. This thesis aimed to identify some of these controversies and raise a flag where more caution should be taken, and to put forward some recommendations and suggest more efficient novel bioequivalence approaches when necessary. Here, 'novel approaches' included implementation of population CPT analysis in demonstrating bioequivalence when conventional PK-endpoint studies and NCPT analysis of data cannot be employed.

The first part of this thesis investigated whether there is any concern to conduct PK-endpoint bioequivalence studies in healthy volunteers of one ethnic or geographical population and extrapolate the results to another. To this purpose, food effect for study drug products were calculated and compared between two ethnic populations, North Americans and Indians. This study demonstrated that the food effect for a reference drug product may not be the same in two different populations. The study also detected some discrepancies between the food effect reported in the product label and the one concluded from bioequivalence studies for generic submissions. In addition, this study showed that the heterogeneity in food effect study results may be different between geographical and ethnic populations. In summary, the results of this study demonstrated that readily extrapolation of bioequivalence study results from one population/region to another may not always be appropriate.

The second part of this thesis detected the gaps in the US FDA 1995 Guidance document for bioequivalence assessment of topical dermatological corticosteroids. The gaps comprised the lack of specific instruction on type of non-linear mixed effect population

modeling method to be implemented and the type of distribution assumptions for PD parameters to be set in dose duration-response characterization of topical corticosteroids. It is essential to characterize the duration-response relationship of these products in a robust manner, because DD_{50}, the dose duration where the comparison of PD effect of generic vs. Ref product is conducted, must be estimated from this relationship. The second part of this thesis, therefore, investigated the outcomes of employing two different non-linear mixed effect algorithms, FOCE and MLEM, and two different assumptions for distribution profile of PD parameters, normal and ln-normal. MLEM algorithm provided better results with lower in values of DD_{50} estimates associated with lower inter-CV% and less shrinkage of the variance. The results also favored ln-normal versus normal distribution assumptions. This study not only recommended updating the FDA Guidance with more specific instructions to ensure consistent approaches would be followed by pharmaceutical manufacturers, but it also called attention to the caveats that may be associated with population modeling. Despite the advantages that the population CPT modeling has to offer, due to its complex nature, it can be difficult to foresee all potential outcomes of compartmental approach.

As has been repeatedly quoted, “all models are wrong, but some are useful” [484]. Although highly challenging, population modeling has played an important role in the bioequivalence world, from product development to regulatory standards development especially for non-oral dosage forms and complex drug products where conventional BE approaches are not appropriate. The last part of this thesis implemented population CPT modeling to develop a novel bioequivalence approach for topical dermatological drug products. In this project, the local exposure of drug in the skin was measured by stripping the outermost layer of the skin and the data was fitted using population CPT modeling. Our novel approach circumvented the need for numerous measurements, assumptions and sophisticated calculations and yielded the PK parameters relevant for topical BA and BE assessment, the rate and extent of exposure in the skin. Our proposed approach was successful in concluding both BE and BIE for topical cream formulations of acyclovir.

In summary, this thesis brings some of the outstanding challenges in the current practice of bioequivalence to the attention of scientists and drug manufacturers involved in generic drug development, but more importantly to the attention of regulatory agencies. It is

important that regulatory agencies take initiative for questioning the reliability and accuracy of BE studies in certain fields, such as those highlighted in the context of this thesis, and set requirements that pharmaceutical applicants should meet in their generic submissions. These requirements could be one additional pivotal study whose results can be used only for information purposes. Based on the trend observed in the myriad of generic applications, regulators can then decide whether more stringent requirements need to be set or not. For instance, presently the US FDA require both fasted and fed PK-endpoint bioequivalence studies for the approval of almost all generic oral dosage forms. In addition to fed and fasted bioequivalence studies, the US FDA and HC could ask one pivotal food effect bioavailability study, to ensure whether the food effect in one ethnic population is similar to the food effect which was previously demonstrated in the targeted population where the drug is intended to be marketed, i.e., North America. Based on the pharmacological effect of drug in question, they can then decide whether the extrapolation of bioequivalence study results for that particular drug would be safe or not.

This thesis also highlighted both the challenges and the advantages of population CPT modeling in bioequivalence assessment, by illustrating how population CPT modeling can undermine the reliability of bioequivalence study results when there is no pre-specifications, and demonstrating how it can be useful in developing alternative bioequivalence methods when conventional bioequivalence methods are not applicable.

REFERENCES

1. Katzung, B.G., M. Kruidering-Hall, and A.J. Trevor, *Pharmacokinetics*, in *Katzung & Trevor's Pharmacology: Examination & Board Review, 12e*. 2019, McGraw-Hill Education: New York, NY.
2. Franklin, M.R., *Drug Absorption, Distribution, Metabolism, and Excretion*, in *Remington Essentials of Pharmaceutics* L. Felton, Editor. 2012, Pharmaceutical Press. p. 739-754.
3. Jr., A.J.A., *Drug Absorption and Bioavailability*, in *Principles of Clinical Pharmacology*. 2007, Elsevier Inc. p. 37-49.
4. Thomas, V.H., et al., *The road map to oral bioavailability: an industrial perspective.* Expert Opin Drug Metab Toxicol, 2006. **2**(4): p. 591-608.
5. Estudante, M., et al., *Intestinal drug transporters: an overview.* Adv Drug Deliv Rev, 2013. **65**(10): p. 1340-56.
6. Chan, L.M., S. Lowes, and B.H. Hirst, *The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability.* Eur J Pharm Sci, 2004. **21**(1): p. 25-51.
7. Hilal-Dandan, R. and L.L. Brunton, *Pharmacokinetics: The Dynamics of Drug Absorption, Distribution, Metabolism, and Elimination*, in *Goodman and Gilman's Manual of Pharmacology and Therapeutics, 2e*. 2016, McGraw-Hill Education: New York, NY.
8. A, E.-K. and V. M, *Oral Absorption, Intestinal Metabolism and Human Oral Bioavailability*, in *Topics on Drug Metabolism*, P. J., Editor. 2012. p. 1-34.
9. *US-FDA, CDER: Guidance for Industry, Changes to an Approved NDA or ANDA. April 2004. Last accessed 13/Jan/2018, at: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm077097.pdf.*
10. *Crank, J. (1975) The Mathematics of Diffusion. 2nd Edition, Oxford University Press, London.*
11. Ho, R.H. and R.B. Kim, *Drug Transporters*, in *Handbook of Drug-Nutrient Interactions*, J.I. Boullata and V.T. Armenti, Editors. 2010, Humana Press: Totowa, NJ. p. 45-84.
12. Herkenne, C., et al., *In Vivo Methods for the Assessment of Topical Drug Bioavailability.* Pharmaceutical Research, 2007. **25**(1): p. 87.
13. Yacobi, A., et al., *Current challenges in bioequivalence, quality, and novel assessment technologies for topical products.* Pharm Res, 2014. **31**(4): p. 837-46.
14. Hwa, C., E.A. Bauer, and D.E. Cohen, *Skin biology.* Dermatol Ther, 2011. **24**(5): p. 464-70.
15. Chang, R.-K., et al., *Generic Development of Topical Dermatologic Products: Formulation Development, Process Development, and Testing of Topical Dermatologic Products.* The AAPS Journal, 2013. **15**(1): p. 41-52.
16. Prausnitz, M.R. and R. Langer, *Transdermal drug delivery.* Nat Biotechnol, 2008. **26**(11): p. 1261-8.
17. Alexander, A., et al., *Approaches for breaking the barriers of drug permeation through transdermal drug delivery.* Journal of Controlled Release, 2012. **164**(1): p. 26-40.
18. Moser, K., et al., *Passive skin penetration enhancement and its quantification in vitro.* European Journal of Pharmaceutics and Biopharmaceutics, 2001. **52**(2): p. 103-112.
19. Baron, J.M., et al., *Expression of Multiple Cytochrome P450 Enzymes and Multidrug Resistance-Associated Transport Proteins in Human Skin Keratinocytes.* Journal of Investigative Dermatology, 2001. **116**(4): p. 541-548.
20. Pailler-Mattei, C., et al., *Interpretation of the human skin biotribological behaviour after tape stripping.* Journal of The Royal Society Interface, 2011.

21. Elias, P.M., *Epidermal Lipids, Barrier Function, and Desquamation.* Journal of Investigative Dermatology, 1983. **80**(1, Supplement): p. S44-S49.
22. Pyo, S.M. and H.I. Maibach, *Skin Metabolism: Relevance of Skin Enzymes for Rational Drug Design.* Skin Pharmacology and Physiology, 2019. **32**(5): p. 283-294.
23. Elias, P.M., *Epidermal lipids, membranes, and keratinization.* Int J Dermatol, 1981. **20**(1): p. 1-19.
24. Caron, D., et al., *Correlation between the drug penetration and the blanching effect of topically applied hydrocortisone creams in human beings.* J Am Acad Dermatol, 1990. **23**(3 Pt 1): p. 458-62.
25. Harding, C.R., *The stratum corneum: structure and function in health and disease.* Dermatol Ther, 2004. **17 Suppl 1**: p. 6-15.
26. Holbrook, K.A. and G.F. Odland, *Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis.* J Invest Dermatol, 1974. **62**(4): p. 415-22.
27. Brody, I., *Ultrastructure of the stratum corneum.* Int J Dermatol, 1977. **16**(4): p. 245-56.
28. Rawlings, A.V., et al., *Stratum corneum moisturization at the molecular level.* J Invest Dermatol, 1994. **103**(5): p. 731-41.
29. Law, R.M. and D.T.S. Law, *Dermatologic Drug Reactions and Common Skin Conditions*, in *Pharmacotherapy: A Pathophysiologic Approach, 10e*, J.T. DiPiro, et al., Editors. 2017, McGraw-Hill Education: New York, NY.
30. Chapman, S.J. and A. Walsh, *Desmosomes, corneosomes and desquamation. An ultrastructural study of adult pig epidermis.* Arch Dermatol Res, 1990. **282**(5): p. 304-10.
31. Chapman, S.J., et al., *Lipids, proteins and corneocyte adhesion.* Arch Dermatol Res, 1991. **283**(3): p. 167-73.
32. T.J., F. and L. P.A., *The skin as a barrier: Structure and function*, in *Biochemical Modulation of Skin Reactions: Transdermals, Topicals, Cosmetics*, K. A.F. and W. J.J., Editors. 2000, CRC Press: New York.
33. Sewell, M.J., C.N. Burkhart, and D.S. Morrell, *Dermatological Pharmacology*, in *Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e*, L.L. Brunton, R. Hilal-Dandan, and B.C. Knollmann, Editors. 2017, McGraw-Hill Education: New York, NY.
34. *Danish Ministry of the Environment, Environmental Protection Agency (2009). Dermal Absorption of Pesticides - Evaluation of Variability and Prevention. Last access 11 Aug 2019 at: https://www2.mst.dk/udgiv/publications/2009/978-87-7052-980-8/pdf/978-87-7052-981-5.pdf.*
35. *Sun H and Zhao H: Physiologic Drug Distribution and Protein Binding. In Shargel L, Yu Andrew BC (eds). Applied Biopharmaceutics and Pharmacokinetics. 7th ed. The McGraw-Hill Inc. 2016.*
36. Burton, M.E., *Applied pharmacokinetics & pharmacodynamics : principles of therapeutic drug monitoring*. 2006, Baltimore: Lippincott Williams & Wilkins.
37. Markey, S.P., *Chapter 11 - Pathways of Drug Metabolism*, in *Principles of Clinical Pharmacology (Third Edition)*, A.J. Atkinson, et al., Editors. 2012, Academic Press. p. 153-172.
38. Kadlubar, S. and F.F. Kadlubar, *Enzymatic Basis of Phase I and Phase II Drug Metabolism*, in *Enzyme- and Transporter-Based Drug-Drug Interactions: Progress and Future Challenges*, K.S. Pang, A.D. Rodrigues, and R.M. Peter, Editors. 2010, Springer New York: New York, NY. p. 3-25.
39. Pereira de Sousa, I. and A. Bernkop-Schnurch, *Pre-systemic metabolism of orally administered drugs and strategies to overcome it.* J Control Release, 2014. **192**: p. 301-9.

40. Gonzalez, M.-C., et al., *Cytochrome P450 4A11 expression in human keratinocytes: effects of ultraviolet irradiation.* British Journal of Dermatology, 2001. **145**(5): p. 749-757.
41. Wiegand, C., et al., *Dermal Xenobiotic Metabolism: A Comparison between Native Human Skin, Four in vitro Skin Test Systems and a Liver System.* Skin Pharmacology and Physiology, 2014. **27**(5): p. 263-275.
42. Ademola, J.I., et al., *Skin Penetration and Metabolism: Comparative Evaluation of Skin Equivalent, Cell Culture, and Human Skin.* Journal of Toxicology: Cutaneous and Ocular Toxicology, 1993. **12**(2): p. 129-138.
43. Hu, T., et al., *Xenobiotic metabolism gene expression in the EpiDermin vitro 3D human epidermis model compared to human skin.* Toxicol In Vitro, 2010. **24**(5): p. 1450-63.
44. Saeki, M., et al., *mRNA expression of multiple cytochrome p450 isozymes in four types of cultured skin cells.* Int Arch Allergy Immunol, 2002. **127**(4): p. 333-6.
45. Ahmad, N. and H. Mukhtar, *Cytochrome P450: A Target for Drug Development for Skin Diseases.* Journal of Investigative Dermatology, 2004. **123**(3): p. 417-425.
46. Afaq, F. and H. Mukhtar, *Effects of solar radiation on cutaneous detoxification pathways.* J Photochem Photobiol B, 2001. **63**(1-3): p. 61-9.
47. Schuessler, M., et al., *Skin is an autonomous organ in synthesis, two-step activation and degradation of vitamin D(3): CYP27 in epidermis completes the set of essential vitamin D(3)-hydroxylases.* Steroids, 2001. **66**(3-5): p. 399-408.
48. Conney, A.H., *Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture.* Cancer Res, 1982. **42**(12): p. 4875-917.
49. Kao, J. and M.P. Carver, *Cutaneous metabolism of xenobiotics.* Drug Metab Rev, 1990. **22**(4): p. 363-410.
50. Mukhtar, H., R. Agarwal, and D.R. Bickers, *Cutaneous metabolism of xenobiotics and steroid hormones*, in *Pharmacology of the Skin*, H. Mukhtar, Editor. 1991, CRC Press: Boca Raton, FL. p. 89–109.
51. Duell, E.A., et al., *Human skin levels of retinoic acid and cytochrome P-450-derived 4-hydroxyretinoic acid after topical application of retinoic acid in vivo compared to concentrations required to stimulate retinoic acid receptor-mediated transcription in vitro.* J Clin Invest, 1992. **90**(4): p. 1269-74.
52. Nakashima, E., P.K. Noonan, and L.Z. Benet, *Transdermal bioavailability and first-pass skin metabolism: a preliminary evaluation with nitroglycerin.* J Pharmacokinet Biopharm, 1987. **15**(4): p. 423-37.
53. Higo, N., et al., *Cutaneous metabolism of nitroglycerin in vitro. I. Homogenized versus intact skin.* Pharm Res, 1992. **9**(2): p. 187-90.
54. Gysler, A., et al., *Skin penetration and metabolism of topical glucocorticoids in reconstructed epidermis and in excised human skin.* Pharm Res, 1999. **16**(9): p. 1386-91.
55. Lombardi Borgia, S., et al., *In vitro skin absorption and drug release - a comparison of six commercial prednicarbate preparations for topical use.* Eur J Pharm Biopharm, 2008. **68**(2): p. 380-9.
56. Hammell, D.C., et al., *A duplex "Gemini" prodrug of naltrexone for transdermal delivery.* Journal of Controlled Release, 2004. **97**(2): p. 283-290.
57. Mavon, A., V. Raufast, and D. Redoules, *Skin absorption and metabolism of a new vitamin E prodrug, delta-tocopherol-glucoside: in vitro evaluation in human skin models.* J Control Release, 2004. **100**(2): p. 221-31.

