A physiologically based biopharmaceutical analysis of zolpidem

Dissertation

zur Erlangung des Doktorgrades

der Naturwissenschaften

Vorgelegt beim Fachbereich 14

(Biochemie, Chemie und Pharmazie)

Der Johann Wolfgang Goethe – Universität

von Rafael Leal Monteiro Paraiso

aus Sao Francisco, Minas Gerais – Brasilien

Frankfurt am Main, 2021

Von Fachbereich für Biochemie, Chemie und Pharmazie der Johann Wolfgang Goethe – Universität als Dissertation angenommen

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Datum der Disputation: 11th of June 2021

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CHAPTER 1

Introduction

1 - Introduction

1.1 - Zolpidem

Zolpidem is a non-benzodiazepine hypnotic agent which has been used in medical practice to induce sleep in adults (Salvà and Costa, 1995). Zolpidem tartrate is the salt form used to manufacture the reference tablets Ambien® and Stilnox® by Sanofi-Aventis ("FDA," 1992). Chemically, zolpidem is N,N,6-trimethyl-2-p-tolylimidazo[1,2-a] pyridine-3-acetamide L-(+)-tartrate (2:1). It has the following chemical structure (Figure 1):

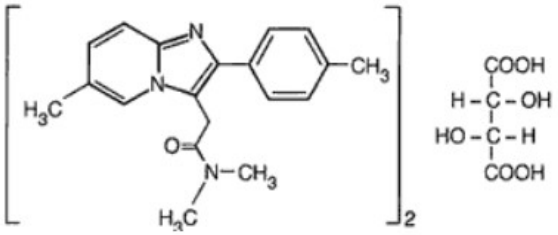


Figure 1. Chemical structure of zolpidem tartarate.

Zolpidem is an imidazopyridine which acts at the benzodiazepine ω1-receptor subtype exhibiting hypnotic-sedative action due to specific agonist binding on the α1- gamma-aminobutyric acid type A ($GABA_A$) receptor. This receptor is mainly associated with sedation, whereas other subtypes are responsible for various other effects of $GABA_{ergics}$ ligands, such as muscle relaxation, anxiolysis and memory damage (de Haas et al., 2010). The selective binding of zolpidem explains why this drug is strongly sedative in its action, with lesser anxiolytic, miorelaxant and anticonvulsivant activities (Salvà and Costa, 1995).

Zolpidem is one of most frequently prescribed hypnotics in the world (Norman et al., 2017). The reason for this high prescription rate is, first, because the compound is rapidly absorbed, which makes it very suitable for rapid sleep induction. Second, its short half-life (2.4h) is responsible for fewer residual effects the next day ("hangover" effect) compared with other drugs used as hypnotics (Vermeeren, 2004; de Haas et al., 2010). Although the short half-life can be of benefit for most of the patients due to the absence of the hangover effect, for others this characteristic may result in an earlier awakening time than desired. Therefore, Sanofi developed a modified release formulation consisting of two drug layers. In the first layer is the immediate

release that is responsible for the fast sleep induction, while the second one is the modified release part that is responsible for maintaining the release of the drug over the desired period of sleep (Weinling et al., 2006).

1.2 – BCS

The Biopharmaceutics Classification System (BCS) was proposed by Amidon and co-workers (1995) (Amidon et al., 1995). According to this concept, the solubility and permeability are the main factors which control the rate and the extent of drug absorption. Therefore, in this system the drugs are classified in four classes based on their aqueous solubility and intestinal permeability characteristics, as showed in Figure 2 (Amidon et al., 1995).

BCS I High solubility and high permeability	BCS II Low solubility and high permeability
BCS III High solubility and low permeability	BCS IV Low solubility and low permeability

Figure 2. The Biopharmaceutics Classification System (BCS).

The drugs which belongs to Class I are quickly and completely absorbed with an extent of absorption greater than 90% (SUPAC, 1995). As long as the dissolution of the drug is rapid, the limiting step for the absorption of Class I drugs is the rate of gastric emptying (Amidon et al., 1995; Colo et al., 2015; Paraiso et al., 2019a). The limiting step for oral absorption of BCS II drugs has been categorized into two types: dissolution rate-limited and solubility-limited. Therefore, to improve the oral absorption of these compounds it is often necessary to develop bioenabling formulations such as amorphous solid dispersions, lipid based formulations, micro or nanosized formulations, etc. (Sugano et al., 2007). For BCS III drugs, the limiting step will be the permeation of the drug through the intestinal membrane. The rate and extent of absorption of this group of drugs can be highly variable due to the influence of gastrointestinal transit, luminal

content and membrane permeability. The drugs from BCS IV are drugs that do not have either high solubility or high permeability. Thus, they have poor absorption, often with high variability in both the rate and extent of absorption, making them more problematic for oral administration. As for BCS Class II drugs, it is often necessary to develop bioenabling formulations to improve the biovailability of Class IV drugs (Ono and Sugano, 2014).

The BCS classification system is popular in pharmaceutical development due to its simplicity of approach, but it is of particular significance for regulatory submissions. Various regulatory agencies have adopted it for BCS-based biowaiver applications of Class I and III drugs, which reduces the number of bridging studies required for New Drug Applications (NDA) as well as obviating the need for a pharmacokinetic proof of bioequivalence for Amended New Drug Applications (ANDA) i.e. generic drug applications.

1.3 - Developability Classification System

The developability classification system (DCS) was proposed by Butler and Dressman (2010). As for the BCS classification this system categorizes drugs based on their solubility and permeability characteristics, as shown in figure 3. This modified classification system is referred to as the Developability Classification System (DCS) to emphasize that it is aimed at addressing solubility and permeability issues in a way that is more meaningful to product development (Butler and Dressman, 2010).

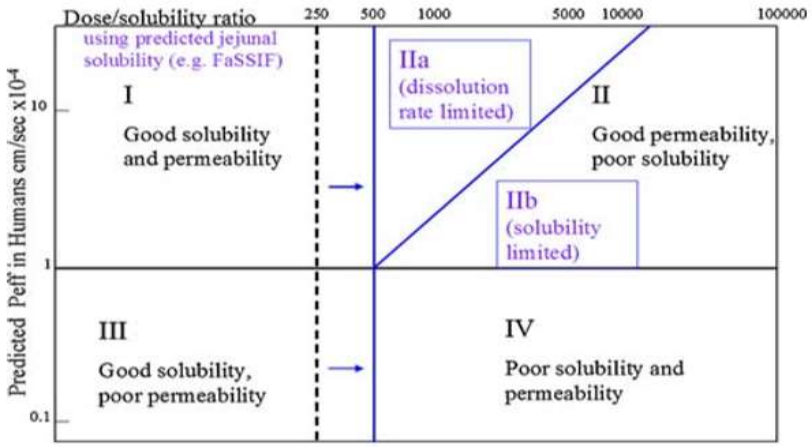


Figure 3. Developability classification system (DCS) with modifications from BCS. Reproduced with permission from Butler & Dressman, 2010.

One of the advantages of DCS is that for Class II compounds, which exhibit low solubility and high permeability, there is a subdivision into two classes: Class IIa drugs, for which the limiting step for drug absorption is the dissolution rate, and Class IIb drugs, for which the limiting step is the solubility of the drug. This sub-classification is of particular interest because compounds belonging to the DCS II are very common in drug discovery programs and can often be successfully delivered orally only with a bioenabling formulation which helps to increase the dissolution rate and/or solubility (Butler and Dressman, 2010).

The boundary between Class IIa and IIb is represented by the solubility limited absorbable dose (SLAD), representing the dose above which absorption is limited by solubility. DCS evaluation uses both a higher volume of gastrointestinal (GI) fluid — 500 mL instead of 250 mL — as well as using the biorelevant media, e.g. fasted state simulated gastric fluid (FaSSGF) and fasted state simulated intestinal fluid (FaSSIF) instead of the simple buffers which are used to determine the classification of drugs according to the BCS (Butler and Dressman, 2010).

1.4 - Drug dissolution

The dissolution experiment has evolved over the years to become one of the most important tools in pharmaceutical testing. It provides assurance that the dosage form disintegrates and that its contents disaggregate with subsequent dissolution in the dissolution medium, resulting in a solution of the drug that can absorbed from the small intestine. Therefore, from a patient's perspective, dissolution is an extremely critical test guaranteeing the quality of the drug product (Klein, 2010; Mirza et al., 2005).

A simplified description of the dissolution mechanism that occurs in the gastrointestinal tract is shown in Figure 4. This basically consists of four stages: (I) it starts with the hydration of the pharmaceutical formulation, (II) initial disintegration of the formulation matrix is followed by (III) dispersion of the drug into the medium with subsequent (IV) dissolution of drug in the gastrointestinal fluids. In certain cases, often involving weak bases, precipitation may occur after drug dissolution. The overall dissolution rate is limited by the slowest of the three first steps. (Hermans et al., 2017). If the first or the second step is rate-limiting, the overall dissolution rate is said to be disintegration controlled and the cohesive properties of the formulation will be an

important determinant of the overall process. It should also be recognized that disintegration can yield drug particles directly and/or via a granular intermediate. If the third step is the rate-limiting, the mechanism is dispersion controlled and the physical/chemical form of the drug, together with the physicochemical properties of the excipients will determine the dissolution rate. (Hermans et al., 2017). The fourth step, dissolution of the drug from the dispersed particles, will be limited by the wetting and solubility properties of the drug and the composition and hydrodynamics of the GI fluids.