58. Beall, H.D. and K.B. Sloan, *Topical delivery of 5-fluorouracil (5-FU) by 1,3-bisalkylcarbonyl-5-FU prodrugs.* Int J Pharm, 2002. **231**(1): p. 43-9.
59. Morris, A.P., K.R. Brain, and C.M. Heard, *Skin permeation and ex vivo skin metabolism of O-acyl haloperidol ester prodrugs.* International Journal of Pharmaceutics, 2009. **367**(1): p. 44-50.
60. Sung, K.C., J.-Y. Fang, and O. Yoa-Pu Hu, *Delivery of nalbuphine and its prodrugs across skin by passive diffusion and iontophoresis.* Journal of Controlled Release, 2000. **67**(1): p. 1-8.
61. Doh, H.J., et al., *Synthesis and evaluation of Ketorolac ester prodrugs for transdermal delivery.* J Pharm Sci, 2003. **92**(5): p. 1008-17.
62. Lin, J.H., M. Chiba, and T.A. Baillie, *Is the Role of the Small Intestine in First-Pass Metabolism Overemphasized?* Pharmacological Reviews, 1999. **51**(2): p. 135-158.
63. Thummel, K.E., *Gut instincts: CYP3A4 and intestinal drug metabolism.* The Journal of clinical investigation, 2007. **117**(11): p. 3173-3176.
64. Watkins, P.B., et al., *Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man.* J Clin Invest, 1987. **80**(4): p. 1029-36.
65. Kolars, J.C., et al., *Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes.* J Clin Invest, 1992. **90**(5): p. 1871-8.
66. Hebert, M.F., et al., *Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction.* Clinical Pharmacology & Therapeutics, 1992. **52**(5): p. 453-457.
67. Wu, C.-Y., et al., *Differentiation of absorption and first-pass gut and hepatic metabolism in humans: Studies with cyclosporine.* Clinical Pharmacology & Therapeutics, 1995. **58**(5): p. 492-497.
68. Fromm, M.F., et al., *Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin.* Hepatology, 1996. **24**(4): p. 796-801.
69. Paine, M.F., et al., *First-pass metabolism of midazolam by the human intestine.* Clin Pharmacol Ther, 1996. **60**(1): p. 14-24.
70. Sun, H. and H. Zhao, *Drug Elimination and Hepatic Clearance*, in *Applied Biopharmaceutics & Pharmacokinetics, 7e*, L. Shargel and A.B.C. Yu, Editors. 2016, McGraw-Hill Education: New York, NY.
71. Ducharme, M.P., *Drug Elimination, Clearance, and Renal Clearance*, in *Applied Biopharmaceutics & Pharmacokinetics, 7e*, L. Shargel and A.B.C. Yu, Editors. 2016, McGraw-Hill Education: New York, NY.
72. Hurst, S., et al., *Impact of physiological, physicochemical and biopharmaceutical factors in absorption and metabolism mechanisms on the drug oral bioavailability of rats and humans.* Expert Opinion on Drug Metabolism & Toxicology, 2007. **3**(4): p. 469-489.
73. Gibson, G.G. and P. Skett, *Factors affecting drug metabolism: internal factors*, in *Introduction to Drug Metabolism*. 1986, Springer US: Boston, MA. p. 113-146.
74. Gibson, G.G. and P. Skett, *Factors affecting drug metabolism: external factors*, in *Introduction to Drug Metabolism*. 1994, Springer US: Boston, MA. p. 133-156.
75. Scherrmann, J.M., *Transporters in absorption, distribution, and elimination.* Chem Biodivers, 2009. **6**(11): p. 1933-42.
76. Konig, J., F. Muller, and M.F. Fromm, *Transporters and drug-drug interactions: important determinants of drug disposition and effects.* Pharmacol Rev, 2013. **65**(3): p. 944-66.
77. Szakacs, G., et al., *The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME-Tox).* Drug Discov Today, 2008. **13**(9-10): p. 379-93.

78. Cao, X., et al., *Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model.* Pharm Res, 2006. **23**(8): p. 1675-86.
79. Bleasby, K., et al., *Expression profiles of 50 xenobiotic transporter genes in humans and pre-clinical species: a resource for investigations into drug disposition.* Xenobiotica, 2006. **36**(10-11): p. 963-88.
80. Thiebaut, F., et al., *Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues.* Proc Natl Acad Sci U S A, 1987. **84**(21): p. 7735-8.
81. Shitara, Y., et al., *Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles in hepatic clearance and intestinal absorption.* Biopharm Drug Dispos, 2013. **34**(1): p. 45-78.
82. Kunta, J.R. and P.J. Sinko, *Intestinal drug transporters: in vivo function and clinical importance.* Curr Drug Metab, 2004. **5**(1): p. 109-24.
83. Giacomini, K.M., et al., *Membrane transporters in drug development.* Nat Rev Drug Discov, 2010. **9**(3): p. 215-36.
84. Benet, L.Z., *The drug transporter-metabolism alliance: uncovering and defining the interplay.* Mol Pharm, 2009. **6**(6): p. 1631-43.
85. Benet, L.Z., C.L. Cummins, and C.Y. Wu, *Unmasking the dynamic interplay between efflux transporters and metabolic enzymes.* International Journal of Pharmaceutics, 2004. **277**(1): p. 3-9.
86. Wacher, V.J., C.Y. Wu, and L.Z. Benet, *Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy.* Mol Carcinog, 1995. **13**(3): p. 129-34.
87. Konig, J., et al., *A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane.* Am J Physiol Gastrointest Liver Physiol, 2000. **278**(1): p. G156-64.
88. Chen, C., et al., *Differential interaction of 3-hydroxy-3-methylglutaryl-coa reductase inhibitors with ABCB1, ABCC2, and OATP1B1.* Drug Metab Dispos, 2005. **33**(4): p. 537-46.
89. Lau, Y.Y., et al., *Effect of OATP1B Transporter Inhibition on the Pharmacokinetics of Atorvastatin in Healthy Volunteers.* Clinical Pharmacology & Therapeutics, 2007. **81**(2): p. 194-204.
90. Lam, J.L., et al., *In vitro and in vivo correlation of hepatic transporter effects on erythromycin metabolism: Characterizing the importance of transporter-enzyme interplay.* Drug Metabolism and Disposition, 2006. **34**(8): p. 1336.
91. Lau, Y.Y., et al., *Multiple Transporters Affect the Disposition of Atorvastatin and Its Two Active Hydroxy Metabolites: Application of in Vitro and ex Situ Systems.* Journal of Pharmacology and Experimental Therapeutics, 2006. **316**(2): p. 762.
92. Shitara, Y., et al., *Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A.* J Pharmacol Exp Ther, 2003. **304**(2): p. 610-6.
93. Shitara, Y., et al., *In vitro and in vivo correlation of the inhibitory effect of cyclosporin A on the transporter-mediated hepatic uptake of cerivastatin in rats.* Drug Metab Dispos, 2004. **32**(12): p. 1468-75.
94. Kajosaari, L.I., et al., *Metabolism of repaglinide by CYP2C8 and CYP3A4 in vitro: effect of fibrates and rifampicin.* Basic Clin Pharmacol Toxicol, 2005. **97**(4): p. 249-56.
95. Nies, A.T., M. Schwab, and D. Keppler, *Interplay of conjugating enzymes with OATP uptake transporters and ABCC/MRP efflux pumps in the elimination of drugs.* Expert Opin Drug Metab Toxicol, 2008. **4**: p. 545–568.

96. Kivisto, K.T., M. Niemi, and M.F. Fromm, *Functional interaction of intestinal CYP3A4 and P-glycoprotein.* Fundam Clin Pharmacol, 2004. **18**: p. 621–626.
97. Lin, J.H. and A.Y. Lu, *Interindividual variability in inhibition and induction of cytochrome P450 enzymes.* Annu Rev Pharmacol Toxicol, 2001. **41**: p. 535-67.
98. Paine, M.F., et al., *The human intestinal cytochrome P450 "pie".* Drug Metab Dispos, 2006. **34**(5): p. 880-6.
99. Spatzenegger, M. and W. Jaeger, *Clinical importance of hepatic cytochrome P450 in drug metabolism.* Drug Metab Rev, 1995. **27**(3): p. 397-417.
100. Xie, F., X. Ding, and Q.-Y. Zhang, *An update on the role of intestinal cytochrome P450 enzymes in drug disposition.* Acta pharmaceutica Sinica. B, 2016. **6**(5): p. 374-383.
101. Nelson, D.R., et al., *P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature.* Pharmacogenetics, 1996. **6**(1): p. 1-42.
102. Ingelman-Sundberg, M., *The human genome project and novel aspects of cytochrome P450 research.* Toxicol Appl Pharmacol, 2005. **207**(2 Suppl): p. 52-6.
103. Wilkinson, G.R., *Drug metabolism and variability among patients in drug response.* N Engl J Med, 2005. **352**(21): p. 2211-21.
104. Zanger, U.M. and M. Schwab, *Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation.* Pharmacol Ther, 2013. **138**(1): p. 103-41.
105. Kolars, J.C., et al., *CYP3A gene expression in human gut epithelium.* Pharmacogenetics, 1994. **4**(5): p. 247-59.
106. de Waziers, I., et al., *Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues.* J Pharmacol Exp Ther, 1990. **253**(1): p. 387-94.
107. Shimada, T., et al., *Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians.* J Pharmacol Exp Ther, 1994. **270**(1): p. 414-23.
108. Lasker, J.M., et al., *Characterization of CYP2C19 and CYP2C9 from human liver: respective roles in microsomal tolbutamide, S-mephenytoin, and omeprazole hydroxylations.* Arch Biochem Biophys, 1998. **353**(1): p. 16-28.
109. Penner, N., C. Woodward, and C. Prakash, *Drug Metabolizing Enzymes and Biotransformation Reactions*, in *ADME-Enabling Technologies in Drug Design and Development*, Z. Donglu and S. Sekhar, Editors. 2013, John Wiley & Sons, Inc. . p. 545-565.
110. Zou, P. and L.X. Yu, *Pharmacodynamic Endpoint Bioequivalence Studies*, in *FDA Bioequivalence Standards*, L.X. Yu and B.V. Li, Editors. 2014, Springer New York: New York, NY. p. 217-241.
111. Holford, N.H. and L.B. Sheiner, *Kinetics of pharmacologic response.* Pharmacol Ther, 1982. **16**(2): p. 143-66.
112. Wright, D.F., H.R. Winter, and S.B. Duffull, *Understanding the time course of pharmacological effect: a PKPD approach.* Br J Clin Pharmacol, 2011. **71**(6): p. 815-23.
113. Holford, N. and L. Sheiner, *Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models.* Clinical Pharmacokinetics, 1981. **6**(6): p. 429–453.
114. Mathangi Gopalakrishnan, M., V. Kumar, and I. M., *Relationship Between Pharmacokinetics and Pharmacodynamics*, in *Applied Biopharmaceutics & Pharmacokinetics*, A.B.C.Y. Leon Shargel, Editor. 2016, McGraw-Hill Education. p. 635-679.

115. Al-Numani, D., P. Colucci, and M.P. Ducharme, *Rethinking bioequivalence and equivalence requirements of orally inhaled drug products.* Asian Journal of Pharmaceutical Sciences, 2015. **10**(6): p. 461-471.
116. Nallagundla, S., S. Patnala, and I. Kanfer, *Application of an Optimized Tape Stripping Method for the Bioequivalence Assessment of Topical Acyclovir Creams.* AAPS PharmSciTech, 2018. **19**(4): p. 1567-1573.
117. Dayneka, N.L., V. Garg, and W.J. Jusko, *Comparison of four basic models of indirect pharmacodynamic responses.* J Pharmacokinet Biopharm, 1993. **21**(4): p. 457-78.
118. Williams, R.L., et al., *Where are we now and where do we go next in terms of the scientific basis for regulation on bioavailability and bioequivalence?* European Journal of Drug Metabolism and Pharmacokinetics, 2000. **25**(1): p. 7-12.
119. Chen, M.-L., *Fundamentals of Bioequivalence*, in *FDA Bioequivalence Standards*, L.X. Yu and B.V. Li, Editors. 2014, Springer New York: New York, NY. p. 29-53.
120. Chen, M.L., et al., *Bioavailability and bioequivalence: an FDA regulatory overview.* Pharm Res, 2001. **18**(12): p. 1645-50.
121. *US-FDA Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations March 2003. Last accessed 10 March 2018 at https://www.fda.gov/ohrms/dockets/ac/03/briefing/3995B1_07_GFI-BioAvail-BioEquiv.pdf.*
122. Benet, L.Z. and P. Zia-Amirhosseini, *Basic principles of pharmacokinetics.* Toxicol Pathol, 1995. **23**(2): p. 115-23.
123. *US-FDA, CDER: CFR Code of Federal Regulations Title 21 Part 314.3. Definitions. https://www.ecfr.gov/cgi-bin/text-idx?SID=f0d908c3d43c0ba3c1ff14f093311c7a&mc=true&node=se21.5.314_13&rgn=div8. 2019.*
124. *US-FDA Guidance for Industry: Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs - General Considerations March 2014. Last accessed 9 August 2018 at https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM389370.pdf.*
125. *US-FDA, CDER: CFR Code of Federal Regulations Title 21 Part 320.25(d). https://www.ecfr.gov/cgi-bin/text-idx?SID=6722cff82178638756eddd26e3dff335&mc=true&node=se21.5.320_125&rgn=div8. 2019*
126. *US-FDA, CDER: Guidance for Industry, Clinical Drug Interaction Studies - Study Design, Data Analysis, and Clinical Implications. October 2017. Last accessed 2 June 2019, at: https://www.fda.gov/media/82734/download.*
127. *US-FDA Guidance for Industry: Food-Effect Bioavailability and Fed Bioequivalence Studies. December 2002. Last accessed 2 June 2019 at: https://www.fda.gov/media/70945/download.*
128. *US-FDA Guidance for Industry: Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA December 2013. Last accessed 8 September 2019 at: https://www.fda.gov/media/87219/download.*
129. Kanfer, I. and L. Shargel, *Introduction-Bioequivalence Issues*, in *Generic drug product development : bioequivalence issues*, I. Kanfer and L. Shargel, Editors. 2008, Informa Healthcare: New York. p. 1-14.
130. *Health Canada Guidance Document: Conduct and Analysis of Comparative Bioavailability Studies. May 2017. Last access 6 June 2018 at: https://www.canada.ca/content/dam/hc-sc/documents/services/drugs-health-products/drug-products/applications-*

submissions/guidance-documents/bioavailability-bioequivalence/conduct-analysis-comparative.pdf.

131. *European Medicines Agency. Note for Guidance on the Investigation of Bioavailability and Bioequivalence. December 2000. Last access 3 June 2019 at: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-note-guidance-investigation-bioavailability-bioequivalence_en.pdf.*
132. Chen, M.-L., *An Alternative Approach for Assessment of Rate of Absorption in Bioequivalence Studies.* Pharmaceutical Research, 1992. **9**(11): p. 1380-1385.
133. Schall, R. and H.G. Luus, *Comparison of absorption rates in bioequivalence studies of immediate release drug formulations.* Int J Clin Pharmacol Ther Toxicol, 1992. **30**(5): p. 153-9.
134. Bois, F.Y., et al., *Bioequivalence: performance of several measures of extent of absorption.* Pharm Res, 1994. **11**(5): p. 715-22.
135. Endrenyi, L. and P. Al-Shaikh, *Sensitive and specific determination of the equivalence of absorption rates.* Pharm Res, 1995. **12**(12): p. 1856-64.
136. Chen, M.L., L. Lesko, and R.L. Williams, *Measures of exposure versus measures of rate and extent of absorption.* Clin Pharmacokinet, 2001. **40**(8): p. 565-72.
137. Chen, M.-L., et al., *Using Partial Area for Evaluation of Bioavailability and Bioequivalence.* Pharmaceutical Research, 2011. **28**(8): p. 1939-1947.
138. Seng Yue, C., et al., *Opportunities and Challenges Related to the Implementation of Model-Based Bioequivalence Criteria.* Clin Pharmacol Ther, 2019. **105**(2): p. 350-362.
139. Chen, M.-L., et al., *Challenges and opportunities in establishing scientific and regulatory standards for assuring therapeutic equivalence of modified release products: workshop summary report.* The AAPS journal, 2010. **12**(3): p. 371-377.
140. *European Medicines Agency. EMA Guideline. Note for Guidance on the Investigation of Bioavailbility and Bioequivalence. Last access 20 August 2019 at: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-note-guidance-investigation-bioavailability-bioequivalence_en.pdf.*
141. *Davit B, Conner D: United States of America. In Kanfer I, Shargel L (eds). Generic Drug Product Develoment International Regulatory Requirements for Bioequivalence. Volume 201. Informa Healthcare USA Inc. 2010.*
142. Davit, B., D. Conner, and L. Shargel, *Drug Product Performance, In Vivo: Bioavailability and Bioequivalence*, in *Applied Biopharmaceutics & Pharmacokinetics, 7e*, L. Shargel and A.B.C. Yu, Editors. 2016, McGraw-Hill Education: New York, NY.
143. *US-FDA Guidance for Industry: SUPAC-MR: Modified Release Solid Oral Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing and In Vivo Bioequivalence Documentation, September 1997. Last access 13/Jan/2018 at: https://www.fda.gov/downloads/Drugs/Guidances/ucm070640.pdf.*
144. Felton, L.A. and D.W. Raisch, *The New Drug Approval Process*, in *Remington Essentials of Pharmaceutics* L. Felton, Editor. 2012, Pharmaceutical Press. p. 93-99.
145. Tamimi, N.A. and P. Ellis, *Drug development: from concept to marketing!* Nephron Clin Pract, 2009. **113**(3): p. c125-31.
146. Campen, L.V., *The Product Development Process*, in *Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice.* , Y. Qiu, Y. Chen, and G. Zhang, Editors. 2009, Elsevier Inc: Burlington, MA. p. 845-860.
147. Hoff, S.F. and Y. Chen, *Product Registration and Drug Approval Process in the United States*, in *Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice.* , Y. Qiu, Y. Chen, and G. Zhang, Editors. 2009, Elsevier Inc: Burlington, MA. p. 861-883.

148. Ames, T.W., et al., *ANDA Regulatory Approval Process*, in *Generic drug product development : solid oral dosage forms*, L. Shargel and I. Kanfer, Editors. 2005, New York : Marcel Dekker: New York. p. 211-225.
149. Yu, A., et al., *Bioequivalence History*, in *FDA Bioequivalence Standards*, L.X. Yu and B.V. Li, Editors. 2014, AAPS Press: New York. p. 1-28.
150. *US-FDA Guidance for Industry: SUPAC-IR: Immediate Release Solid Oral Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation. November 1995. Last accessed 15 September 2019 at: https://www.fda.gov/media/70949/download.*
151. *Health Canada Therapeutic Products Directorate Policy: Bioequivalence of Proportional Formulations: Solid Oral Dosage. Last accessed 19 September 2019 at: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/pol/bioprop_pol-eng.pdf.*
152. *Health Canada Guidance Document: Post-Notice of Compliance (NOC) Changes: Quality Document. September 2009. Last access 12 Oct 2019 at: https://www.canada.ca/content/dam/hc-sc/documents/services/drugs-health-products/drug-products/applications-submissions/guidance-documents/post-notice-compliance-changes/quality-document/quality-document.pdf.*
153. *US-FDA, CDER: CFR Code of Federal Regulations Title 21 Part 320 Bioavailability and Bioequivalence Requirements. https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=d400d15e7fb0055b34a2393591062a8c&mc=true&n=sp21.5.320.b&r=SUBPART&ty=HTML. 2018.*
154. *US-FDA, CDER: Guidance for Industry, Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action. April 2003. Last accessed 14 Oct 2019, at: file:///C:/Users/deniz/Desktop/2003guidance_redo_10-02-13(2).pdf.*
155. *US-FDA Guidance for Industry: Topical Dermatologic Corticosteroids: In Vivo Bioequivalence, 02/June/1995. Last accessed 05/Aug/2016, at: http://www.fda.gov/ohrms/dockets/dockets/04p0206/04p-0206-ref0001-08-FDA-Guidance-for-Industry-06-1995-vol3.pdf.*
156. Shah, V.P., et al., *Bioequivalence of topical dermatological dosage forms-methods of evaluation of bioequivalence.* Pharm Res, 1998. **15**(2): p. 167-71.
157. *European Medicines Agency Guideline on the Investigation of Bioequivalence. August 2010. Last access 6/June/2018 at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf.*
158. *US-FDA Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered. Drug Products — General. Considerations March 2003. Last accessed 27 May 2019 at: https://www.ipqpubs.com/wp-content/uploads/2014/04/BABEOld.pdf.*
159. *European Medicines Agency Guideline on the Investigation of Bioequivalence. August 2010. Last access 6 August 2019 at https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf.*
160. Lionberger, R.A., et al., *Use of partial AUC to demonstrate bioequivalence of Zolpidem Tartrate Extended Release formulations.* Pharm Res, 2012. **29**(4): p. 1110-20.
161. Stier, E.M., et al., *Use of Partial Area under the Curve Metrics to Assess Bioequivalence of Methylphenidate Multiphasic Modified Release Formulations.* The AAPS Journal, 2012. **14**(4): p. 925-926.

162. Fourie Zirkelbach, J., et al., *Use of partial AUC (PAUC) to evaluate bioequivalence--a case study with complex absorption: methylphenidate.* Pharm Res, 2013. **30**(1): p. 191-202.
163. Gillespie, W.R., *Noncompartmental versus compartmental modelling in clinical pharmacokinetics.* Clin Pharmacokinet, 1991. **20**(4): p. 253-62.
164. Ozdin, D., et al., *Revisiting FDA's 1995 Guidance on Bioequivalence Establishment of Topical Dermatologic Corticosteroids: New Research Based Recommendations.* J Pharm Pharm Sci, 2018. **21**(1): p. 413-428.
165. Colucci, P., et al., *Performance of Different Population Pharmacokinetic Algorithms.* Therapeutic Drug Monitoring, 2011. **33**(5): p. 583-591.
166. Plan, E.L., et al., *Performance comparison of various maximum likelihood nonlinear mixed-effects estimation methods for dose-response models.* Aaps j, 2012. **14**(3): p. 420-32.
167. Staatz, C.E. and S.E. Tett, *Comparison of two population pharmacokinetic programs, NONMEM and P-PHARM, for tacrolimus.* Eur J Clin Pharmacol, 2002. **58**(9): p. 597-605.
168. Bennett, J.E. and J.C. Wakefield, *A comparison of a Bayesian population method with two methods as implemented in commercially available software.* J Pharmacokinet Biopharm, 1996. **24**(4): p. 403-32.
169. Gibaldi, M. and D. Perrier, *Noncompartmental Analysis Based on Statistical Moment Theory*, in *Pharmacokinetics*, M. Gibaldi and D. Perrier, Editors. 2007, Informa Healthcare USA, Inc.: New York. p. 409-416.
170. Colucci, P., J. Turgeon, and M.P. Ducharme, *How critical is the duration of the sampling scheme for the determination of half-life, characterization of exposure and assessment of bioequivalence?* J Pharm Pharm Sci, 2011. **14**(2): p. 217-26.
171. Benet, L.Z. and R.A. Ronfeld, *Volume Terms in Pharmacokinetics.* Journal of Pharmaceutical Sciences, 1969. **58**(5): p. 639-641.
172. DiStefano, J.J., 3rd and E.M. Landaw, *Multiexponential, multicompartmental, and noncompartmental modeling. I. Methodological limitations and physiological interpretations.* Am J Physiol, 1984. **246**(5 Pt 2): p. R651-64.
173. Ozdin, D., I. Kanfer, and M. Ducharme, *Novel Approach for the Bioequivalence Assessment of Topical Cream Formulations: Model-Based Analysis of Tape Stripping Data Correctly Concludes BE and BIE.* 2019. **Accepted.**
174. Auclair, B., et al., *Novel pharmacokinetic modelling of transdermal nitroglycerin.* Pharm Res, 1998. **15**(4): p. 614-9.
175. Auclair, B., et al., *Population pharmacokinetics of nitroglycerin and of its two metabolites after a single 24-hour application of a nitroglycerin transdermal matrix delivery system.* Ther Drug Monit, 1998. **20**(6): p. 607-11.
176. *Center for Drug Evaluation and Research, FDA. Approval Package for ANDA 75-710. Rockville, MD. 2008.*
177. Borsadia, S., et al., *Factors to be considered in the evaluation of bioavailability and bioequivalence of topical formulations.* Skin Pharmacol, 1992. **5**(3): p. 129-45.
178. Guy, R.H., J. Hadgraft, and H.I. Maibach, *A pharmacokinetic model for percutaneous absorption.* International Journal of Pharmaceutics, 1982. **11**(2): p. 119-129.
179. Alberti, I., et al., *Assessment and Prediction of the Cutaneous Bioavailability of Topical Terbinafine, In Vivo, in Man.* Pharmaceutical Research, 2001. **18**(10): p. 1472-1475.
180. Seng Yue, C., et al., *Novel population pharmacokinetic method compared to the standard noncompartmental approach to assess bioequivalence of iron gluconate formulations.* J Pharm Pharm Sci, 2013. **16**(3): p. 424-40.