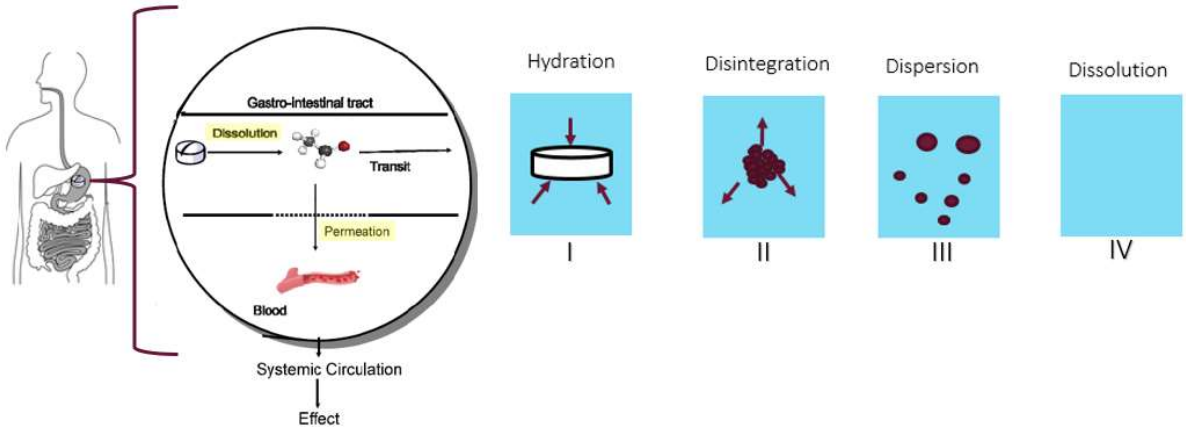


Figure 4. Drug dissolution process in the gastrointestinal tract.

1.4.1 - Quality Control dissolution media

The Quality Control (QC) dissolution media which are specified in the pharmacopeia (e.g., hydrochloric acid 0,1N pH1.2, acetate buffer pH 4.5, 50-mM phosphate buffer pH 6.8) have been used for solubility and dissolution experiments for decades and are referenced in the majority of USP monographs for quality control of pharmaceutical solid oral dosage form (Gray et al., 2009). Besides their advantage in terms of simple preparation, these media can be valuable and provide simple and reasonably accurate assessments of in vivo dissolution rate, especially for highly soluble (BCS I and III) compounds. However, for poorly soluble drugs BCS II and IV, the use of these media can be challenging as they fail to take several key aspects of the gastrointestinal fluids into consideration (Dressman, 2014; Gray et al., 2009).

In the past, compendial media have been used in formulation development and in quality control of solid oral dosage forms. Since the development of the biorelevant media, which attempt to more closely mimic the gastrointestinal environment, the QC media have been used

more in quality control for clinical batch release, stability studies and to support post approval changes (Hermans et al., 2017).

1.4.2 - Biorelevant media

Due to the failure of the aqueous buffer dissolution media to accurately represent the gastrointestinal tract (GIT) environment, different dissolution media simulating the GI tract content have been proposed in the literature (Dressman JB, Amidon GL, Reppas C, 1998; Fotaki and Vertzoni, 2010a; Lennernäs and Abrahamsson, 2005). These media contain solubilizing agents present in the intestine such as lecithin, bile salts and lipolysis products, which usually lead to an increase the solubility and consequently to an improvement in the dissolution rate of poorly soluble drugs. Biorelevant media have been widely used for characterization of the in vivo solubility and dissolution to predict the absorption of drugs (Fotaki and Vertzoni, 2010a; Jantratid and Dressman, 2007; Klein, 2010).

For drugs belonging to BCS class I and III and are thus highly soluble, dissolution is not rate limiting to oral absorption. In contrast to these compounds, for drugs from BCS class II and IV, which are poorly soluble compounds, the choice of the medium is expected to play an important role in dissolution because it may be influenced by various gastrointestinal environment characteristics such as pH, buffer capacity, and ionic strength as well as solubilization due the presence of surfactants and/or food components (Dressman and Reppas, 2000).

Markopoulos and co-workers (2015) proposed a classification of Levels of dissolution media from 0 to 3. This classification reflects the changes in media composition according to the complexity of the drug/formulation combination, going from Level 0 for combinations requiring only a low complexity in terms of dissolution media design to Level 3 for combinations requiring a higher complexity or to answer specific dissolution-related questions (Markopoulos et al., 2015a).

Level 0 media are simple aqueous solutions in which the pH is adjusted to represent the pH in of specific gastrointestinal segment. For this level, the main purpose is to maintain the pH during the experiment. As long as this condition is maintained, the buffer capacity of the media may not be relevant. This case typically arises for highly soluble drugs in immediate release

dosage forms, and may also be applicable to simple osmotic pump dosage forms. In Level I media, both the pH and buffer capacity are adjusted to better simulate the lumen conditions. This Level may be appropriate for enteric coated dosage forms, or for ionizable drugs which are highly soluble in the ionized state. In the Level II media, bile salts, dietary lipids and digestion products are added into the media to better describe the gastrointestinal content. The bile components will have an influence on the wetting and solubilization of the drug in the intestine and are therefore appropriate when poorly soluble drugs are to be tested. Dietary lipids and their digestion products are additionally included in Level II media to complete the description of the solubilization capacity of the luminal fluids in the fed state. With these additions and appropriate adjustments to the pH and buffer capacity, differences between the fasted and fed states related with gastrointestinal content are addressed. Level III media, which can have the most complex composition, are used to address the possible influence of proteins, the viscosity of the media and/or enzymes that are present in the luminal content of the GIT segment on the release of the drug from the dosage form (Markopoulos et al., 2015a). Using the Levels concept, different levels of dissolution media can be applied in various dissolution set-ups e.g. USP apparatus and transfer models to guide formulation development, quality control for stability studies and batch release of the finished drug product.

1.4.3 - Quality Control (QC) Dissolution Method

The Quality Control (QC) dissolution test is a very important in vitro test to determine batch-to-batch quality, consistency and performance of a drug product. Moreover, QC dissolution testing can be used to detect variations during routine product manufacturing and changes during formulation shelf life that might negatively impact in product performance leading to inefficacy of the drug in the treatment. Additionally, it can be used for different purposes in pharmaceutical drug development. (Grady et al., 2018; Gray et al., 2009; Gray, 2018)

In the formulation development phase, dissolution tests are used to validate initial screening among potential formulations and to help in the selection of the best candidate formulation. These will be translated into QC dissolution methods, which should be able to identify product variations related to changes in the API and/or the excipients raw materials or

critical quality attributes specific to the manufacturing process. For example, if there have been meaningful changes in the critical attributes of the API (e.g., particle morphology or state of hydration), or in key excipients, for example, surfactants, granulation aids, disintegrants, lubricants, and so forth, or e.g. if the product has been under-granulated or over-granulated, under-compressed or over-compressed (Grady et al., 2018; Gray et al., 2009). As well as the QC method needing to be discriminative, it should also be robust and simple to run in the QC environment of the pharmaceutical company.

Accordingly, for the majority of immediate release and modified release drug products (Table 1), QC dissolution is performed with USP dissolution apparatus 1 or 2, under conditions that have been established during product development. Although USP apparatus 3 is more appropriate for the evaluation of sustained release or release targeting dissolution in the colon release and the apparatus IV can be useful for poorly soluble drugs for which it is necessary to maintain the sink conditions, these apparatus have not been widely applied in the QC setting. With regard to media selection, dissolution media at Level 0 i.e. simple buffers or acid solutions are often used for QC purposes. The most common dissolution media described in USP monographs is HCl 0.1N with a pH of 1.2, but, depending the API properties, other pH values may be chosen is e.g. pH 4.5 or 6.8. In some cases, to maintain the sink condition of the media during the experiment, it is necessary to add surfactants at concentrations that do not exceed the regulatory agencies specifications (Grady et al., 2018).

Table 1. USP apparatus used in quality control for the different drug formulations.

USP apparatus	**Example applications**
I – Rotating basket	Solid oral dosage forms
II – Paddle	Solid oral dosage forms
III – Reciprocating cylinder	Bead-type modified-release drug formulation
IV – Flow-through cell	Modified-release drug formulation (poorly soluble actives); soft gelatin capsules

Although the lack of a biorelevant dissolution method to adequately reflect human physiology in QC testing often leads to data that are disconnected from *in vivo* results, thus limiting the value of the testing, QC dissolution has been widely accepted up till now by regulatory agencies.

1.4.4 - Biorelevant dissolution tests

Biorelevant testing aims to mimic the physiological environment of the gastrointestinal tract in an *in vitro* dissolution method. Biorelevant dissolution testing, designed with appropriate simulated media and hydrodynamics, is useful from the early stages of drug discovery and development, where it is used to identify the biopharmaceutical performance of the compound (i.e. solubility problems, food effect, precipitation in the small intestine), through to the later stages of development, where it is used to assist in identifying suitable formulation strategies and to establish *in vitro-in vivo* correlations that will lead to reduction of the number of animal experimentation, bioavailability and bioequivalence studies needed to bring the drug product to market (Fotaki and Vertzoni, 2010b; Grady et al., 2018; Jantratid and Dressman, 2007; Klein, 2010).

Biorelevant dissolution methods are effective in screening drug dissolution behavior in media that model different *in vivo* environments (e.g., gastric, intestinal, and colonic) or those that model the influence of food (e.g. fed vs. fasted state). They can also be helpful in modeling the effects of dosing a drug product to achlorhydric patients or those receiving proton pump inhibitors, or the effect of drug precipitation (Grady et al., 2018).