181. David Fleisher, et al., *Drug Absorption with Food*, in *Handbook of Drug-Nutrient Interactions*, J.I. Boullata and V.T. Armenti, Editors. 2010, Humana Press. p. 209-241.
182. *Health Canada Guidance Document: Comparative Bioavailability Standards: Formulations Used for Systemic Effects. June 2018. Last access 16 Oct 2019 at: https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/bio/comparative-bioavailability-standards-formulations-used-systemic-effects.pdf.*
183. *US-FDA, CDER: CFR Code of Federal Regulations Title 21 Part 314 Subpart C. Abbreviated Applications. Last accessed 21 August 2019 at: https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=8fc8e6970793eeb679f664b180b5d6b9&mc=true&n=sp21.5.314.c&r=SUBPART&ty=HTML#se21.5.314_194.*
184. *Health Canada. Section C.08.002.1 Food and Drug Regulations (FDR). Last accessed 21 August 2019 at: https://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._870/section-C.08.002.1.html.*
185. Karim, A., et al., *Food-induced changes in theophylline absorption from controlled-release formulations. Part II. Importance of meal composition and dosing time relative to meal intake in assessing changes in absorption.* Clin Pharmacol Ther, 1985. **38**(6): p. 642-7.
186. Karim, A., et al., *Food-induced changes in theophylline absorption from controlled-release formulations. Part I. Substantial increased and decreased absorption with Uniphyl tablets and Theo-Dur Sprinkle.* Clin Pharmacol Ther, 1985. **38**(1): p. 77-83.
187. Lagas, M. and J.H. Jonkman, *Influence of food on the rate and extent of absorption of theophylline after single dose oral administration of a controlled release tablet.* Int J Clin Pharmacol Ther Toxicol, 1985. **23**(8): p. 424-6.
188. Lagas, M. and J.H. Jonkman, *Greatly enhanced bioavailability of theophylline on postprandial administration of a sustained release tablet.* Eur J Clin Pharmacol, 1983. **24**(6): p. 761-7.
189. Davit, B.M. and D.P. Conner, *Food Effects on Drug Bioavailability: Implications for New and Generic Drug Development*, in *Biopharmaceutics Applications in Drug Development*, R. Krishna and L. Yu, Editors. 2008, Springer US: Boston, MA. p. 317-335.
190. Lionberger, R.A., *FDA critical path initiatives: opportunities for generic drug development.* The AAPS journal, 2008. **10**(1): p. 103-109.
191. Dale P. Conner, a.B.M.D., *Bioequivalence and Drug Product Assessment, In Vivo* in *Generic Drug Product Development Solid Oral Dosage Forms*, a.I.K. Leon Shargel, Editor. 2005, Marcel Dekker, Inc. p. 227-255.
192. *US-FDA, CDER: Guidance for Industry, Bioequivalence Recommendations for Specific Products. June 2010. Last accessed 21 Oct 2019, at: https://www.fda.gov/media/71401/download.*
193. *US FDA. Product-Specific Guidances for Generic Drug Development. https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075207.htm. Accessed 15/June/2018.*
194. *US-FDA, CDER: Guidance for Industry, : Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations, 1997. Last accessed13/Jan/2018, at: https://www.fda.gov/downloads/drugs/guidances/ucm070239.pdf*
195. *US-FDA, CDER: Guidance for Industry, Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. Guidance for Industry. December 2017. Last access 21 Oct 2019 at: https://www.fda.gov/media/70963/download.*
196. *US FDA. Product Specific Draft Guidance on Cholestyramine oral powder. Last access 18 Oct 2019 at:*

https://www.accessdata.fda.gov/drugsatfda_docs/psg/Cholestyramine_oral%20powder_74557%20and%2074558_RV06-15.pdf.
197. *US FDA. Product Specific Draft Guidance on Calcium Acetate. Last access 18 Oct 2019 at: https://www.accessdata.fda.gov/drugsatfda_docs/psg/calcium%20acetate_oral%20capsule_RLD%20021160_RV03-17.pdf.*
198. Braddy, A.C., et al., *Survey of international regulatory bioequivalence recommendations for approval of generic topical dermatological drug products.* Aaps j, 2015. **17**(1): p. 121-33.
199. *US FDA. Product Specific Draft Guidance on Acyclovir Topical Ointment. Last access 18 Oct 2019 at: https://www.accessdata.fda.gov/drugsatfda_docs/psg/PSG_018604.pdf.*
200. Bigger, J.T., Jr. and E.B. Leahey, Jr., *Quinidine and digoxin. An important interaction.* Drugs, 1982. **24**(3): p. 229-39.
201. *The Medicare Prescription Drug, Improvement and Modernization Act of 2003; Title XI—access to affordable pharmaceuticals. http://www.gpo.gov/fdsys/pkg/PLAW-108publ173/pdf/PLAW-108publ173.pdf. Accessed 3 Feb 2018.*
202. *US FDA. Product-Specific Guidances for Generic Drug Development. Last access 20 Oct 2019 at: https://www.accessdata.fda.gov/scripts/cder/psg/index.cfm.*
203. *US-FDA Guidance for Industry: Statistical Approaches to Establishing Bioequivalence, January 2001. Last access 12/Aug/2018 at: https://www.fda.gov/downloads/drugs/guidances/ucm070244.pdf*
204. el-Tahtawy, A.A., et al., *Evaluation of bioequivalence of highly variable drugs using clinical trial simulations. II: Comparison of single and multiple-dose trials using AUC and Cmax.* Pharm Res, 1998. **15**(1): p. 98-104.
205. el-Tahtawy, A.A., A.J. Jackson, and T.M. Ludden, *Evaluation of bioequivalence of highly variable drugs using Monte Carlo simulations. I. Estimation of rate of absorption for single and multiple dose trials using Cmax.* Pharm Res, 1995. **12**(11): p. 1634-41.
206. Makhlouf, F.T., S.C. Grosser, and D.J. Schuirmann, *Basic Statistical Considerations*, in *FDA Bioequivalence Standards*, L.X. Yu and B.V. Li, Editors. 2014, Springer New York: New York, NY. p. 55-93.
207. Davit, B., et al., *International guidelines for bioequivalence of systemically available orally administered generic drug products: a survey of similarities and differences.* Aaps j, 2013. **15**(4): p. 974-90.
208. *US FDA. Product Specific Draft Guidance on Warfarin Sodium. Last access 18 Oct 2019 at: https://www.accessdata.fda.gov/drugsatfda_docs/psg/Warfarin_Sodium_tab_09218_RC12-12.pdf.*
209. Jiang, W., et al., *A Bioequivalence Approach for Generic Narrow Therapeutic Index Drugs: Evaluation of the Reference-Scaled Approach and Variability Comparison Criterion.* The AAPS journal, 2015. **17**(4): p. 891-901.
210. Haidar, S.H., et al., *Bioequivalence approaches for highly variable drugs and drug products.* Pharm Res, 2008. **25**(1): p. 237-41.
211. *US FDA. Product Specific Draft Guidance on Progesterone. Last access 18 Oct 2019 at: https://www.accessdata.fda.gov/drugsatfda_docs/psg/Progesterone_caps_19781_RC02-11.pdf.*
212. *Orange Book (2018) Approved drug products with therapeutic equivalence evaluations, 38th edn. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Office of Generic Drugs, 2018. Accessed 10 August 2018 at https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/UCM071436.pdf.*

213. Hauck, W.W. and S. Anderson, *A new statistical procedure for testing equivalence in two-group comparative bioavailability trials.* J Pharmacokinet Biopharm, 1984. **12**(1): p. 83-91.
214. Brodie, B.B., W.M. Heller, and S. Bazel, *Bioavailability of drugs*. 1972, Basel: Karger.
215. Dighe, S.V. and W.P. Adams, *Bioequivalence: a United States regulatory perspective*, in *Pharmaceutical Bioequivalence*, P.G. Welling, F.L.S. Tse, and S.V. Dighe, Editors. 1991, Marcel Dekker, Inc. : New York p. 347-380.
216. Skelly, J.P., *A history of biopharmaceutics in the Food and Drug Administration 1968-1993.* The AAPS journal, 2010. **12**(1): p. 44-50.
217. Schuirmann, D.J., *A comparison of the Two One-Sided Tests Procedure and the Power Approach for assessing the equivalence of average bioavailability.* Journal of Pharmacokinetics and Biopharmaceutics, 1987. **15**(6): p. 657-680.
218. Senn, S., *Statistical Issues in Drug Development*. 2 ed. 2007: John Wiley & Sons, Ltd.
219. Gaddis, M.L. and G.M. Gaddis, *Introduction to biostatistics: Part 1, Basic concepts.* Ann Emerg Med, 1990. **19**(1): p. 86-9.
220. Gaddis, G.M. and M.L. Gaddis, *Introduction to biostatistics: Part 3, Sensitivity, specificity, predictive value, and hypothesis testing.* Ann Emerg Med, 1990. **19**(5): p. 591-7.
221. Potvin, D., et al., *Sequential design approaches for bioequivalence studies with crossover designs.* Pharm Stat, 2008. **7**(4): p. 245-62.
222. Phillips, K.F., *Power of the two one-sided tests procedure in bioequivalence.* Journal of Pharmacokinetics and Biopharmaceutics, 1990. **18**(2): p. 137-144.
223. Diletti, E., D. Hauschke, and V.W. Steinijans, *Sample size determination for bioequivalence assessment by means of confidence intervals.* Int J Clin Pharmacol Ther Toxicol, 1992. **30 Suppl 1**: p. S51-8.
224. Liu, J.-p. and S.-C. Chow, *Sample size determination for the two one-sided tests procedure in bioequivalence.* Journal of Pharmacokinetics and Biopharmaceutics, 1992. **20**(1): p. 101-104.
225. Fuglsang, A., *Sequential bioequivalence approaches for parallel designs.* The AAPS journal, 2014. **16**(3): p. 373-378.
226. Herring., C., *Biostatistics*, in *Applied Biopharmaceutics & Pharmacokinetics, 7e*, L. Shargel and A.B.C. Yu, Editors. 2016, McGraw-Hill Education: New York, NY.
227. Field, A., *Everything you ever wanted to know about statistics. Confidence Interval*, in *Discovering Statistics Using SPSS*. 2009, SAGE Publications Ltd. p. 43-48.
228. Estudante, M., et al., *Intestinal drug transporters: An overview.* Advanced Drug Delivery Reviews, 2013. **65**(10): p. 1340-1356.
229. Buggins, T.R., P.A. Dickinson, and G. Taylor, *The effects of pharmaceutical excipients on drug disposition.* Adv Drug Deliv Rev, 2007. **59**(15): p. 1482-503.
230. Ashiru, D.A., R. Patel, and A.W. Basit, *Polyethylene glycol 400 enhances the bioavailability of a BCS class III drug (ranitidine) in male subjects but not females.* Pharm Res, 2008. **25**(10): p. 2327-33.
231. Garcia-Arieta, A., *Interactions between active pharmaceutical ingredients and excipients affecting bioavailability: impact on bioequivalence.* Eur J Pharm Sci, 2014. **65**: p. 89-97.
232. Panakanti, R. and A.S. Narang, *Impact of excipient interactions on drug bioavailability from solid dosage forms.* Pharm Res, 2012. **29**(10): p. 2639-59.
233. Zhang, W., et al., *The Effects of Pharmaceutical Excipients on Gastrointestinal Tract Metabolic Enzymes and Transporters—an Update.* The AAPS Journal, 2016. **18**(4): p. 830-843.
234. Williams, R.L., et al., *Effects of Formulation and Food on the Absorption of Hydrochlorothiazide and Triamterene or Amiloride from Combination Diuretic Products.* Pharmaceutical Research, 1987. **4**(4): p. 348-352.

235. Trevor, A.J., B.G. Katzung, and M. Kruidering-Hall, *Drug Metabolism*, in *Katzung & Trevor's Pharmacology: Examination & Board Review, 11e*. 2015, McGraw-Hill Education: New York, NY.
236. Barry, M. and J. Feely, *Enzyme induction and inhibition.* Pharmacol Ther, 1990. **48**(1): p. 71-94.
237. *Enzymes and Enzyme Kinetics*, in *Integrative Medical Biochemistry Examination and Board Review*, M.W. King, Editor. 2014 McGraw-Hill Education
238. Kenyon, C.J., et al., *The use of pharmacoscintigraphy to elucidate food effects observed with a novel protease inhibitor (saquinavir).* Pharm Res, 1998. **15**(3): p. 417-22.
239. Kakuda, T.N. and R.W. Falcon, *Effect of food and ranitidine on saquinavir pharmacokinetics and gastric pH in healthy volunteers.* Pharmacotherapy, 2006. **26**(8): p. 1060-8.
240. Carver, P.L., et al., *Meal composition effects on the oral bioavailability of indinavir in HIV-infected patients.* Pharm Res, 1999. **16**(5): p. 718-24.
241. Li, L.Y., et al., *Intestinal metabolism promotes regional differences in apical uptake of indinavir: coupled effect of P-glycoprotein and cytochrome P450 3A on indinavir membrane permeability in rat.* J Pharmacol Exp Ther, 2002. **301**(2): p. 586-93.
242. Custodio, J.M., C.Y. Wu, and L.Z. Benet, *Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption.* Adv Drug Deliv Rev, 2008. **60**(6): p. 717-33.
243. Konishi, T., et al., *Inhibitory effect of a bitter melon extract on the P-glycoprotein activity in intestinal Caco-2 cells.* Br J Pharmacol, 2004. **143**(3): p. 379-87.
244. Konishi, T., et al., *A bitter melon extract inhibits the P-glycoprotein activity in intestinal Caco-2 cells: monoglyceride as an active compound.* Biofactors, 2004. **22**(1-4): p. 71-4.
245. Ingels, F., et al., *Simulated intestinal fluid as transport medium in the Caco-2 cell culture model.* Int J Pharm, 2002. **232**(1-2): p. 183-92.
246. Chen, X.W., et al., *Herb-drug interactions and mechanistic and clinical considerations.* Curr Drug Metab, 2012. **13**(5): p. 640-51.
247. Xu, H., et al., *Disposition of naringenin via glucuronidation pathway is affected by compensating efflux transporters of hydrophilic glucuronides.* Mol Pharm, 2009. **6**(6): p. 1703-15.
248. Ducharme, M.P., L.H. Warbasse, and D.J. Edwards, *Disposition of intravenous and oral cyclosporine after administration with grapefruit juice.* Clin Pharmacol Ther, 1995. **57**(5): p. 485-91.
249. Ducharme, M.P., et al., *Trough concentrations of cyclosporine in blood following administration with grapefruit juice.* Br J Clin Pharmacol, 1993. **36**(5): p. 457-9.
250. Edwards, D.J., et al., *6',7'-Dihydroxybergamottin in grapefruit juice and Seville orange juice: effects on cyclosporine disposition, enterocyte CYP3A4, and P-glycoprotein.* Clin Pharmacol Ther, 1999. **65**(3): p. 237-44.
251. Spahn-Langguth, H. and P. Langguth, *Grapefruit juice enhances intestinal absorption of the P-glycoprotein substrate talinolol.* Eur J Pharm Sci, 2001. **12**(4): p. 361-7.
252. Egashira, K., et al., *Pomelo-induced increase in the blood level of tacrolimus in a renal transplant patient.* Transplantation, 2003. **75**(7): p. 1057.
253. Guo, L.Q., et al., *Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity.* Drug Metab Dispos, 2000. **28**(7): p. 766-71.
254. Malhotra, S., et al., *Seville orange juice-felodipine interaction: comparison with dilute grapefruit juice and involvement of furocoumarins.* Clin Pharmacol Ther, 2001. **69**(1): p. 14-23.
255. Grenier, J., et al., *Pomelo juice, but not cranberry juice, affects the pharmacokinetics of cyclosporine in humans.* Clin Pharmacol Ther, 2006. **79**(3): p. 255-62.