Biorelevant dissolution methods commonly utilize nonstandard media and experimental conditions, such as physiologically relevant media (e.g. Fasted State Simulating Gastric Fluid (FaSSIF), Fasted State Simulating Intestinal Fluid (FaSSIF), Fed State Simulating Intestinal Fluid (FeSSIF), and so forth), non-sink conditions, biphasic media (i.e. including an immiscible layer such as octanol to mimic permeability), multiple compartmental apparatus to reflect the change in environmental conditions as the dosage form moves through the GI tract, or to mimic a combination of dissolution and drug absorption. (Fotaki and Vertzoni, 2010a; Grady et al., 2018; Stillhart et al., 2019).

1.5 - Physiologically based pharmacokinetic PBPK model

In 1937, Theorel was the first scientist to introduce the concept of physiologically-based pharmacokinetic (PBPK) model (Teorel, 1937). PBPK models are mathematical models defined by a collection of differential equations which describe the mass transport of the drug throughout various organs and tissues in order to adequately reflect the physiological processes to which a drug may be subjected (Lin and Wong, 2017; Miller et al., 2019). While classical compartmental pharmacokinetic models (Figure 5a) lump the organs in pre-determined compartments and simply describe absorption as a first-order process, PBPK models (Figure 5b) differ from this approach by attempting to incorporate physiological processes in the pharmacokinetic description more mechanistically (Miller et al., 2019). In PBPK models, the most important organs are taken in account as individual compartments connected by the blood stream, enabling a description of the how the blood flow carries a drug into and out of specific tissues and organs. In oral PBPK models, the gastrointestinal segment is typically described as a series of compartments through which a drug transits. These models provide an useful approach to translate preclinical absorption data to the humans (Lin and Wong, 2017).

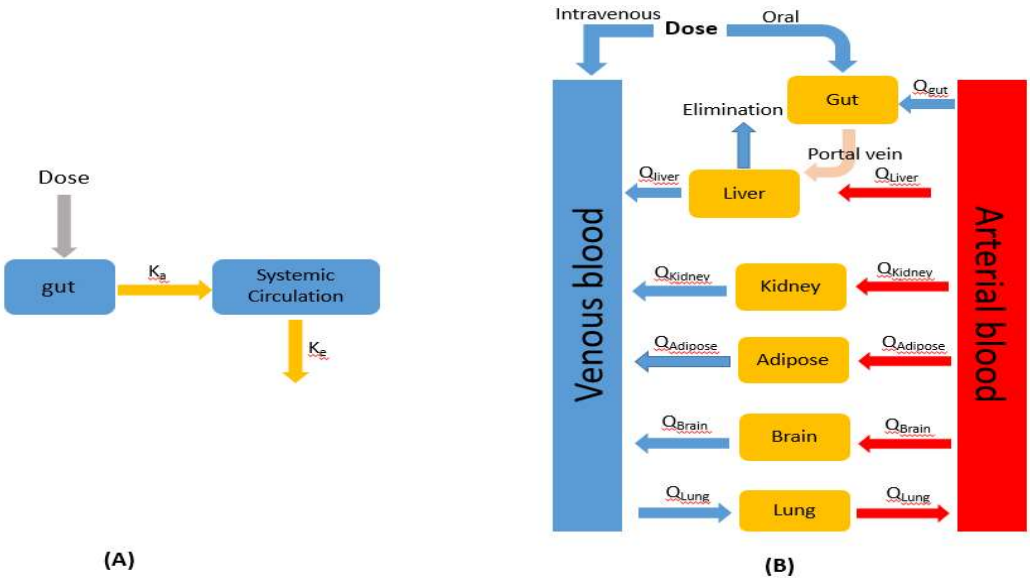


Figure 5. Compartmental model and a mechanistic, physiologically-based pharmacokinetic (PBPK) model. (A) Scheme of compartment model, in this model a drug is inputted into the gastrointestinal compartment, and absorption into the systemic circulation compartment is governed by the absorption rate constant (k_a). The elimination phase it is described by the elimination rate constant (k_e); (B) In the whole-body PBPK model, major organs/tissues are represented by compartments, connected by blood flows (Q). Intravenous (IV) dosing inputs drugs directly into venous blood, whereas oral dosing inputs drug into the gut compartment.

Although the first idea of PBPK model dates back to 1937, it was not until recently that the use of PBPK models in drug discovery research rapidly increased in popularity (Miller et al., 2019). This increase is due to advances in *in silico* software and *in vitro* assays. With the improvement of *in vitro* experiments, more relevant information about ADME process (absorption, distribution, metabolism and excretion) can be obtained.

The use of PBPK modeling in pharmaceutical industry has rapidly expanded in recent times and has been used in sophisticated mechanistic applications to prediction of food effects, drug-drug interactions, pharmacokinetic profiles in healthy and special populations, and the assessment of population variability (Hermans et al., 2017; Paraiso et al., 2019a; Stillhart et al., 2019; Tistaert et al., 2019).

1.6 - Absorption models

1.6.1 - Mixing tank model

The mixing tank (MT) model was introduced by Dressman and Fleisher (1986), in this model the GI tract is considered as a single, well-stirred compartment with mass transfer following linear transit kinetics (Dressman & Fleisher, 1986). This approach was one of the first models to integrate the dissolution and permeation in an oral PBPK model to describe the oral absorption process (Figure 6). Therefore, this was the first model that allowed the characterization of drugs with dissolution rate limited absorption.

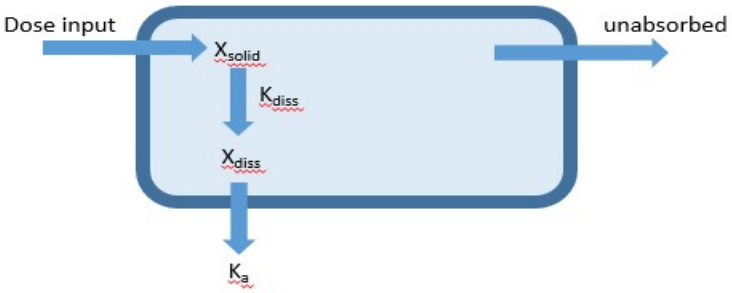


Figure 6. Mixing tank model which represents the gastrointestinal (GI) tract as a single, well-stirred compartment. k_a is the absorption rate constant, X_{diss} is the amount of drug dissolved in the GI tract. k_{diss} is the dissolution rate constant, and X_{solid} is the dose that has been placed into the GI tract. The oral absorption rate is governed by k_a and X_{diss} and the dissolution rate is governed by X_{solid} and k_{diss}.

In terms of hydrodynamics, the MT model assumes that the GI contents are well-stirred with instantaneous dilution of the inputted dose resulting in a uniform distribution of the dissolved and undissolved drug. To handle drug dissolution, the MT model uses a modified Noyes-Whitney equation, while a first order absorption rate constant (k_a) is used to account for drug mass transport through the intestinal mucosa. This parameter can be estimated from effective permeability (P_{eff}) obtained from *in vitro* permeability experiments or from *in silico* structure-based predictions.

The validation of the ability of MT model to investigate the factors limiting drug oral absorption was performed with griseofulvin and digoxin. For griseofulvin, increasing the dissolution rate and gastrointestinal transit time did not have impact on the oral absorption, leading to the conclusion that for this drug the limiting step for absorption is the drug solubility. For digoxin, increasing the dissolution rate by particle size reduction, demonstrated significant improvement in drug absorption, showing that for this drug the dissolution rate was the limiting step to absorption. Therefore, it was shown that the MT model could successfully discriminate between dissolution rate-limited and dose/solubility limited absorption (Dressman & Fleisher, 1986).

Although it provided a break-through in the use of modeling to identify the rate-limiting step to absorption, the MT model did not take other processes that can have impact in the oral absorption into account, such as gut metabolism, hepatic first-pass metabolism, and drug chemical instability. Furthermore, the assumption that the GI tract acts as a single, homogeneous compartment underestimates the heterogeneity of this organ.

1.6.2 - Maximum Absorbable Dose (MAD)

The maximum absorbable dose concept was derived from the MT model. MAD was introduced by Johnson and Swindell (1996) with the purpose of guiding the API particle size specification to reduce the variability of the oral absorption of poorly soluble drugs. (Swindell, 1996).

The MAD equation estimates the maximum amount of a drug that can be absorbed in the gastrointestinal tract during the GI residence time, assuming that this value is reached when the drug reaches its saturation concentration in the gastrointestinal fluid. Because of its simplicity,

the MAD approach enables rapid assessment of the potential extent of oral bioavailability from a small information set (Duxin Sun et. al, 2004). It was an important advance because it combined, in a conceptually simple way, four key factors that impact the extent of absorption: solubility, absorption rate constant, gastrointestinal fluid volume and time available for absorption, as shown in Equation 1:

$$MAD = K_a C_s V \int dt \qquad \text{Equation 1}$$

where K_a is the absorption constant, C_s is the drug solubility, V is the volume of fluid in the GI tract and t is the time available for absorption.

Due to its model, this approach has often been used in the screening of new drug candidates in early stages of pharmaceutical drug development. However, models such as these cannot be used explore more complex circumstances such as pH-dependent oral absorption and food effects (Curatolo, 1998; Swindell, 1996).

1.6.3 – Compartmental absorption and transit model

The compartmental absorption and transit (CAT) model was described by Yu and Amidon (1999). In this model the gastrointestinal tract (GIT) is described as a series of compartments as opposed to a single compartment used by the previous absorption models (Yu and Amidon, 1999).

While a multiple compartment approach had been used previously to describe effects such as gastric emptying, Yu and Amidon (1999) additionally utilized multiple compartments to represent different sections of the small intestine (Figure 7). According to this model the small intestine is divided in seven compartments. The first one is represents the duodenum, the next two by the jejunum and the final four by the ileum (Yu and Amidon, 1999).

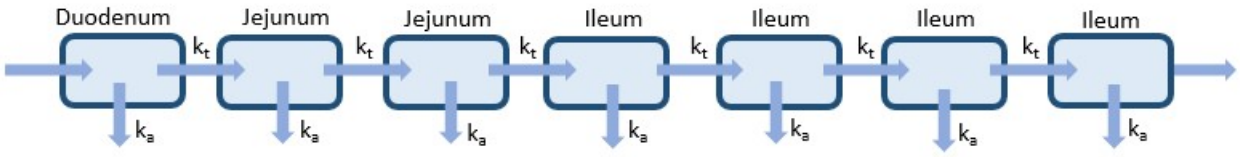


Figure 7. The compartmental absorption and transit (CAT). In this model, seven well-stirred compartments are used to describe absorption and transit through the small intestine.

The transit of a drug through each small intestine compartment in this model is controlled by a transit rate constant (k_t), while the movement of drug through the CAT model can be mathematically represented by the following equation:

$$\frac{dYn}{dt} = k_t \times Y_{n-1} - k_t \times Y_n - k_a \times Y_n \qquad \text{Equation 2}$$

where Y_n refers to the amount of a drug in a specific compartment, Y_{n-1} refers to the amount of a drug in the previous compartment, k_t is the transit rate constant, and k_a is the absorption rate constant.

An advantage of the CAT model is its mathematical simplicity, as a single rate constant (k_t) is used to describe the transit of a drug through different regions of the small intestine. Yu later improved the model making possible to represent the dissolved and undissolved drugs in each compartment (Yu, 1999). In the latter version, a rate constant describing dissolution governs the movement of a drug from an "undissolved compartment" into a "dissolved drug compartment" in each region of the intestine. This addition allowed the CAT model to capture dissolution rate-limited absorption.

The CAT model set the framework for further oral absorption models incorporating additional features and properties (Agoram et al., 2001; Jamei et al., 2009a).

1.6.4 - Advanced Compartmental Absorption and Transit Model (ACAT)

The ACAT model for oral absorption (Figure 8) is an extension of the CAT model and is implemented in the GastroPlus® software platform (Agoram et al., 2001; Lin and Wong, 2017). In this model, there are new compartments describing different drug states: unreleased drug still

in the formulation, undissolved but already released drug, dissolved drug, degraded, metabolized and absorbed drug. Regarding to the gastrointestinal segments, in this model there are seven compartments representing the small intestine, a compartment representing the stomach to allow the incorporation of gastric emptying and a compartment representing the colon to explore the possibility of colonic absorption. Further, assignment of characteristics such as pH variation in each segment, effective surface area, transporter expression, and GI transit time to each specific compartment enables the ACAT model to account for GI heterogeneity (Agoram et al., 2001; Kostewicz et al., 2014; Lin and Wong, 2017; Sager et al., 2015). Finally, gut and liver metabolism were added to the ACAT model, improving predictions of the extent of oral absorption of drugs that undergo significant gut and liver first-pass metabolism, such as propranolol (Lin and Wong, 2017).

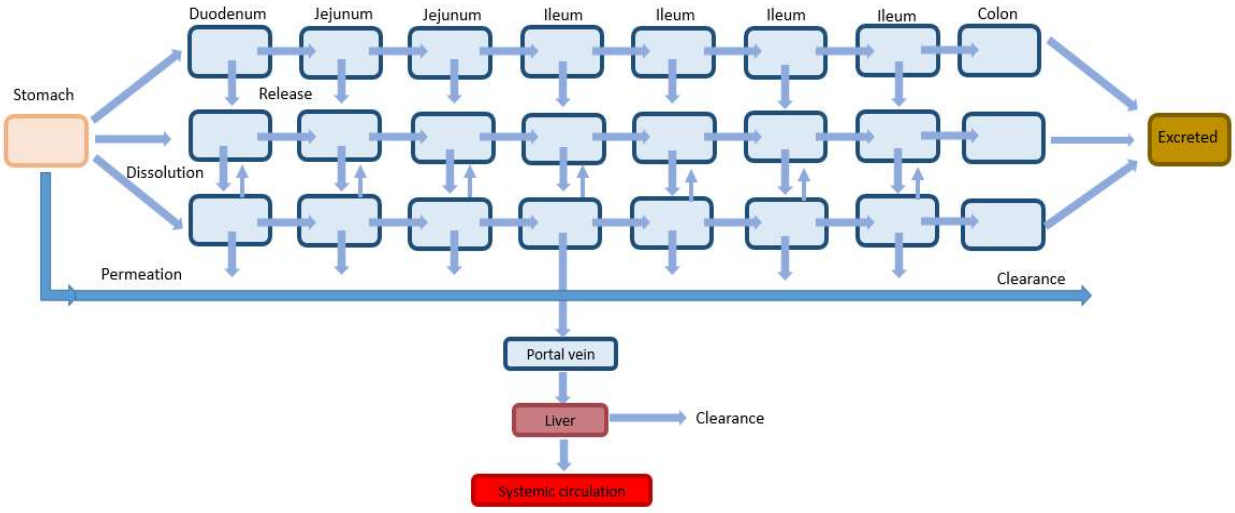


Figure 8. The Advanced Compartment Absorption and Transit (ACAT) model. In this representative ACAT model which it is an extension of CAT model, additional compartments are added to characterize features such as the stomach, colon absorption, drug release from the formulation, and first-pass metabolism in the liver and the gut (shown by the Clearance arrows).

The ACAT platform is built with equations that mostly describe linear kinetics, while to characterize saturable metabolism and carrier-mediated transport, Michaelis-Menten nonlinear kinetics are used (Agoram et al., 2001; Kostewicz et al., 2014). The data related to drug, formulation and physiological parameters can be obtained from *in vitro* experiments, *in vivo* trials or *in silico* predictions.

As such, the ACAT model not only allows for investigation of dissolution-rate limited absorption, but also allows exploration of the approximation GI location of drug release, dissolution, passive and carrier-mediated absorption, saturable metabolism and efflux (Agoram et al., 2001). Furthermore, it provides the possibility to explore the influence of pH, food effect and diseases in the drug absorption (Andreas et al., 2016; Hermans et al., 2017; Paraiso et al., 2019a; Stillhart et al., 2019).

1.6.5 - Advanced dissolution, absorption and metabolism (ADAM)

Like the ACAT model, the ADAM model is an extension of the CAT absorption model. The ADAM model is implemented in the Simcyp® Population-Based Simulator software platform. The background and mathematical principles of the ADAM model structure have been described elsewhere (Jamei et al., 2009b). Briefly, this model divides the GI tract into nine anatomically defined segments to describe the stomach, seven small intestine segments and the colon. The fluid dynamics are integrated into the model as they are fundamental to mechanistically modelling the oral absorption process of the drug following release from the formulation. dissolution, precipitation, (super)-saturation, luminal degradation, permeability, metabolism, active and passive transport and the transit from one segment to the next are all taken into account, as shown in Figure 9 (Darwich et al., 2010; Jamei et al., 2009b; Kostewicz et al., 2014).

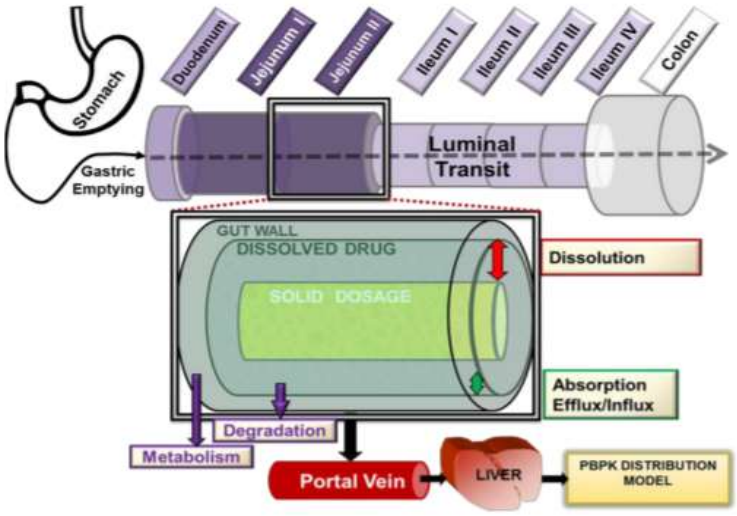


Figure 9. Schematic representation of the Advanced Dissolution, Absorption, and Metabolism (ADAM) model within the Simcyp population based simulator. Reproduce with permission from Pathak et al., 2017 (Pathak et al., 2017).

In the ADAM model structure, the population mean and inter-individual variability of regional luminal pH and bile salt concentrations in the fasted and fed states are taken into consideration. The model assumes that the absorption in the stomach is negligible compared with the small intestine compartments, and that the movement of liquid and solid drug through segments of the GIT is described by first-order kinetics. It is also assumed that drug metabolism in the colon is insignificant (Jamei et al., 2009b).

To better describe dissolution of spherical particles under both sink and non-sink conditions, the diffusion layer model (DLM) developed by Wang and Flanagan is implemented in the ADAM model (Jamei et al., 2009b; Wang and Flanagan, 1999):

$$DR(t) = -NS\frac{D_{eff}}{h_{eff(t)}}4\pi a(t)\big(a(t)+h_{eff}(t)\big)\big(S_{surf}(t)-C_{bulk}(t)\big) \qquad \text{Equation 3}$$

where DR(t) is the rate of dissolution at time t; N is the number of particles in a given particle size bin; S is an empirical scalar; D_{eff} is the effective diffusion coefficient; h_{eff} (t) is the thickness of the hydrodynamic boundary layer at time t; a(t) is the particle radius at time t; C_{bulk}(t) is the concentration of dissolved drug in bulk solution at time t and S_{surf}(t) is the saturation solubility at the particle surface at time t.