256. Lilja, J.J., et al., *Itraconazole increases but grapefruit juice greatly decreases plasma concentrations of celiprolol.* Clin Pharmacol Ther, 2003. **73**(3): p. 192-8.
257. Lilja, J.J., L. Juntti-Patinen, and P.J. Neuvonen, *Orange juice substantially reduces the bioavailability of the beta-adrenergic-blocking agent celiprolol.* Clin Pharmacol Ther, 2004. **75**(3): p. 184-90.
258. Lilja, J.J., K. Raaska, and P.J. Neuvonen, *Effects of orange juice on the pharmacokinetics of atenolol.* Eur J Clin Pharmacol, 2005. **61**(5-6): p. 337-40.
259. Neuhofel, A.L., et al., *Lack of bioequivalence of ciprofloxacin when administered with calcium-fortified orange juice: a new twist on an old interaction.* J Clin Pharmacol, 2002. **42**(4): p. 461-6.
260. Tapaninen, T., P.J. Neuvonen, and M. Niemi, *Grapefruit juice greatly reduces the plasma concentrations of the OATP2B1 and CYP3A4 substrate aliskiren.* Clin Pharmacol Ther, 2010. **88**(3): p. 339-42.
261. Bailey, D.G., *Fruit juice inhibition of uptake transport: a new type of food-drug interaction.* Br J Clin Pharmacol, 2010. **70**(5): p. 645-55.
262. Dresser, G.K., et al., *Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine.* Clin Pharmacol Ther, 2002. **71**(1): p. 11-20.
263. Dresser, G.K., R.B. Kim, and D.G. Bailey, *Effect of grapefruit juice volume on the reduction of fexofenadine bioavailability: possible role of organic anion transporting polypeptides.* Clin Pharmacol Ther, 2005. **77**(3): p. 170-7.
264. Roth, M., B.N. Timmermann, and B. Hagenbuch, *Interactions of green tea catechins with organic anion-transporting polypeptides.* Drug Metab Dispos, 2011. **39**(5): p. 920-6.
265. Gupta, S.K., et al., *Effect of food on the pharmacokinetics of cyclosporine in healthy subjects following oral and intravenous administration.* J Clin Pharmacol, 1990. **30**(7): p. 643-53.
266. Gupta, S.K. and L.Z. Benet, *High-fat meals increase the clearance of cyclosporine.* Pharm Res, 1990. **7**(1): p. 46-8.
267. McLean, A.J., et al., *Reduction of first-pass hepatic clearance of propranolol by food.* Clin Pharmacol Ther, 1981. **30**(1): p. 31-4.
268. Melander, A., et al., *Enhancement of the bioavailability of propranolol and metoprolol by food.* Clin Pharmacol Ther, 1977. **22**(1): p. 108-12.
269. Olanoff, L.S., et al., *Food effects on propranolol systemic and oral clearance: support for a blood flow hypothesis.* Clin Pharmacol Ther, 1986. **40**(4): p. 408-14.
270. Walle, T., et al., *Food-induced increase in propranolol bioavailability--relationship to protein and effects on metabolites.* Clin Pharmacol Ther, 1981. **30**(6): p. 790-5.
271. Chen, M.L., et al., *A modern view of excipient effects on bioequivalence: case study of sorbitol.* Pharm Res, 2007. **24**(1): p. 73-80.
272. Goole, J., et al., *The effects of excipients on transporter mediated absorption.* Int J Pharm, 2010. **393**(1-2): p. 17-31.
273. Mountfield, R.J., et al., *Potential inhibitory effects of formulation ingredients on intestinal cytochrome P450.* Int J Pharm, 2000. **211**(1-2): p. 89-92.
274. Bravo Gonzalez, R.C., et al., *In vitro investigation on the impact of the surface-active excipients Cremophor EL, Tween 80 and Solutol HS 15 on the metabolism of midazolam.* Biopharm Drug Dispos, 2004. **25**(1): p. 37-49.
275. Christiansen, A., et al., *Effects of non-ionic surfactants on cytochrome P450-mediated metabolism in vitro.* Eur J Pharm Biopharm, 2011. **78**(1): p. 166-72.

276. Zhu, S., et al., *Effects of polyoxyethylene (40) stearate on the activity of P-glycoprotein and cytochrome P450.* Eur J Pharm Sci, 2009. **37**(5): p. 573-80.
277. Johnson, B.M., W.N. Charman, and C.J. Porter, *An in vitro examination of the impact of polyethylene glycol 400, Pluronic P85, and vitamin E d-alpha-tocopheryl polyethylene glycol 1000 succinate on P-glycoprotein efflux and enterocyte-based metabolism in excised rat intestine.* AAPS PharmSci, 2002. **4**(4): p. E40.
278. Ren, X., et al., *Nonionic surfactants are strong inhibitors of cytochrome P450 3A biotransformation activity in vitro and in vivo.* Eur J Pharm Sci, 2009. **36**(4-5): p. 401-11.
279. Martin, P., et al., *Mediation of in vitro cytochrome p450 activity by common pharmaceutical excipients.* Mol Pharm, 2013. **10**(7): p. 2739-48.
280. Ashiru-Oredope, D.A., et al., *The effect of polyoxyethylene polymers on the transport of ranitidine in Caco-2 cell monolayers.* Int J Pharm, 2011. **409**(1-2): p. 164-8.
281. Hanke, U., et al., *Commonly used nonionic surfactants interact differently with the human efflux transporters ABCB1 (p-glycoprotein) and ABCC2 (MRP2).* Eur J Pharm Biopharm, 2010. **76**(2): p. 260-8.
282. Zhao, G., et al., *Enhanced intestinal absorption of etoposide by self-microemulsifying drug delivery systems: roles of P-glycoprotein and cytochrome P450 3A inhibition.* Eur J Pharm Sci, 2013. **50**(3-4): p. 429-39.
283. Cornaire, G., et al., *Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo.* Int J Pharm, 2004. **278**(1): p. 119-31.
284. Tomaru, A., et al., *Effects of Cremophor EL on the absorption of orally administered saquinavir and fexofenadine in healthy subjects.* Drug Metab Pharmacokinet, 2015. **30**(3): p. 221-6.
285. Huang, J., et al., *Effect of pluronic F68 block copolymer on P-glycoprotein transport and CYP3A4 metabolism.* Int J Pharm, 2008. **356**(1-2): p. 351-3.
286. Ma, L., et al., *Effects of Pluronic F68 and Labrasol on the intestinal absorption and pharmacokinetics of rifampicin in rats.* Arch Pharm Res, 2011. **34**(11): p. 1939-43.
287. Guan, Y., et al., *Effect of pluronic P123 and F127 block copolymer on P-glycoprotein transport and CYP3A metabolism.* Arch Pharm Res, 2011. **34**(10): p. 1719-28.
288. Yamagata, T., et al., *Effect of excipients on breast cancer resistance protein substrate uptake activity.* J Control Release, 2007. **124**(1-2): p. 1-5.
289. Yamagata, T., et al., *Improvement of the oral drug absorption of topotecan through the inhibition of intestinal xenobiotic efflux transporter, breast cancer resistance protein, by excipients.* Drug Metab Dispos, 2007. **35**(7): p. 1142-8.
290. Yamagata, T., et al., *Characterization of the inhibition of breast cancer resistance protein-mediated efflux of mitoxantrone by pharmaceutical excipients.* Int J Pharm, 2009. **370**(1-2): p. 216-9.
291. Engel, A., et al., *Pharmaceutical excipients influence the function of human uptake transporting proteins.* Mol Pharm, 2012. **9**(9): p. 2577-81.
292. Gardiner, S.J. and E.J. Begg, *Pharmacogenetics, drug-metabolizing enzymes, and clinical practice.* Pharmacol Rev, 2006. **58**(3): p. 521-90.
293. Evans, W.E. and M.V. Relling, *Pharmacogenomics: translating functional genomics into rational therapeutics.* Science, 1999. **286**(5439): p. 487-91.
294. Nagata, K. and Y. Yamazoe, *Genetic polymorphism of human cytochrome p450 involved in drug metabolism.* Drug Metab Pharmacokinet, 2002. **17**(3): p. 167-89.
295. Kleinschmidt, K.C. and K.A. Delaney, *Biochemical and Metabolic Principles*, in *Goldfrank's Toxicologic Emergencies*, R.S. Hoffman, et al., Editors. 2014, McGraw-Hill Education.

296. Yasuda, S., L. Zhang, and S.-M. Huang, *The Role of Ethnicity in Variability in Response to Drugs: Focus on Clinical Pharmacology Studies.* Clinical Pharmacology & Therapeutics, 2008. **84**(3): p. 417-423.
297. Ramamoorthy, A., et al., *Racial/ethnic differences in drug disposition and response: review of recently approved drugs.* Clin Pharmacol Ther, 2015. **97**(3): p. 263-73.
298. Relling, M.V., et al., *Lower prevalence of the debrisoquin oxidative poor metabolizer phenotype in American black versus white subjects.* Clinical Pharmacology & Therapeutics, 1991. **50**(3): p. 308-313.
299. Evans, W.E., et al., *Genetic basis for a lower prevalence of deficient CYP2D6 oxidative drug metabolism phenotypes in black Americans.* J Clin Invest, 1993. **91**(5): p. 2150-4.
300. Bradford, L.D., *CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants.* Pharmacogenomics, 2002. **3**(2): p. 229-43.
301. de Morais, S.M., et al., *The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans.* J Biol Chem, 1994. **269**(22): p. 15419-22.
302. Goldstein, J.A., et al., *Frequencies of the defective CYP2C19 alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations.* Pharmacogenetics, 1997. **7**(1): p. 59-64.
303. Xie, H.G., et al., *Molecular basis of ethnic differences in drug disposition and response.* Annu Rev Pharmacol Toxicol, 2001. **41**: p. 815-50.
304. Sohn, D.R., et al., *Disposition kinetics and metabolism of omeprazole in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation recruited from an Oriental population.* J Pharmacol Exp Ther, 1992. **262**(3): p. 1195-202.
305. Chang, M., et al., *Interphenotype differences in disposition and effect on gastrin levels of omeprazole--suitability of omeprazole as a probe for CYP2C19.* Br J Clin Pharmacol, 1995. **39**(5): p. 511-8.
306. Pershing, L.K., et al., *Comparison of skin stripping, in vitro release, and skin blanching response methods to measure dose response and similarity of triamcinolone acetonide cream strengths from two manufactured sources.* J Pharm Sci, 2002. **91**(5): p. 1312-23.
307. Limdi, N.A., et al., *Influence of CYP2C9 and VKORC1 1173C/T genotype on the risk of hemorrhagic complications in African-American and European-American patients on warfarin.* Clin Pharmacol Ther, 2008. **83**(2): p. 312-21.
308. *FDA, Warfarin Label, 2018. [Online]. Last accessed 26 December 2018, at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/009218s118lbl.pdf.*
309. Maeda, K., et al., *Effects of organic anion transporting polypeptide 1B1 haplotype on pharmacokinetics of pravastatin, valsartan, and temocapril.* Clinical Pharmacology & Therapeutics, 2006. **79**(5): p. 427-439.
310. Mwinyi, J., et al., *Evidence for Inverse Effects of OATP-C (SLC21A6) *5 and *1b Haplotypes on Pravastatin Kinetics.* Clinical Pharmacology & Therapeutics, 2004. **75**(5): p. 415-421.
311. Kalliokoski, A., et al., *Effects of the SLCO1B1*1B haplotype on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide.* Pharmacogenet Genomics, 2008. **18**(11): p. 937-42.
312. Link, E., et al., *SLCO1B1 variants and statin-induced myopathy--a genomewide study.* N Engl J Med, 2008. **359**(8): p. 789-99.
313. Nies, A.T., et al., *Genetics is a major determinant of expression of the human hepatic uptake transporter OATP1B1, but not of OATP1B3 and OATP2B1.* Genome Med, 2013. **5**(1): p. 1.

314. Nozawa, T., et al., *Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis.* J Pharmacol Exp Ther, 2002. **302**(2): p. 804-13.
315. Lee, W., et al., *Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry.* J Biol Chem, 2005. **280**(10): p. 9610-7.
316. Zamber, C.P., et al., *Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine.* Pharmacogenetics, 2003. **13**(1): p. 19-28.
317. Morisaki, K., et al., *Single nucleotide polymorphisms modify the transporter activity of ABCG2.* Cancer Chemother Pharmacol, 2005. **56**(2): p. 161-72.
318. Elsby, R., C. Hilgendorf, and K. Fenner, *Understanding the Critical Disposition Pathways of Statins to Assess Drug–Drug Interaction Risk During Drug Development: It's Not Just About OATP1B1.* Clinical Pharmacology & Therapeutics, 2012. **92**(5): p. 584-598.
319. Peng, K.-w., et al., *Ethnic Variability in the Expression of Hepatic Drug Transporters: Absolute Quantification by an Optimized Targeted Quantitative Proteomic Approach.* Drug Metabolism and Disposition, 2015. **43**(7): p. 1045-1055.
320. *FDA, Rosuvastatin Label, 2018. [Online]. Last accessed 16 December 2018, at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021366s016lbl.pdf.*
321. *FDA, Tacrolimus Label [Online]. Last accessed 20 December 2018, at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/050709s031lbl.pdf.*
322. *FDA, Tegretol® -XR Carbamazepine Tablets Label, [Online]. Last accessed 28 March 2019, at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/016608s105,018281s053,018927s046,020234s038lbl.pdf.*
323. Andrews, P.A., M. Sen, and R.W.S. Chang, *Racial variation in dosage requirements of tacrolimus.* The Lancet, 1996. **348**(9039): p. 1446.
324. Mancinelli, L.M., et al., *The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups.* Clin Pharmacol Ther, 2001. **69**(1): p. 24-31.
325. Lamba, J.K., et al., *Genetic contribution to variable human CYP3A-mediated metabolism.* Adv Drug Deliv Rev, 2002. **54**(10): p. 1271-94.
326. Palma-Aguirre, J.A., et al., *Bioavailability of oral cyclosporine in healthy Mexican volunteers: evidence for interethnic variability.* J Clin Pharmacol, 1997. **37**(7): p. 630-4.
327. Lindholm, A., et al., *Demographic factors influencing cyclosporine pharmacokinetic parameters in patients with uremia: racial differences in bioavailability.* Clin Pharmacol Ther, 1992. **52**(4): p. 359-71.
328. Johnson, J.A., *Influence of race or ethnicity on pharmacokinetics of drugs.* J Pharm Sci, 1997. **86**(12): p. 1328-33.
329. Sowunmi, A., et al., *Ethnic differences in nifedipine kinetics: comparisons between Nigerians, Caucasians and South Asians.* Br J Clin Pharmacol, 1995. **40**(5): p. 489-93.
330. Lou, Y.C., *Differences in drug metabolism polymorphism between Orientals and Caucasians.* Drug Metab Rev, 1990. **22**(5): p. 451-75.
331. Ahsan, C.H., et al., *Ethnic differences in the pharmacokinetics of oral nifedipine.* Br J Clin Pharmacol, 1991. **31**(4): p. 399-403.
332. Chávez-Teyes, L., G. Castañeda-Hernández, and F.J. Flores-Murrieta, *Pharmacokinetics of Midazolam in Mexicans Evidence for Interethnic Variability.* Clinical Drug Investigation, 1999. **17**(3): p. 233-239.