The DLM model assumes that there is a hydrodynamic boundary layer around the fine particle of the drug and that diffusion of dissolved drug through this layer into bulk solution is the rate limiting step for the dissolution of drug (Jamei et al., 2009b).

Besides describing the absorption process in formulation development in fasted and fed states and mechanistically describing the influence of drug particle size on dissolution, the model is useful for evaluating the impact of inter- and intra-subject variability in gastrointestinal physiology, pathology and genetic and other sources of variability in intestinal gut wall metabolism and transport.

1.7 - Physiologically Based Biopharmaceutics Models (PBBM)

In the last decade, with advances in the pharmaceutical sciences it has become possible to develop and improve the physiologically pharmacokinetics (PBPK) models which link the human physiology with physicochemical properties of the drug to support drug development and regulatory decisions (Kostewicz et al., 2014; Miller et al., 2019; Stillhart et al., 2019).

While PBPK modeling approaches have become a very important tool to predict and simulate the absorption, distribution, metabolism and elimination (ADME) of drugs, the parameters inputted into the PBPK models to describe the drug and its formulation remained quite basic for a long time. To achieve better prediction of the *in vivo* performance of the drug and its formulation, more detailed information regarding the in the form of a biopharmaceutics analysis is necessary (Miller et al., 2019; Paraiso et al., 2019a; Stillhart et al., 2019).

Recently, the concept of physiologically based biopharmaceutics models (PBBM) has been proposed. With this approach is possible to capture the interaction between the physiology (using PBPK) and the drug formulation by implementation of critical formulation or manufacturing aspects that are relevant to the drug release and dissolution from the drug product. From the moment that these critical elements of formulation are defined, PBBM can be used for formulation development predicting the impact of critical material attributes (CMA) and critical process parameters (CPPs) and, when combined with virtual BE simulations, can make it possible to establish a "safe space" of specifications for the drug product (Grady et al., 2018; Gray, 2018). Therefore, this approach may become very useful to establish the clinical relevance of product quality from the pharmaceutical development through to the regulatory approval

(Grady et al., 2018). Such an approach should be useful to both the pharmaceutical companies, (saving money by limiting the number of *in vivo* studies required for approval), for the regulatory agencies (to support regulatory decisions) and for the patients, as new and better drug products can be brought to the market faster.

1.7.1 - Application of PBBM

The PBBM model approach has many different applications during the course of drug discovery and drug formulation development. Simulations using this strategy in drug discovery step, in which only very limited *in vitro* data are available, can be used to assess the potential bioavailability of drug candidates by providing an *in vivo* context to the available *in vitro* data (Grady et al., 2018; Miller et al., 2019; Stillhart et al., 2019). In the early drug development stage PBBM have been used to estimate the oral absorption and pharmacokinetics of drug candidates in humans. In later stages, when more *in vitro* and some *in vivo* data become available, detailed simulations can be performed using more refined PBBM models to inform decisions about dosage form selection and formulation optimization. Further, these models may be an ideal tool to explore more complex phenomenon such as pH and food effects (Li et al., 2017; Miller et al., 2019; Paraiso et al., 2019a; Rowland et al., 2011; Stillhart et al., 2019).

The applications of PBBM to guide drug form selection and formulation optimization as well as to investigate pH, food effects and to set clinically relevant dissolution specification are of high value for internal pharmaceutical company decisions and may become useful in submissions to regulatory agencies.

1.7.2 - pH and food effect

As mentioned previously, PBBM can be a useful tool to investigate the effect of GI pH and food on oral drug absorption (Paraiso et al., 2019a; Parrott et al., 2020; Stillhart et al., 2019; Tistaert et al., 2019). Of the two phenomena, pH effects are much easier to deal with using PBBM models. As in the absorption model (ADAM or ACAT) the gastrointestinal tract is described as different compartments, a pH effect can be simulated by simply adjusting the pH in the GI compartment of interest (Parrott et al., 2020). For example, to evaluate the effect of higher

stomach pH in patients taking Antacid Reducing Agents (ARAs) it is only necessary to adjust the pH in the stomach compartment. This change of pH basically affects the solubility of the drug in the specific compartment, and the subsequent simulation with the PBBM model will indicate its effect on oral absorption (Amitava Mitra et. al., 2020; Parrott et al., 2020; Tistaert et al., 2019).

Modeling the food effect on drug exposure is more complex. After food intake, various changes in GI transit and gastric pH occur (Welling, 1996). Further, bile secretions from the gall bladder aid in the dissolution and poorly soluble compounds, improving the absorption of these compounds (Glomme et al., 2007). These changes in the gastrointestinal tract can lead to a negative or positive food effect, depending on the drug characteristics. The food effect is interest because on the one hand it can lead to an improvement in oral absorption (positive effect), potentially enabling the administration of a lower dose if given in the fed state, but on the other hand it can lead to a decrease in oral absorption (negative food effect), potentially leading to lack of efficacy of the drug. Therefore, the prognosis of the food effect using PBBM is a very important tool in formulation development.

Despite the high degree of challenge associated with estimating the effect of food on a drug's oral bioavailability, there are many publications in the open literature showing the usefulness of the PBBM approach for this purpose (Abuhelwa et al., 2017; Andreas et al., 2017; Paraiso et al., 2019a; Parrott et al., 2020; Tistaert et al., 2019).

1.7.3 - Setting clinically relevant dissolution specifications

A clinically relevant dissolution specification can be set by comparing *in vitro* dissolution experiment data with *in vivo* clinical (PK) data, and thus establishing an *in vitro in vivo* correlation or relationship (IVIVC or IVIVR). Although it is often not possible to establish a 1:1 correlation of dissolution results with the PK data, by applying a PBBM approach it may be possible to use the dissolution method to guarantee an acceptable clinical performance via a demonstrated PK "safe space" (Grady et al., 2018; Mcallister et al., 2020; Stillhart et al., 2019).

Sometimes the concept of "biorelevant" dissolution methods is confused with the concept of "biopredictive" dissolution methods. On the one hand, a "biorelevant "method represent a set of testing conditions to reflect *in vivo* dissolution by designing the method to closely mimic the relevant biological fluid and physiological environment. This may or may not always result in

the method being biopredictive. On the other hand, a "biopredictive" method is a set of testing conditions for which the *in vitro* dissolution profiles are capable of predicting pharmacokinetic profiles (Grady et al., 2018). These are typically based on classical or mechanistic IVIVC. Therefore, both the QC and biorelevant dissolution methods can be characterized as clinically relevant as long as they can exhibit some elements of IVIVC/R (Cardot et al., 2016). Since biorelevant dissolution experiments are typically more complex and thus more geared to internal decisions during drug formulation, it is desirable to develop simpler but biopredictive QC dissolution tests that can be used day-in, day-out for batch release.

The concept of a clinically relevant dissolution specification (CRDS) was introduced within the context of Quality by Design (QbD) and proposed to further the understanding of process and formulation variations on PK parameters, ultimately assuring that only "good" product is released, and unacceptable product is rejected. The BCS classification of drugs can be useful to develop clinically relevant dissolution method. For BCS I and III drugs, a Q of 80% dissolved in 15 min (BCS III) or 30 min (BCS I) in 500 mL 0.01 N HCl with 50 rpm agitation in the USP Apparatus 2 should be considered clinically relevant without actual *in vivo* data (Grady et al., 2018; Hermans et al., 2017). This relationship is possible because for these highly soluble compounds dissolution in the intestinal fluids it is not the limiting step for the drug absorption. For BCS II and IV compounds, which are poorly soluble, establishing clinical relevance of the dissolution method it is more challenging. Specifications for these drugs should ideally be set using IVIVC to link the *in vitro* dissolution data with one PK study. But it is necessary to run more *in vivo* studies to build this relationship (Hermans et al., 2017; Mcallister et al., 2020). As a solution to this quandary, many authors have proposed the use of PBBM approach to set CRDS. As mentioned before, the PBBM approach can also be beneficial to help mechanistically understand the important factors for formulation performance *in vivo*, which can then be used to guide the development of the *in vitro* dissolution tests (Grady et al., 2018; Hermans et al., 2017; Mcallister et al., 2020; Stillhart et al., 2019).

Therefore, PBBM can nowadays provide a useful approach to link *in vitro* with *in vivo* performance. With the increased adoption of PBBM in recent publications, we expect that best practices in development and verification of these models will be established that will eventually be accepted by the regulatory agencies (Grady et al., 2018; Mcallister et al., 2020; Miller et al., 2019; Parrott et al., 2020; Stillhart et al., 2019).

CHAPTER 2

Objectives of the thesis

2.1 Main objective

The main objective of the doctoral studies was to couple data from *in vitro* experiments with *in silico* tools to describe the *in vivo* behavior of zolpidem immediate release formulation when administered with or without food using a PBBM approach. This case example serves to illustrate the usefulness of the PBBM approach to internal development campaigns in the pharmaceutical industry as well as to help pave the way to acceptance of the approach by regulatory authorities.

2.2 Specific objectives

- Chapter 3 illustrates the use of *in vitro* (biorelevant dissolution) and *in silico* tools to better understand the *in vivo* behavior of the immediate release (IR) formulation of zolpidem. For this, a PBBM model for zolpidem was established. Then, the dissolution behavior of immediate release zolpidem formulations in biorelevant media simulating the fasting and fed states was integrated into the model.
- Chapter 4 describes the creation of a PBBM/PD model for zolpidem in Simcyp® using dissolution, pharmacokinetic and pharmacodynamic data, which was then used to establish a "safe space" for the dissolution of zolpidem from the commercial immediate release (IR) formulation.

CHAPTER 3

In vitro-in silico investigation of the negative food effect of zolpidem when administered as immediate release (IR) tablets.

3.1 - Introduction

It is important to gauge the impact of the co-administration of drug and food during the drug development stage because interactions between food and the drug can lead to variation in the plasma concentration and subsequently in the therapeutic effect of the drug. In general, food can have a positive effect (higher plasma levels), negative effect (lower and/or later plasma levels) or no significant effect on drug absorption. In some cases, a positive food effect can lead to drug toxicity but in other cases this effect is necessary to improve the absorption of the drug to achieve the therapeutic effect. On the other hand, a negative food effect may increase the risk of treatment failure, due to insufficient concentration at the site of action (Fleisher et al., 1999).

In the literature several categories of food-drug interactions are described. For example, food can have impact on drug exposure due to inhibition of the cytochrome P450 enzymes that are responsible for the metabolism of drugs, resulting in an increase in the systemic exposure of the drug administered. Other examples are by delaying the gastric emptying time, which can lead to a slower absorption rate, and by increasing the blood flow in the gut and liver which can lead to more efficient absorption of the drug (Burggraaf et al., 1996; Fleisher et al., 1999; Schmidt and Dalhoff, 2002; Singh, 1999). Furthermore, food effects may be mediated physicochemically by the interaction between the food and drug formulation, for example, an increase in gastric pH induced by food ingestion may change the rate of release of drug from the dosage form (Greenblatt et al., 2001; Hanley et al., 2011). Likewise, improvement of the drug solubility due to the increased bile secretion in response to food ingestion may lead to improved absorption for drugs with poor solubility. Yet other examples include a potential negative impact of higher luminal viscosity on disintegration of the drug formulation, complexation of the drug with food components or catalysis of drug degradation by food components, all of which can lead to a reduction in the amount of drug available for absorption (Radwan et al., 2012).

The biopharmaceutical classification system (BCS) proposed by Amidon and co-workers (1995) has been a useful tool to predict food effect in the drug absorption. (Amidon et. al., 1995). Usually, for drugs that belong to BCS class I, which exhibit high solubility and high permeability, food intake is not expected to induce changes in the extent of absorption because the solubility and dissolution of the drug are not the limiting step for drug absorption (Fleisher et al., 1999). Nevertheless, the slower gastric emptying time in the fed state may lead to some drugs

belonging to this class exhibiting a lower peak concentration (C_{max}) of the drug, reflecting a decrease in the absorption rate or even a reduction in the amount absorbed (Andreas et al., 2017; David J Greenblatt et al., 2013; Rostami-Hodjegan, 2002; Souliman et al., 2006; Stillings et al., 2000).

According to the BCS and Developability Classification System (DCS), zolpidem is described as a class I drug (Colo et al., 2015). For drugs which are used in treatment of sleep disorders, like zolpidem, a fast onset of action is highly desirable (Greenblatt, 2010). As food intake can have an impact on the gastric emptying time, the rate and potentially the extent of absorption of drugs with short elimination half-lives, such as zolpidem, can be decreased if the drug product is administered in the fed state, leading to treatment failure (Eller and Della-Coletta, 1990; Greenblatt et al., 1978; Locniskar et al., 1984; Ms et al., 2007). In particular, for zolpidem the labeling of the commercial product describes zolpidem as having a negative food effect. This applies to the immediate release (IR), modified release (MR) and sublingual (SL) formulations of zolpidem (Andreas et al., 2017; FDA, 2007; Paraiso et al., 2019).

Typically, it is difficult to predict if the co-administration of drug with food will have impact on the rate and extent of drug absorption, since the interactions of drug and food are manifold (Welling, 1996). However, more recent publications have supported the use of biorelevant media in dissolution experiments coupled with PBBM models to simulate the effect of food intake on the plasma profile. In this approach, the physicochemical properties of the drug together with biorelevant dissolution data and physiological parameters are combined with PBPK modeling to predict the plasma profile of the drug. The objective of this chapter was thus to use PBBM modeling to describe the *in vivo* behavior of immediate release tablets of zolpidem and to explain the negative food effect of this drug.

3.2 - Materials and methods

3.2.1 – Materials

Zolpidem active pharmaceutical ingredient (API) and 10 mg immediate release (Stilnox®, Sanofi Aventis, France) film-coated tablets were obtained commercially (Lot #70662). FaSSIF, FeSSIF & FaSSGF Powder® (Lot #01-1512-05-NP) and FaSSIF-V2 Powder® (Lot no. 03-1610-02) were kindly donated by biorelevant.com (Surrey, United Kingdom). Glyceryl monooleate (GMO) was also donated to the Goethe University by biorelevant.com. Lipofundin® MCT 20% was purchased from B. Braun (Melsungen, Germany). Methylhydroxypropylcellulose (HPMC, Hypromellosum E4M Prem) was purchased from Fagron GmbH & Co. KG (Barsbüttel, Germany). Maleic acid, sodium dihydrogen phosphate dyhydrate, dodecyl sulfate sodium, acetonitrile and methanol HPLC grade were obtained from Merck KGaA (Darmstadt, Germany), while sodium acetate trihydrate, sodium chloride, potassium dihydrogen phosphate, sodium hydroxide, di-sodium hydrogen phosphate dodecahydrate, tris-(hydroxymethyl) aminomethane, D(+)-glucose, acetic acid 100%, hydrochloric acid 37%, hydrochloric acid 1 N, orthophosphoric acid 85% and sodium hydroxide 1 N were purchased from VWR (Leuven, Belgium) and were of analytical grade. Sodium oleate (N 82% fatty acids) was obtained from Sigma-Aldrich (Steinheim, Germany).

3.2.2 - *In Vivo* Studies

To verify the influence of food in the pharmacokinetic parameters of zolpidem a clinical study was performed in 30 healthy, male volunteers under fasting and fed state conditions conducted by Sanofi-Aventis. To assist the set-up and verification of the PBBM model, the company kindly provided the study protocol, the mean pharmacokinetic profiles and the summary statistics for the pharmacokinetics. The clinical study was performed in accordance with good clinical practices and the declaration of Helsinki.

The study was conducted in an open-label, 2x2 cross-over study design with a wash-out period of one week. The subjects received a zolpidem (Stilnox®) 10mg immediate release tablet ingested together with 240 mL of water either in the fasted state or 20 min after consuming an

evening meal, which consisted of a roast beef sandwich on whole wheat bread, a banana and skim milk. The tablets were administered at 10 p.m. and, because of the hypnotic effect of the drug, the subjects were asked to assume a supine position in preparation for sleep and remain so until the following day at 10 a.m. The pharmacokinetic profile of zolpidem was determined by collecting blood samples at pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12h post dose.

3.2.3 - Biorelevant Dissolution Tests

The compositions of the biorelevant dissolution media used for this work to simulate the fasted and fed states are summarized in Tables 2 and 3, respectively. To better simulate the gastrointestinal environment, the Level II biorelevant media contain bile components, dietary lipids and lipid digestion products, as well as osmolality adjusting agents (Markopoulos et al., 2015b). Table 3 depicts the Level II dissolution media used to simulate the stomach environment in different periods after food ingestion i.e. $FeSSGF_{early}$ (representing the gastric environment in the first 75 minutes after ingestion of the meal), $FeSSGF_{middle}$ (75-165 min), $FeSSGF_{late}$ (165 min and longer after meal ingestion), as well as FeSSIF-V2, simulating the upper intestine in the fed state. The dissolution media simulating fasted and fed state were prepared according to previously published procedures (Markopoulos et al., 2015b).

Biorelevant dissolution tests were performed using calibrated USP 2 dissolution test apparatus (Erweka DT 80, Heusenstamm, Germany) at 37 ± 0.5° C in 500 mL of each medium. To simulate the fasted state release was investigated at 50 rpm, while the fed state testing (see media in Table 2) was conducted at 75 rpm.

Table 2. Composition and physicochemical characteristics of Level II biorelevant media for simulating the environment of gastric and the small intestine in the fasted state.

Gastrointestinal segment	Stomach	Intestine (proximal gut)
Biorelevant media	Level II FaSSGF	Level II FaSSIF – V2
HCl	q.s. pH 1.6	-
Maleic acid (mM)	-	19.1
NaOH (mM)	-	34.8
Sodium taurocholate (mM)	0.08	3
Sodium chloride	-	-
Lecithin	0.02	0.2
Sodium oleate	-	-
Sodium chloride	34.2	68.6
Osmolality	121	180
Buffer capacity (HCl) [(mmol/l)/ΔpH]	n.a.	10
pH	1.6	6.5

Table 3. Composition and physicochemical characteristics of Level II biorelevant media for simulating the environment of the stomach and the small intestine in the fed state.

Gastrointestinal segment	Stomach			Proximal Intestine
Biorelevant media	Level II $FeSSGF_{early}$	Level II $FeSSGF_{middle}$	Level II $FeSSGF_{late}$	Level II FeSSIF – V2
Acetic acid (mM)	-	18.31	-	-
Sodium acetate (mM)	-	32.98	-	-
Orthophosphoric acid (mM)	-	-	5.5	-
Sodium dihydrogen phosphate (mM)	-	-	32	-
Maleic acid (mM)	47	-	-	71.9
Sodium hydroxide (mM)	-	-	-	102.4
Lipofundin / buffer	17.5 : 82.5	8.75 : 91.25	4.38 : 95.62	-
Sodium taurocholate (mM)	-	-	-	10
Lecithin (mM)	-	-	-	2
Glyceryl monooleate (mM)	-	-	-	5
Sodium oleate (mM)	-	-	-	0.8
Sodium chloride (mM)	270.1	181.7	127.5	125.5
Osmolality (mOsm/kg)	559	400	300	390
Buffer capacity (HCl) [(mmol/l)/ΔpH]	21	25	25	25
pH	6.4	5.0	3.0	5.8

The experimental conditions used to evaluate the effect of fat content and viscosity on release are shown in Tables 4 and 5, respectively (see Sections 3.2.3.1 and 3.2.3.2). Sampling for all dissolution tests was performed at 5, 10, 20, 30, 40, 50, 60, 120 and 180 min. Due to the

slow drug release in the study with high viscosity, additional sampling times of 4 and 6 h were necessary to determine the extent of release.