333. Ramamoorthy, A., et al., *Racial/ethnic differences in drug disposition and response: Review of recently approved drugs.* Clinical Pharmacology & Therapeutics, 2015. **97**(3): p. 263-273.
334. Birmingham, B.K., et al., *Rosuvastatin pharmacokinetics and pharmacogenetics in Caucasian and Asian subjects residing in the United States.* Eur J Clin Pharmacol, 2015. **71**(3): p. 329-40.
335. Lee, E., et al., *Rosuvastatin pharmacokinetics and pharmacogenetics in white and Asian subjects residing in the same environment.* Clin Pharmacol Ther, 2005. **78**(4): p. 330-41.
336. *Simeprevir Clinical Pharmacology and Biopharmaceutics Review. NDA 205-123. Last accessed 16 December 2018, at https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/205123Orig1s000ClinPharmR.pdf.*
337. García-Arieta, A. and J. Gordon, *Bioequivalence Requirements in the European Union: Critical Discussion.* The AAPS Journal, 2012. **14**(4): p. 738-748.
338. Vaithianathan, S., et al., *Effect of Common Excipients on the Oral Drug Absorption of Biopharmaceutics Classification System Class 3 Drugs Cimetidine and Acyclovir.* J Pharm Sci, 2016. **105**(2): p. 996-1005.
339. Vaithianathan, S., et al., *Reply to "On the Effect of Common Excipients on the Oral Absorption of Class 3 Drugs".* J Pharm Sci, 2016. **105**(4): p. 1355-7.
340. *CDER FDA. Inactive Ingredient Search for Approved Products. Last accessed 7 February 2019, at http://www.accessdata.fda.gov/scripts/cder/iig/index.Cfm.*
341. *World Health Organization. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. WHO. In: WHO Technical Report Series, Vol. 937, WHO Press, Geneva. 2006. WHO Expert Committee on Specifications for Pharmaceutical Preparations, pp. 347–390.*
342. van Os, S., M. Relleke, and P.M. Piniella, *Lack of bioequivalence between generic risperidone oral solution and originator risperidone tablets.* Int J Clin Pharmacol Ther, 2007. **45**(5): p. 293-9.
343. Singh, G.J., et al., *Development of in vivo bioequivalence methodology for dermatologic corticosteroids based on pharmacodynamic modeling.* Clin Pharmacol Ther, 1999. **66**(4): p. 346-57.
344. *US-FDA, CDER: Guidance for Industry, Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. February 2012. Last accessed 1 February 2017, at: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm*
345. *FDA Draft Guidance on Lidocaine. Guidelines, bioequivalence recommendation for specific products. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm086293.pdf. Accessed 8 February 2018.*
346. *Draft Guidance on Diclofenac Sodium. Guidelines, bioequivalence recommendation for specific products. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM244644.pdf. Accessed 8 February 2018.*
347. *US FDA. Product Specific Draft Guidance on Estradiol. Last access 18 Oct 2019 at: https://www.accessdata.fda.gov/drugsatfda_docs/psg/Estradiol_draft_Transdermal%20film*

%20ER_RLD%2020375%20%20%20%20%20%20%20%20%20%20%20%20%2021674_RC10-18.pdf.
348. *Stoughton RB. Vasoconstrictor assay: specific applications. In: Maibach HI, Surber C, editors. Topical corticosteroids. Basel: Karger; 1992. p. 42-53.*
349. Stoughton, R.B., *The vasoconstrictor assay in bioequivalence testing: practical concerns and recent developments.* Int J Dermatol, 1992. **31 Suppl 1**: p. 26-8.
350. Mc, K.A. and R.B. Stoughton, *Method for comparing percutaneous absorption of steroids.* Archives of Dermatology, 1962. **86**(5): p. 608-610.
351. Pershing, L.K., et al., *Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching bioassay.* Pharm Res, 1992. **9**(1): p. 45-51.
352. Hauck, W.W., *Bioequivalence studies of topical preparations: statistical considerations.* Int J Dermatol, 1992. **31 Suppl 1**: p. 29-33.
353. Locke, C.S., *An exact confidence interval from untransformed data for the ratio of two formulation means.* J Pharmacokinet Biopharm, 1984. **12**(6): p. 649-55.
354. Shah, V.P. and R.L. Williams, *Challenges in Evaluating Bioequivalence of Topical Dermatological Drug Products*, in *Topical Drug Bioavailability, Bioequivalence, and Penetration*, V.P. Shah, H.I. Maibach, and J. Jenner, Editors. 2014, Springer. p. 101-112.
355. Weiss, S.C., *Conventional topical delivery systems.* Dermatologic Therapy, 2011. **24**(5): p. 471-476.
356. Braddy, A.C. and D.P. Conner, *Bioequivalence for Topical Drug Products*, in *FDA Bioequivalence Standards*, L.X. Yu and B.V. Li, Editors. 2014, Springer New York: New York, NY. p. 335-367.
357. Raney, S.G., et al., *Pharmacokinetics-Based Approaches for Bioequivalence Evaluation of Topical Dermatological Drug Products.* Clinical Pharmacokinetics, 2015. **54**(11): p. 1095-1106.
358. Flynn, G.L., et al., *Assessment of value and applications of in vitro testing of topical dermatological drug products.* Pharm Res, 1999. **16**(9): p. 1325-30.
359. Franz, T.J., P.A. Lehman, and S.G. Raney, *Use of excised human skin to assess the bioequivalence of topical products.* Skin Pharmacol Physiol, 2009. **22**(5): p. 276-86.
360. Corcuff, P., F. Fiat, and A.M. Minondo, *Ultrastructure of the human stratum corneum.* Skin Pharmacol Appl Skin Physiol, 2001. **14 Suppl 1**: p. 4-9.
361. *United States Food and Drug Administration. Critical path: opportunities for generic drugs. Section 4.3.3: bioequivalence of topical dermatological products. 2007. http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/CriticalPathOpportunitiesReports/ucm077250.htm#dermatological. Accessed 09 February 2018.*
362. Boix-Montanes, A., *Relevance of equivalence assessment of topical products based on the dermatopharmacokinetics approach.* European Journal of Pharmaceutical Sciences, 2011. **42**(3): p. 173-179.
363. Navidi, W., et al., *Determining bioequivalence of topical dermatological drug products by tape-stripping.* Journal of Pharmacokinetics and Pharmacodynamics, 2008. **35**(3): p. 337.
364. Bunge A, et al., *Final report to food and drug administration (FDA): therapeutic equivalence of topical products, Colorado School of Mines, Golden, Colorado.* 2007.
365. Kalia, Y.N., et al., *Normalization of stratum corneum barrier function and transepidermal water loss in vivo.* Pharm Res, 2000. **17**(9): p. 1148-50.
366. Lademann, J., et al., *Penetration studies of topically applied substances: Optical determination of the amount of stratum corneum removed by tape stripping.* J Biomed Opt, 2006. **11**(5): p. 054026.

367. Weigmann, H., et al., *Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption.* Skin Pharmacol Appl Skin Physiol, 1999. **12**(1-2): p. 34-45.
368. *US-FDA Guidance for Industry: Topical dermatologic drug product NDAs and ANDAs-in vivo bioavailability, bioequivalence, in vitro release, and associated studies, 1998. Bethesda (MD): Center for Drug Evaluation and Research, FDA. .*
369. Wiedersberg, S., C.S. Leopold, and R.H. Guy, *Bioavailability and bioequivalence of topical glucocorticoids.* Eur J Pharm Biopharm, 2008. **68**(3): p. 453-66.
370. Rougier, A., et al., *In vivo correlation between stratum corneum reservoir function and percutaneous absorption.* J Invest Dermatol, 1983. **81**(3): p. 275-8.
371. Pirot, F., et al., *Characterization of the permeability barrier of human skin in vivo.* Proc Natl Acad Sci U S A, 1997. **94**(4): p. 1562-7.
372. Williams, M.L. and P.M. Elias, *The extracellular matrix of stratum corneum: role of lipids in normal and pathological function.* Crit Rev Ther Drug Carrier Syst, 1987. **3**(2): p. 95-122.
373. Golden, G.M., et al., *Stratum corneum lipid phase transitions and water barrier properties.* Biochemistry, 1987. **26**(8): p. 2382-2388.
374. Potts, R.O. and M.L. Francoeur, *The Influence of Stratum Corneum Morphology on Water Permeability.* Journal of Investigative Dermatology, 1991. **96**(4): p. 495-499.
375. Kalia, Y.N., F. Pirot, and R.H. Guy, *Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo.* Biophys J, 1996. **71**(5): p. 2692-700.
376. Pinkus, H., *Examination of the epidermis by the strip method of removing horny layers. I. Observations on thickness of the horny layer, and on mitotic activity after stripping.* J Invest Dermatol, 1951. **16**(6): p. 383-6.
377. van der Valk, P.G. and H.I. Maibach, *A functional study of the skin barrier to evaporative water loss by means of repeated cellophane-tape stripping.* Clin Exp Dermatol, 1990. **15**(3): p. 180-2.
378. Anderson, R.L. and J.M. Cassidy, *Variation in physical dimensions and chemical composition of human stratum corneum.* J Invest Dermatol, 1973. **61**(1): p. 30-2.
379. Pirot, F., et al., *Stratum corneum thickness and apparent water diffusivity: facile and noninvasive quantitation in vivo.* Pharm Res, 1998. **15**(3): p. 492-4.
380. Russell, L.M., S. Wiedersberg, and M. Begoña Delgado-Charro, *The determination of stratum corneum thickness An alternative approach.* European Journal of Pharmaceutics and Biopharmaceutics, 2008. **69**(3): p. 861-870.
381. Flynn, G.L., *General Introduction and Conceptual Differentiation of Topical and Transdermal Drug Delivery Systems*, in *Topical Drug Bioavailability, Bioequivalence, and Penetration*, V.P. Shah and H.I. Maibach, Editors. 1993, Springer US: Boston, MA. p. 369-391.
382. Pershing, L.K., et al., *Assessment of dermatopharmacokinetic approach in the bioequivalence determination of topical tretinoin gel products.* J Am Acad Dermatol, 2003. **48**(5): p. 740-51.
383. *Franz TJ. Study #1, Avita gel 0.025 % vs Retin-A gel 0.025 %. Transcribed presentation to the Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, MD, November 29, 2001. Presentation slides. http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_03_franz.pdf. Accessed 08 Feb 2018.*
384. *Pershing, LK. Bioequivalence assessment of three 0.025% tretinoin gel products: Dermatopharmacokinetic vs. clinical trial methods, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, MD, November 29, 2001.*

385. *D.P. Conner, Differences in DPK Methods, http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_01_conner/index.htm, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, MD, November 29, 2001.*
386. Russell, L.M. and R.H. Guy, *Measurement and prediction of the rate and extent of drug delivery into and through the skin.* Expert Opin Drug Deliv, 2009. **6**(4): p. 355-69.
387. Herkenne, C., et al., *Dermatopharmacokinetic prediction of topical drug bioavailability in vivo.* J Invest Dermatol, 2007. **127**(4): p. 887-94.
388. Alberti, I., et al., *In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum.* J Control Release, 2001. **71**(3): p. 319-27.
389. N'Dri-Stempfer, B., et al., *Improved bioequivalence assessment of topical dermatological drug products using dermatopharmacokinetics.* Pharm Res, 2009. **26**(2): p. 316-28.
390. N'Dri-Stempfer, B., et al., *Optimizing metrics for the assessment of bioequivalence between topical drug products.* Pharm Res, 2008. **25**(7): p. 1621-30.
391. Cordery, S.F., et al., *Topical bioavailability of diclofenac from locally-acting, dermatological formulations.* Int J Pharm, 2017. **529**(1-2): p. 55-64.
392. Corinne Seng Yue, a.M.P.D., *Empirical Models, Mechanistic Models, Statistical Moments, and Noncompartmental Analysis*, in *Applied Biopharmaceutics & Pharmacokinetics*, A.B.C.Y. Leon Shargel, Editor. 2016, McGraw-Hill Education. p. 817-849.
393. Ette, E.I. and P.J. Williams, *Population pharmacokinetics I: background, concepts, and models.* Ann Pharmacother, 2004. **38**(10): p. 1702-6.
394. Mould, D.R. and R.N. Upton, *Basic concepts in population modeling, simulation, and model-based drug development.* CPT Pharmacometrics Syst Pharmacol, 2012. **1**: p. e6.
395. Roy, A. and E.I. Ette, *A pragmatic approach to the design of population pharmacokinetic studies.* Aaps j, 2005. **7**(2): p. E408-20.
396. Wagner, J.G., *Fundamentals of Clinical Pharmacokinetics*. 1975, Hamilton: Drug Intelligence Publications Inc.
397. Joel S. Owen, a.J.F.K., *Population Model Concepts and Terminology*, in *Introduction to Population Pharmacokinetic / Pharmacodynamic Analysis with Nonlinear Mixed Effects Models*. 2014, John Wiley & Sons, Inc. p. 9-27.
398. Sheiner, L.B. and S.L. Beal, *Pharmacokinetic parameter estimates from several least squares procedures: superiority of extended least squares.* J Pharmacokinet Biopharm, 1985. **13**(2): p. 185-201.
399. Sheiner, L.B., *Analysis of pharmacokinetic data using parametric models. II. Point estimates of an individual's parameters.* J Pharmacokinet Biopharm, 1985. **13**(5): p. 515-40.
400. Racine-Poon, A. and J. Wakefield, *Statistical methods for population pharmacokinetic modelling.* Stat Methods Med Res, 1998. **7**(1): p. 63-84.
401. Sheiner, L.B. and S.L. Beal, *Bayesian individualization of pharmacokinetics: simple implementation and comparison with non-Bayesian methods.* J Pharm Sci, 1982. **71**(12): p. 1344-8.
402. Beal, S.L. and L.B. Sheiner, *Part I, Users basic guide. In NONMEM Users Guide*. 1989, San Francisco, CA.
403. Aarons, L., *Software for Population Pharmacokinetics and Pharmacodynamics.* Clinical Pharmacokinetics, 1999. **36**(4): p. 255-264.
404. Liu, X. and Y. Wang, *Comparing the performance of FOCE and different expectation-maximization methods in handling complex population physiologically-based pharmacokinetic models.* Journal of Pharmacokinetics and Pharmacodynamics, 2016. **43**(4): p. 359-370.

405. Sheiner, L.B., B. Rosenberg, and K.L. Melmon, *Modelling of individual pharmacokinetics for computer-aided drug dosage.* Comput Biomed Res, 1972. **5**(5): p. 411-59.
406. Bonate, P.L., *Recommended reading in population pharmacokinetic pharmacodynamics.* Aaps j, 2005. **7**(2): p. E363-73.
407. Sheiner, L.B. and S.L. Beal, *Evaluation of methods for estimating population pharmacokinetic parameters. II. Biexponential model and experimental pharmacokinetic data.* J Pharmacokinet Biopharm, 1981. **9**(5): p. 635-51.
408. Steimer, J.L., et al., *Alternative approaches to estimation of population pharmacokinetic parameters: comparison with the nonlinear mixed-effect model.* Drug Metab Rev, 1984. **15**(1-2): p. 265-92.
409. Sheiner, L.B., *The population approach to pharmacokinetic data analysis: rationale and standard data analysis methods.* Drug Metab Rev, 1984. **15**(1-2): p. 153-71.
410. Sheiner, L.B. and S.L. Beal, *Evaluation of methods for estimating population pharmacokinetics parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data.* J Pharmacokinet Biopharm, 1980. **8**(6): p. 553-71.
411. Ette, E.I. and P.J. Williams, *Population Pharmacokinetics II: Estimation Methods.* Annals of Pharmacotherapy, 2004. **38**(11): p. 1907-1915.
412. Forrest, A., et al., *Development of a population pharmacokinetic model and optimal sampling strategies for intravenous ciprofloxacin.* Antimicrob Agents Chemother, 1993. **37**(5): p. 1065-72.
413. Mentre, F. and R. Gomeni, *A two-step iterative algorithm for estimation in nonlinear mixed-effect models with an evaluation in population pharmacokinetics.* J Biopharm Stat, 1995. **5**(2): p. 141-58.
414. Whiting, B., A.W. Kelman, and J. Grevel, *Population Pharmacokinetics Theory and Clinical Application.* Clinical Pharmacokinetics, 1986. **11**(5): p. 387-401.
415. Beal, S.L., *Population pharmacokinetic data and parameter estimation based on their first two statistical moments.* Drug Metab Rev, 1984. **15**(1-2): p. 173-93.
416. Ludden, T.M., *Population pharmacokinetics.* J Clin Pharmacol, 1988. **28**(12): p. 1059-63.
417. Colucci, P. and M.P. Ducharme, *Appendix A: Applications of Software Packages in Pharmacokinetics*, in *Applied Biopharmaceutics & Pharmacokinetics*, A.B.C.Y. Leon Shargel, Editor. 2016, McGraw-Hill Education. p. 851-874.
418. *D'Argenio DZ, Schumitzky A, Wang X: ADAPT 5 User's Guide: Pharmacokinetic/Pharmacodynamic Systems Analysis Software. Los Angeles, Biomedical Simulations Resource, 2009.*
419. *P-PHARM user's guide, version 1.3, Créteil, France: SIMED, 1994.*
420. Kinowski, J.-M., et al., *A Limited Sampling Model with Bayesian Estimation to Determine Inulin Pharmacokinetics Using the Population Data Modelling Program P-PHARM.* Clinical Drug Investigation, 1995. **9**(5): p. 260-269.
421. Wang, X., A. Schumitzky, and D. D'Argenio, *Nonlinear Random Effects Mixture Models: Maximum Likelihood Estimation via the EM Algorithm.* Computational statistics & data analysis, 2007. **51**: p. 6614-6623.
422. Wang, X., A. Schumitzky, and D.Z. D'Argenio, *Population Pharmacokinetic/Pharmacodyanamic Mixture Models via Maximum a Posteriori Estimation.* Computational statistics & data analysis, 2009. **53**(12): p. 3907-3915.
423. Gomeni, R., G. Pineau, and F. Mentre, *Population kinetics and conditional assessment of the optimal dosage regimen using the P-PHARM software package.* Anticancer Res, 1994. **14**(6a): p. 2321-6.

424. Bauer, R.J., S. Guzy, and C. Ng, *A survey of population analysis methods and software for complex pharmacokinetic and pharmacodynamic models with examples.* Aaps j, 2007. **9**(1): p. E60-83.
425. Beal, S.L. and L.B. Sheiner, *Part VII, Conditional Estimation Methods. In NONMEM Users Guide*. 1989, San Francisco, CA.
426. Powell, J.R. and J.V. Gobburu, *Pharmacometrics at FDA: evolution and impact on decisions.* Clin Pharmacol Ther, 2007. **82**(1): p. 97-102.
427. Lee, J.Y., et al., *Impact of pharmacometric analyses on new drug approval and labelling decisions: a review of 198 submissions between 2000 and 2008.* Clin Pharmacokinet, 2011. **50**(10): p. 627-35.
428. Bhattaram, V.A., et al., *Impact of pharmacometric reviews on new drug approval and labeling decisions--a survey of 31 new drug applications submitted between 2005 and 2006.* Clin Pharmacol Ther, 2007. **81**(2): p. 213-21.
429. Bhattaram, V.A., et al., *Impact of pharmacometrics on drug approval and labeling decisions: a survey of 42 new drug applications.* Aaps j, 2005. **7**(3): p. E503-12.
430. *US-FDA, CDER: Population Pharmacokinetics Guidance for Industry. July 2019. Last access 25 Oct 2019 at: https://www.fda.gov/media/128793/download.*
431. Colucci, P., J.F. Marier, and M.P. Ducharme, *Population pharmacokinetic approaches for assessing bioequivalence.*, in *Generic Drug Product Development – Bioequivalence Issues*, I. Kanfer and L. Shargel, Editors. 2008, Informa Healthcare, Inc.: New York. p. 143–170.
432. Marier, J. and M.P. Ducharme, *A population PK/PD model to determine the bioequivalence of albuterol using rate and extent of absorption derived from FEV1 measurements. .* Clinical Pharmacology & Therapeutics, 2001. **69**(2): p. 59-69.
433. Fradette, C., et al., *The utility of the population approach applied to bioequivalence in patients: comparison of 2 formulations of cyclosporine.* Ther Drug Monit, 2005. **27**(5): p. 592-600.
434. Aghazadeh-Habashi, A. and F. Jamali, *Pharmacokinetics of meloxicam administered as regular and fast dissolving formulations to the rat: influence of gastrointestinal dysfunction on the relative bioavailability of two formulations.* Eur J Pharm Biopharm, 2008. **70**(3): p. 889-94.
435. Jamali, F. and A. Aghazadeh-Habashi, *Rapidly dissolving formulations for quick absorption during pain episodes: ibuprofen.* Int J Clin Pharmacol Ther, 2008. **46**(2): p. 55-63.
436. Sodeyama, M., et al., *The effect of hemorrhagic shock on intestinal amino acid absorption in vivo.* Circ Shock, 1992. **38**(3): p. 153-6.
437. Pabla, D., F. Akhlaghi, and H. Zia, *A comparative pH-dissolution profile study of selected commercial levothyroxine products using inductively coupled plasma mass spectrometry.* Eur J Pharm Biopharm, 2009. **72**(1): p. 105-10.
438. Sachmechi, I., et al., *Effect of proton pump inhibitors on serum thyroid-stimulating hormone level in euthyroid patients treated with levothyroxine for hypothyroidism.* Endocr Pract, 2007. **13**(4): p. 345-9.
439. Trifiro, G., et al., *Drug interactions with levothyroxine therapy in patients with hypothyroidism: observational study in general practice.* Clin Drug Investig, 2015. **35**(3): p. 187-95.
440. Seng Yue, C., et al., *When Bioequivalence in Healthy Volunteers May not Translate to Bioequivalence in Patients: Differential Effects of Increased Gastric pH on the Pharmacokinetics of Levothyroxine Capsules and Tablets.* J Pharm Pharm Sci, 2015. **18**(5): p. 844-55.

441. Li, L., T. Yi, and C.W. Lam, *Inhibition of human efflux transporter ABCC2 (MRP2) by self-emulsifying drug delivery system: influences of concentration and combination of excipients.* J Pharm Pharm Sci, 2014. **17**(4): p. 447-60.
442. Schafer-Korting, M., et al., *Glucocorticoids for human skin: new aspects of the mechanism of action.* Skin Pharmacol Physiol, 2005. **18**(3): p. 103-14.
443. *US-FDA Guidance for Industry: Topical dermatologic drug product NDAs and ANDAs-in vivo bioavailability, bioequivalence, in vitro release, and associated studies, 1998. Bethesda (MD): Center for Drug Evaluation and Research, FDA.*
444. N'Dri-Stempfer, B., et al., *Improved Bioequivalence Assessment of Topical Dermatological Drug Products Using Dermatopharmacokinetics.* Pharmaceutical Research, 2008. **26**(2): p. 316.
445. Alberti, I., et al., *Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo.* Int J Pharm, 2001. **219**(1-2): p. 11-9.
446. Pershing, L.K., J. Corlett, and C. Jorgensen, *In vivo pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human stratum corneum.* Antimicrob Agents Chemother, 1994. **38**(1): p. 90-5.
447. Bashir, S.J., et al., *Cutaneous bioassay of salicylic acid as a keratolytic.* Int J Pharm, 2005. **292**(1-2): p. 187-94.
448. Schwarb, F.P., et al., *Percutaneous absorption of salicylic acid in man after topical administration of three different formulations.* Dermatology, 1999. **198**(1): p. 44-51.
449. Tsai, J., et al., *Distribution of salicylic acid in human stratum corneum following topical application in vivo: a comparison of six different formulations.* Int J Pharm, 1999. **188**(2): p. 145-53.
450. Teichmann, A., et al., *Reservoir function of the stratum corneum: development of an in vivo method to quantitatively determine the stratum corneum reservoir for topically applied substances.* Skin Pharmacol Physiol, 2005. **18**(2): p. 75-80.
451. Jacobi, U., et al., *Lateral spreading of topically applied UV filter substances investigated by tape stripping.* Skin Pharmacol Physiol, 2004. **17**(1): p. 17-22.
452. Gamer, A.O., E. Leibold, and B. van Ravenzwaay, *The in vitro absorption of microfine zinc oxide and titanium dioxide through porcine skin.* Toxicol In Vitro, 2006. **20**(3): p. 301-7.
453. Wissing, S.A. and R.H. Muller, *Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration.* J Control Release, 2002. **81**(3): p. 225-33.
454. Lboutounne, H., et al., *Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(epsilon-caprolactone) nanocapsule encapsulated form and as a digluconate.* J Control Release, 2002. **82**(2-3): p. 319-34.
455. García-Arieta, A., J. Gordon, and H. Potthast, *General Commentary on the Effect of Common Excipients on the Oral Absorption of Class 3 Drugs.* Journal of Pharmaceutical Sciences, 2016. **105**(4): p. 1353-1354.
456. Balram, C., et al., *Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic-genotypic correlates.* British journal of clinical pharmacology, 2003. **56**(1): p. 78-83.
457. Michael Borenstein, et al., *Fixed-Effect Model*, in *Introduction to Meta-Analysis*. 2009, John Wiley & Sons, Ltd. p. 63-68.
458. Michael Borenstein, et al., *Random-Effect versus Random-Effects Models*, in *Introduction to Meta-Analysis*. 2009, John Wiley & Sons, Ltd. p. 77-86.
459. Michael Borenstein, et al., *Random-Effects Model*, in *Introduction to Meta-Analysis*. 2009, John Wiley & Sons, Ltd. p. 69-76.

460. Floren, L.C., et al., *Tacrolimus oral bioavailability doubles with coadministration of ketoconazole.* Clin Pharmacol Ther, 1997. **62**(1): p. 41-9.
461. Hebert, M.F., et al., *Effects of rifampin on tacrolimus pharmacokinetics in healthy volunteers.* J Clin Pharmacol, 1999. **39**(1): p. 91-6.
462. Floren, L.C., et al., *Sirolimus oral bioavailability increases ten-fold with concomitant ketoconazole.* Clinical Pharmacology & Therapeutics, 1999. **65**(2): p. 159.
463. Lown, K.S., et al., *Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine.* Clin Pharmacol Ther, 1997. **62**(3): p. 248-60.
464. Fricker, G., et al., *Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation.* Br J Pharmacol, 1996. **118**(7): p. 1841-7.
465. Boyd, R.A., et al., *Atorvastatin coadministration may increase digoxin concentrations by inhibition of intestinal P-glycoprotein-mediated secretion.* J Clin Pharmacol, 2000. **40**(1): p. 91-8.
466. Welling, P.G., et al., *Influence of diet and fluid on bioavailability of theophylline.* Clin Pharmacol Ther, 1975. **17**(4): p. 475-80.
467. Gonzalez, M.A. and A.B. Straughan, *Effect of meals and dosage-form modification on theophylline bioavailability from a 24-hour sustained-release delivery system.* Clin Ther, 1994. **16**(5): p. 804-14.
468. Heimann, G., J. Murgescu, and U. Bergt, *Influence of food intake on bioavailability of theophylline in premature infants.* European Journal of Clinical Pharmacology, 1982. **22**(2): p. 171-173.
469. Leeds, N.H., et al., *Effect of food on the bioavailability and pattern of release of a sustained-release theophylline tablet.* J Clin Pharmacol, 1982. **22**(4): p. 196-200.
470. Sips, A.P., et al., *Food does not effect in bioavailability of theophylline from Theolin Retard.* Eur J Clin Pharmacol, 1984. **26**(3): p. 405-7.
471. Jonkman, J.H., *Food interactions with once-a-day theophylline preparations: a review.* Chronobiol Int, 1987. **4**(3): p. 449-58.
472. Jonkman, J.H., *Food interactions with sustained-release theophylline preparations. A review.* Clin Pharmacokinet, 1989. **16**(3): p. 162-79.
473. Singh, G.J., et al., *Evaluation of the proposed FDA pilot dose-response methodology for topical corticosteroid bioequivalence testing.* Pharm Res, 1998. **15**(1): p. 4-7.
474. K, R., et al., *FDA Perspective on Current Challenges with the Use of Pharmacodynamic Endpoint Evaluation of Bioequivalence of Topical Dermatologic Corticosteroids.*, in *AAPS*. 2013: San Antonio.
475. Smith, E.W., J.M. Haigh, and R.B. Walker, *Analysis of chromameter results obtained from corticosteroid-induced skin blanching. I: Manipulation of data.* Pharm Res, 1998. **15**(2): p. 280-5.
476. Waring, M.J., et al., *Assessment of corticosteroid-induced skin blanching: evaluation of the Minolta Chromameter CR200.* International Journal of Pharmaceutics, 1993. **94**(1): p. 211-222.
477. Demana, P.H., et al., *Evaluation of the Proposed FDA Pilot Dose-Response Methodology for Topical Corticosteroid Bioequivalence Testing.* Pharmaceutical Research, 1997. **14**(3): p. 303-308.
478. Dutta, S., Y. Matsumoto, and W.F. Ebling, *Is it possible to estimate the parameters of the sigmoid Emax model with truncated data typical of clinical studies?* J Pharm Sci, 1996. **85**(2): p. 232-9.