3.2.3.1 - Biorelevant dissolution to investigate the effect of pH and fat on release of the drug

To evaluate the effect of pH and fat content added in the FeSSGF Level II media on the release profile of zolpidem from the formulation, different percentages of fat were added to the dissolution media, as described in Table 4. In order to simulate the fat content in the stomach after food ingestion, Lipofundin® MCT 20, an emulsion used for parenteral nutrition, was used in various proportions of Lipofundin®/buffer to represent various periods after meal ingestion (Markopoulos et al., 2015b). The concentration of the drug in the Lipofundin®/buffer samples was determined by filtering the sample through a 1.2 μm cellulose acetate syringe filter (Minisart NML, Sartorius, Goettingen, Germany) and centrifuging the filtrate at 14,000 rpm for 10 min. 0.5 mL of supernatant was then diluted with 1 mL of acetonitrile or methanol prior to HPLC analysis.

Table 4. Experimental conditions to simulate the effect of pH and high fat on release of the drug.

Biorelevant media	0.88% fat	1.75% fat	3.5% fat	7.5% fat
$FeSSGF_{late}$ pH 3.0	x	-	-	x
$FeSSGF_{middle}$ pH 5.0	-	x	-	x
$FeSSGF_{early}$ pH 6.4	-	-	x	x

3.2.3.2 - Biorelevant dissolution to investigate the effect of HPMC and pH on release of the drug

To evaluate the effect of the increase in viscosity of the gastric contents following ingestion of a meal on the release profile of zolpidem, HPMC was added in two concentrations 0.5% with viscosity of 20cP and 1.4% viscosity of 120cP (see Table 5), as suggested by Langguth and co-workers (2012) (Langguth et al., 2012). To prepare the higher viscosity media, the proposed amount of HPMC was dispersed in one third of the amount of purified water

needed for media preparation, preheated to 80 ◦C and then, after cooling, sufficient water was added to the HPMC dispersion to make it to volume (Radwan et al., 2012).

Table 5. Experimental conditions to simulate the effect of pH and high viscosity (HPMC) on release of the drug.

Percentage of HPMC added to the media (%)	**0**	**0.5**	**1.4**
$FeSSGF_{late}$ pH 3.0	-	x	x
$FeSSGF_{middle}$ pH 5.0	-	x	x

To determine the concentration of zolpidem in the dissolution samples, these were filtered through a 0.45µ MPTFE filter (ReZist™ 30 syringe filter) and subsequently diluted with mobile phase prior to HPLC analysis.

3.2.4 - Analysis of Samples

The samples were prepared as described in sections 3.2.3.1 and 3.2.3.2 and the concentration of zolpidem was determined by using HPLC (Hitachi Chromaster, Hitachi Ltd., Tokyo; SpectraSystem, ThermoQuest Inc., San Jose, USA). The analyses were run on a PurospherStar C18®, 5 µm, 150 × 4.6 mm column (Merck KGaA, Darmstadt, Germany). Zolpidem was analyzed using a mixture of 60% acetonitrile, 40% water and 0.01% diethylamine as the mobile phase, at a flow rate of 1.5 mL/min. The sample absorbance was measured with a UV detector at a wavelength of 245 nm. The limits of detection (LOD) and quantification (LOQ) were 0.03 µg/mL and 0.09 µg/mL, respectively.

3.2.5 - PBBM model approach

A PBBM model was built using the Simcyp® Simulator (V18.1; Certara, Sheffield, UK) adopting the parameters shown in Table 6. Physicochemical and most of the pharmacokinetics properties of zolpidem were taken from the literature publications specified in the Table.

To describe the dissolution and absorption process, the Advanced Dissolution, Absorption and Metabolism (ADAM) model was used. The structure, i.e. compartment and equations, of the ADAM model has already been described in detail in the literature. (Cristofoletti and Dressman, 2014; Darwich et al., 2010; Jamei et al., 2009b).

To input the dissolution profile into the ADAM module there are three different ways. The first possibility is to input the dissolution profiles simulating the gastric and small intestine environment directly into the platform, the second is to use a Weibull function to fit the data. These two options are considered more empirical because the model will just fit the inputted data. The third option, considered to be more mechanistic, is to enter the solubility, dissolution data and formulation information into the diffusion layer model (DLM) function. The DLM model, which takes into account drug solubility, particle size, bile-micelle partition, and a number of other particle-related parameters, is especially useful for drugs that have solubility problems, namely those assigned to BCS class II and IV.

Since zolpidem is a BCS I class drug with high solubility and permeability, it is not expected to have solubility problems anywhere in the gastrointestinal tract. Thus, for this work, we used the first option, directly entering the dissolution profile into the respective gastrointestinal segments. Thus, the dissolution profiles were entered separately into the stomach (e.g. $FeSSGF_{middle}$) and the small intestine (e.g. FeSSIF-V2) segments.

Simulation of the plasma profiles after oral administration of Stilnox ® 10 mg IR tablets was run with a „middle-out"-approach using pharmacokinetic disposition parameters derived from *in vivo* data and *in vitro* studies (e.g. gut wall permeability, enzyme kinetics and solubility) available in the literature and from dissolution experiments under standard biorelevant conditions (Shebley et al., 2018). The zolpidem elimination phase was described by information about specific CYP enzymes derived from studies with human liver microsomes (HLM) (Von Moltke et al., 1999; Weinling et al., 2006b).

Zolpidem is considered to have low to moderate volume of distribution (V_{ss}) with a value of 0.54 L/kg. Therefore, the minimal PBPK model structure was utilized to predict its plasma profile. Additionally, the unbound fraction of 8% in plasma, reported by Durand et al. (1992), was applied in the model (Durand et al., 1992).

To best match the *in vivo* food effect studies, all simulations represented in this work were carried out using the Simcyp male Healthy Volunteer population in the fasted or fed state as described in Table 6.

Table 6. Parameters used for the zolpidem PBBM model (Simcyp Simulator®V18.1).

Parameters	Value	Reference/Comments
Molecular weight (g/mol)	307.39	Salvà and Costa, 1995
LogP	2.42	Salvà and Costa, 1995
Compound type	Monoprotic base	Salvà and Costa, 1995
pKa	6.16	Salvà and Costa, 1995
Absorption	ADAM model	
Peff	6.5×10^{-4} cm/s	Andreas et al., 2017
Distribution	Minimal PBPK model	
Main binding protein	Albumin	Chetty et al., 2014
Vss (L/kg)	0.68	Chetty et al., 2014
Fu gut	0.035	predicted
Blood to plasma coefficient (B:P)	0.76	Salvà and Costa, 1995
Fu plasma	0.08	Durand et al., 1992
Elimination	Enzyme kinetics	
HLMs (µL/min/pmol enzyme)		Von Moltke et al., 1999
Clint CYP 3A4	0.217	Von Moltke et al., 1999
Clint CYP 2C9	0.147	Von Moltke et al., 1999
Additional Clint	1.460	Von Moltke et al., 1999
CLR (renal clearance) (L/h)	0.18	
Formulation option in the software	Immediate release (IR) with piecewise cubic polynomial interpolation of the observed *in vitro* dissolution data	Default function in Simcyp®
Population modeling	Virtual population trials were conducted using 300 (30x10) male healthy volunteers, age ranged 18-45 years for each study.	FDA approval document
Transit times in ADAM	0.4 h for fasted state mean GET and 1.0 h for fed state mean GET with 38% CV for population in both states. Other GI transit times were also set at the default values	Default values in Simcyp®

A schematic of the steps used to create the model is shown in Figure 10.

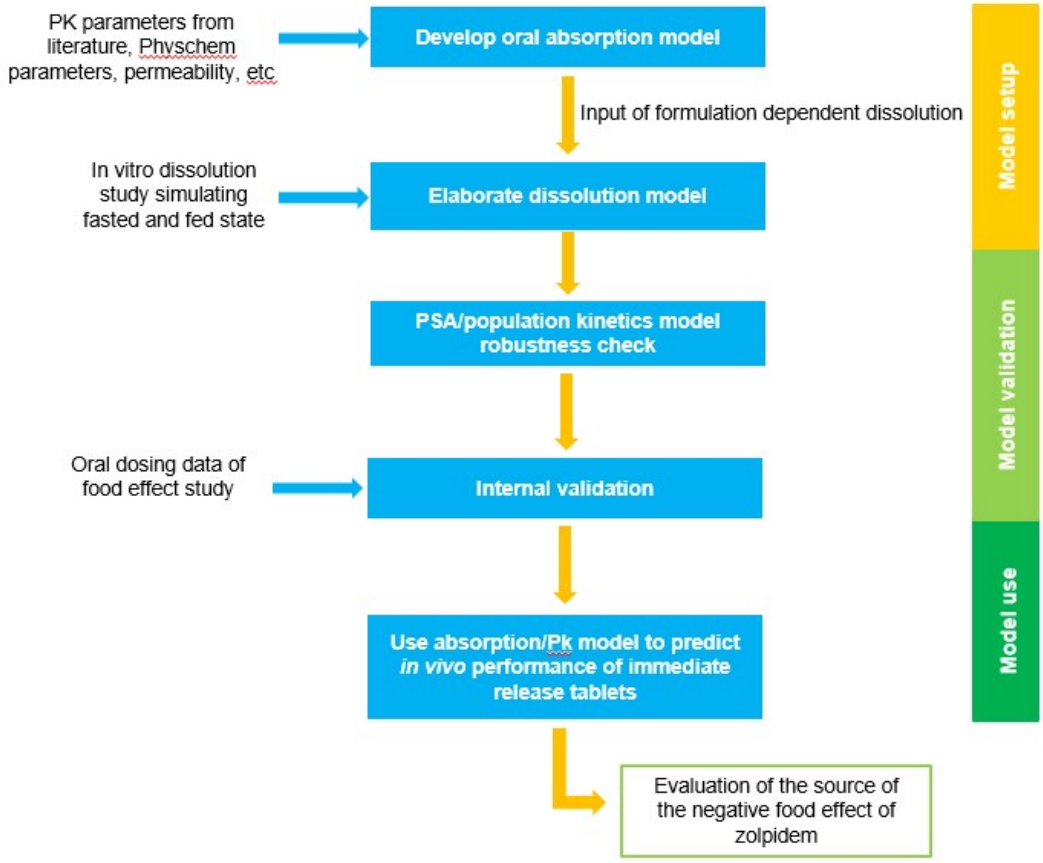


Figure 10. PBBM modeling strategy.