479. *FDA. SUPAC-SS nonsterile semisolid dosage forms, scale-up and postapproval changes: chemistry, manufacturing, and controls; in vitro release testing and in vivo bioequivalence documentation. May 1997. Last access 7 Nov 2019 at: https://www.fda.gov/media/71141/download.*
480. Parry, G.E., et al., *Acyclovir bioavailability in human skin.* J Invest Dermatol, 1992. **98**(6): p. 856-63.
481. de Jalon, E.G., et al., *Topical application of acyclovir-loaded microparticles: quantification of the drug in porcine skin layers.* J Control Release, 2001. **75**(1-2): p. 191-7.
482. Volpato, N.M., et al., *In vitro acyclovir distribution in human skin layers after transdermal iontophoresis1Partially presented at the Symposium on "Transdermal Administration. A Case Study, Iontophoresis", Paris, March 3–4, 1997.1.* Journal of Controlled Release, 1998. **50**(1): p. 291-296.
483. Spruance, S.L. and D.J. Freeman, *Topical treatment of cutaneous herpes simplex virus infections.* Antiviral Research, 1990. **14**(6): p. 305-321.
484. Box, G.E.P., *Science and Statistics.* Journal of the American Statistical Association, 1976. **71**(356): p. 791-799.

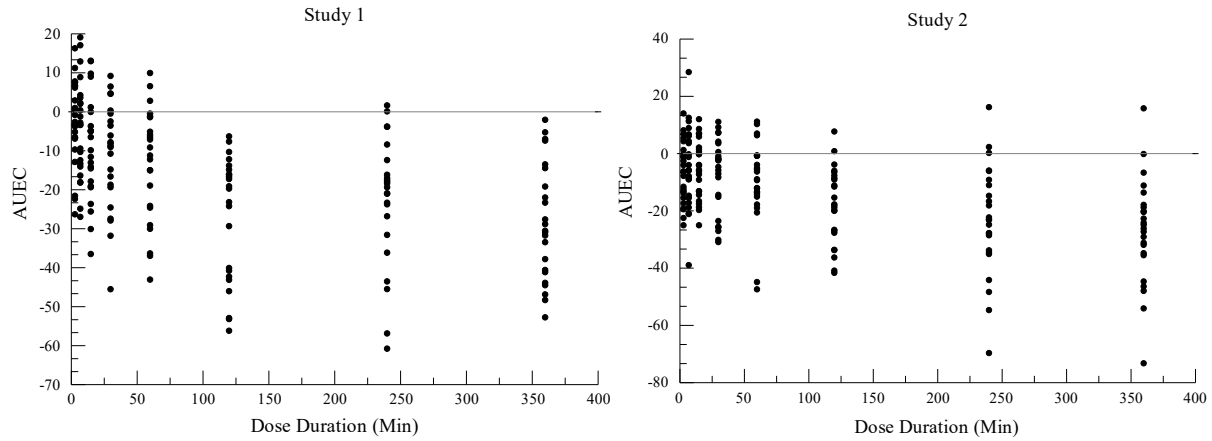
Appendix 1

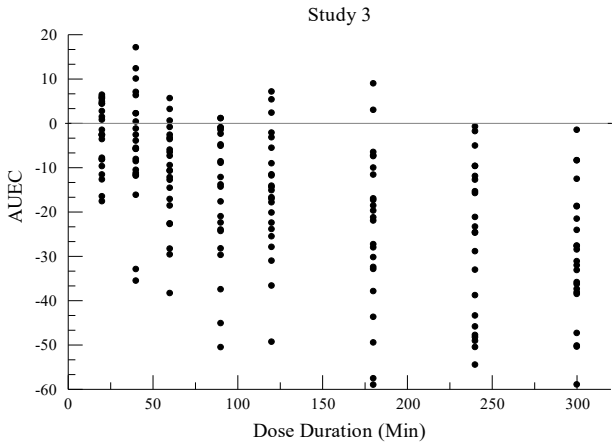
Article #2

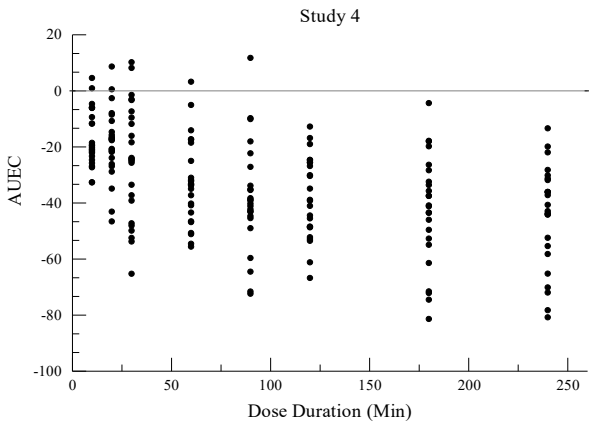
Table 1 Summary of the pilot dose duration- response studies

Study #	Number of subjects	Number of dose durations	Dose durations (min)
Study 1	23	8	3, 7, 15, 30, 60, 120, 240, 360
Study 2	24	8	3, 7, 15, 30, 60, 120, 240, 360
Study 3	23	8	20, 40, 60, 90, 120, 180, 240, 300
Study 4	24	8	10, 20, 30, 60, 90, 120, 180, 240
Study 5	24	8	15, 30, 60, 120, 180, 240, 300, 360
Study 6	16	8	15, 30, 45, 60, 90, 180, 240, 360
Study 7	16	8	15, 30, 45, 60, 90, 180, 240, 360
Study 8	16	8	15, 30, 45, 60, 90, 180, 240, 360
Study 9	24	8	10, 15, 20, 30, 60, 90, 120, 240
Study 10	24	10	5, 10, 20, 30, 45, 60, 120, 240, 300, 360
Study 11	24	8	15, 30, 45, 60, 90, 120, 240, 360

Figure 1. Dose duration-Response observations of each study




Study 3
AUEC
Dose Duration (Min)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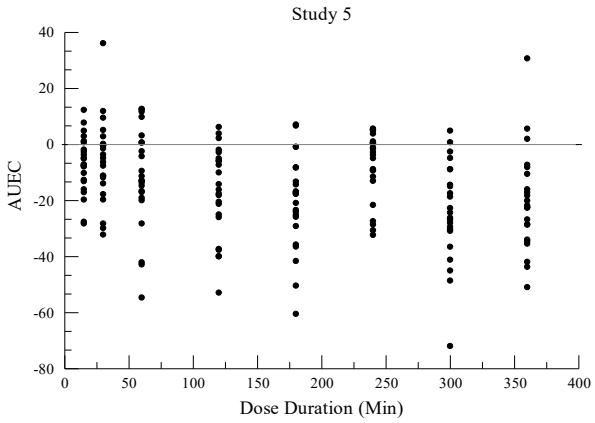
AUEC
Dose Duration (Min)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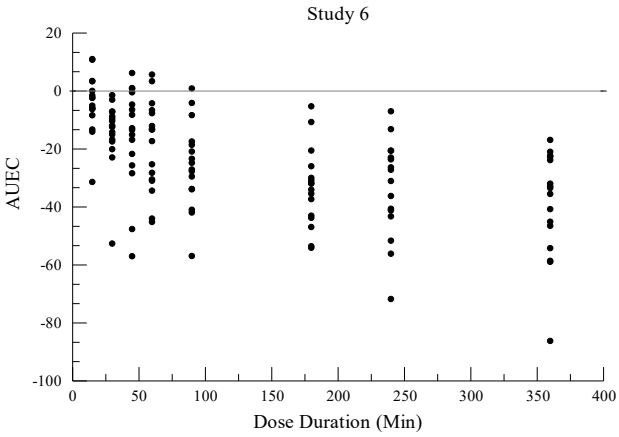
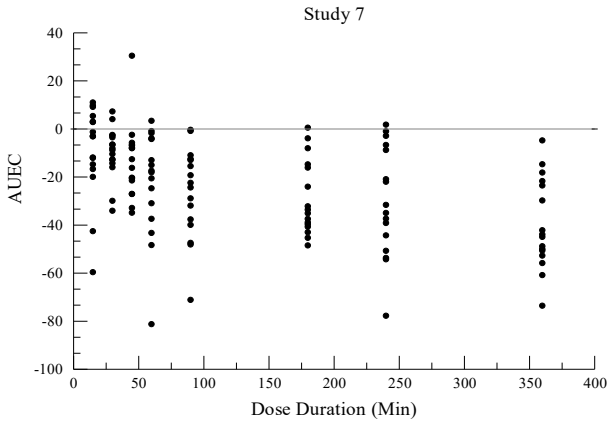
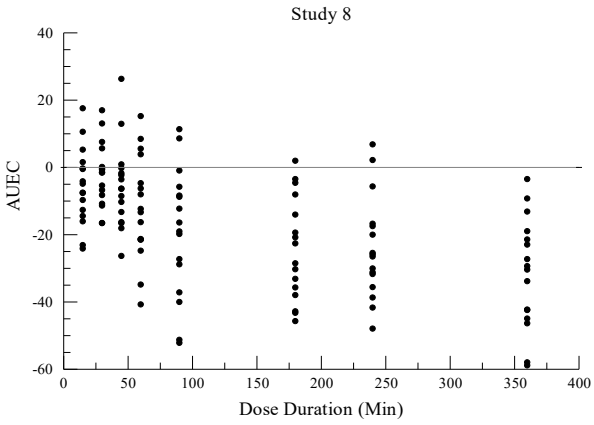
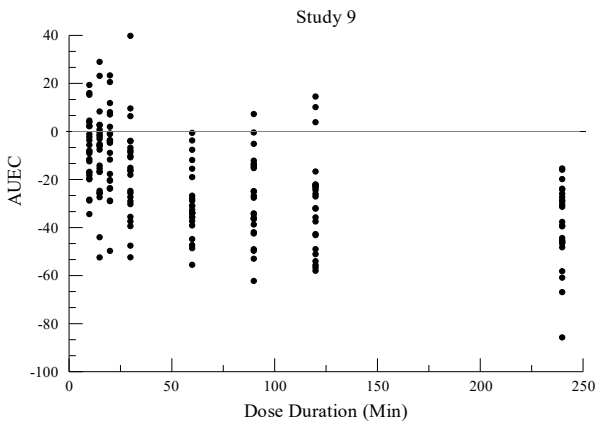
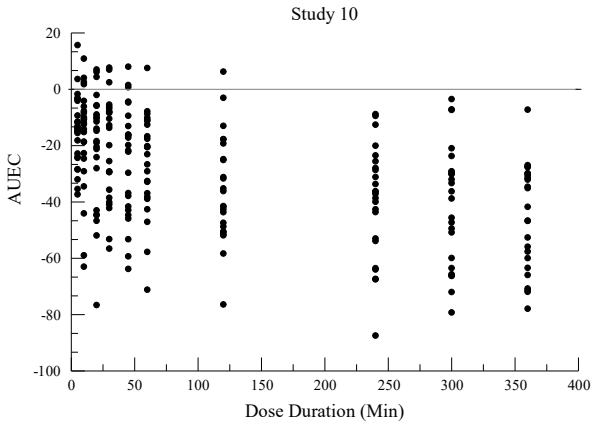
AUEC
Dose Duration (Min)


Study 6
AUEC
Dose Duration (Min)


Study 7
AUEC
Dose Duration (Min)


Study 8
AUEC
Dose Duration (Min)


Study 9
AUEC
Dose Duration (Min)


Study 10
AUEC
Dose Duration (Min)

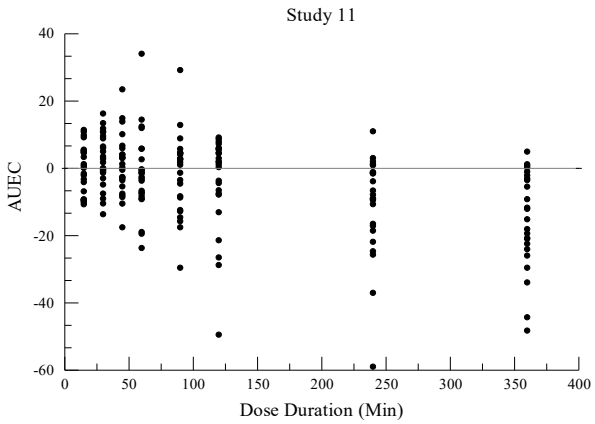

Study 11
AUEC
Dose Duration (Min)

Table 2 A summary of key findings from fittings with FOCE and MLEM algorithms and comparison of their general characteristics

		FOCE	**MLEM**
Fitting Results	Pop* mean estimates	Generally higher	Generally lower
	Inter-CV%$^{\Psi}$	Generally higher	Generally lower
	Variance Shrinkage	More frequent	Less frequent
	Quality of fit	Similar	Similar
Analysis Method	Estimation approach	Estimation of ML	Importance sampling Solving for
	Iteration levels	2 steps: 1st step Pop level, 2nd step Individual level	Repetitive 2 steps until convergence: 1st step (E-step) individual level, 2nd (M-step) Pop level
Consequences to Be Considered	Accuracy of estimates	Less	More
		Approximate ML estimates	True ML estimates
	Dataset	Sparse observational data would not affect the analysis	Sparse observational data should be avoided
	Compliance between Pop and *post-hoc* estimates	Less	More
		More discrepancy between Pop and *post-hoc* estimates in FOCE than in MLEM	
	Application preference	When Pop estimates are required	When individual estimates are required
	Convergence	Automatically	Manually
	Analysis runtime	Shorter	Longer

*Population
$^{\Psi}$Inter-individual variability