3.2.6 - Data Presentation and Statistical Analysis

The dissolution experiments were performed at least in triplicate ($n \geq 3$) and the data are presented as the arithmetic means with standard deviations. The *in vivo* plasma profile data from the food effect studies are presented as means, as no statistics on the variability of the plasma concentrations at each sampling point were available in the Sanofi archives. However, the coefficients of variation for the pharmacokinetic parameters were located and are reported along with the means. The simulated (predicted) plasma profiles using Simcyp® software were compared with *in vivo* (observed) profiles using the average fold error (AFE) and absolute

average fold error (AAFE) (Obach et al., 1997; Poulin and Theil, 2009), whereby the AFE is defined as:

$$AFE = 10^{\frac{1}{n}\sum i \frac{predicted_i}{observed_i}} \quad \text{Equation 4}$$

where n is the number of time points at which the concentration was obtained, and $predicted_i$ and $observed_i$ are the predicted and observed concentrations at a given time point i. The average fold error indicates whether the predicted profile underestimates or overestimates the observed values (as shown in Eq. 4): if the AFE < 1 there is an underestimation and if the AFE > 1 there is an overestimation of the observed value.

The absolute average fold error quantifies the absolute error from the true value (as shown in Eq. 5). If AAFE ≤ 2, the simulation is usually considered acceptable. (Obach et al., 1997; Poulin and Theil, 2009).

$$AAFE = 10^{\frac{1}{n}\sum \left| log \frac{predicted_i}{observed_i} \right|} \quad \text{Equation 5}$$

3.3 - Results

3.3.1 - *In vivo* studies

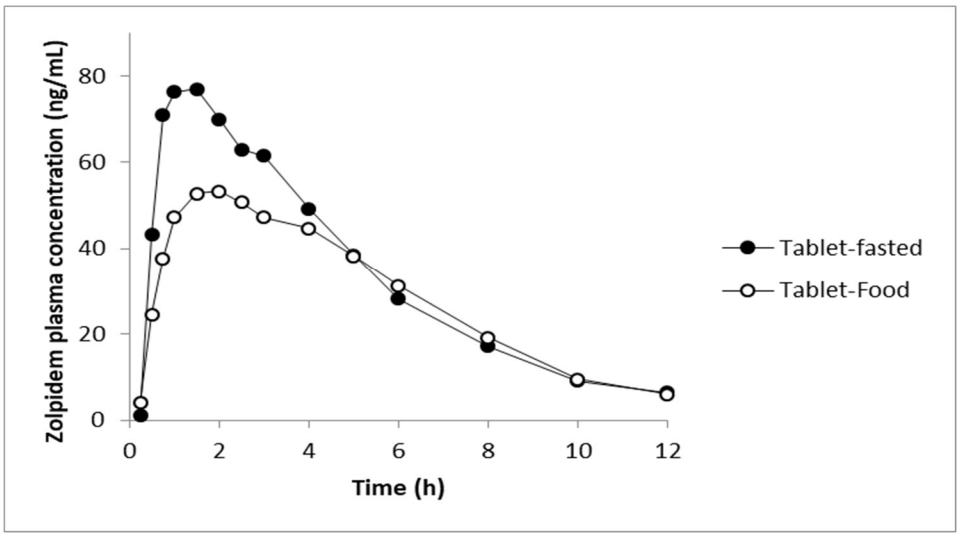


Figure 11. Mean plasma zolpidem concentration after oral administration of 10 mg immediate release Stilnox® under fasting and fed conditions. Reproduced with permission from Paraiso et al. (2019).

Figure 11 depicts the average plasma profiles obtained from food effect studies which zolpidem 10 mg Stilnox® immediate release tablets was administered in 30 healthy male volunteers. The summary statistics for the pharmacokinetic parameters from the study are presented in Table 7. According to the mean profiles obtained, there was a reduction in the values for both AUC and C_{max} and a 60% increase in the time to reach the maximum concentration (T_{max}) was observed in the fed state compared with fasted state administration. The fed/fasted ratios for AUC and C_{max} were 0.86 and 0.76, respectively, showing that in the fed state, AUC and C_{max} were decreased on the average by ~15% and 25%, respectively compared to the fasted state.

Table 7. Summary of descriptive statistics (CV%) of zolpidem PK parameters after a single oral administration of zolpidem 10 mg immediate release (Stilnox®) under fasted and fed conditions.

	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-12h} (ng/mL.h)	Food effect (Fed/Fasted) T_{max}	C_{max}	AUC_{0-12h}
Fasted Mean (CV%)	1.36 (66)	100.1 (42)	398.9 (56)	-	-	-
Fed Mean (CV%)	2.16 (72)	75.5 (43)	344.0 (52)	1.59	0.76	0.86

In Figure 11, it is possible to see that the shape of the mean plasma profile of Stilnox® 10 mg changes significantly between the fed and fasted states. As zolpidem belongs to BCS class I (i.e. is highly soluble and permeable) and has a short half-life (2.4h), the decrease in C_{max} when given with food is unlikely to result from a change in solubility or permeability. One possibility is that the decrease in this parameter is due to the slower gastric emptying rate after food ingestion. The high coefficient of variation for the PK parameters observed in both the fasted and fed arms of the study (Table 7) is in accordance with the broad inter-individual variation in gastric emptying time in healthy volunteers (Andreas et al., 2017; Fleisher et al., 1999; Kaniwa et al., 1988; Koziolek et al., 2014, 2013).

The clinical study was conducted exclusively in healthy male subjects, which is worth noting because previous clinical studies have shown that women tend to exhibit higher zolpidem exposure than men. Interestingly, a gender effect on the pharmacokinetics parameters of zolpidem has been reported not only for immediate-release tablets but also for other types of zolpidem formulation, such as sublingual and modified release tablets (Ambien® CR).(Andreas et al., 2017; David J Greenblatt et al., 2013; David J. Greenblatt et al., 2013; Olubodun et al., 2003)

3.3.2 – Dissolution experiments used to build the PBBM model for zolpidem in the fasted state

3.3.2.1 - Fasted state dissolution

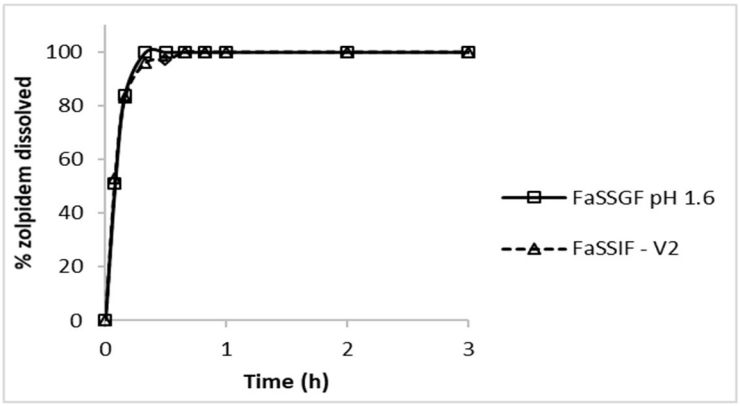


Figure 12. Mean dissolution profiles of 10 mg immediate release Stilnox® tablets in biorelevant Level II dissolution media simulating the fasted state using USP apparatus II. Error bars lie within the symbols in all cases. Reproduced with permission from Paraiso et al. (2019).

Figure 12 depicts the dissolution profile of 10 mg Stilnox® tablets in biorelevant media simulating the gastric (FaSSGF) and upper small intestine (FaSSIF-V2) environment in the fasted state. The rate and extent of dissolution rate was almost superimposable in the two media, indicating that the dissolution of zolpidem tablets is expected to be similar in these two regions of the gastrointestinal tract.

3.3.2.1.1 – Simulation of the observed PK data using the fasted state PBBM model

In order to simulate the plasma profile of zolpidem in fasted state, the PBBM model in Simcyp® was built using the biopharmaceutical, physicochemical and pharmacokinetic drug properties listed in Table 6 and the data obtained from the biorelevant dissolution experiments. The software default values for a healthy, male volunteer population in the fasted state were chosen and the simulation was run twenty times to estimate the 5^{th} and 95^{th} percentiles as well. Figure 13 depicts the predicted and observed mean plasma concentration profiles of zolpidem in the fasted state along with the percentiles.

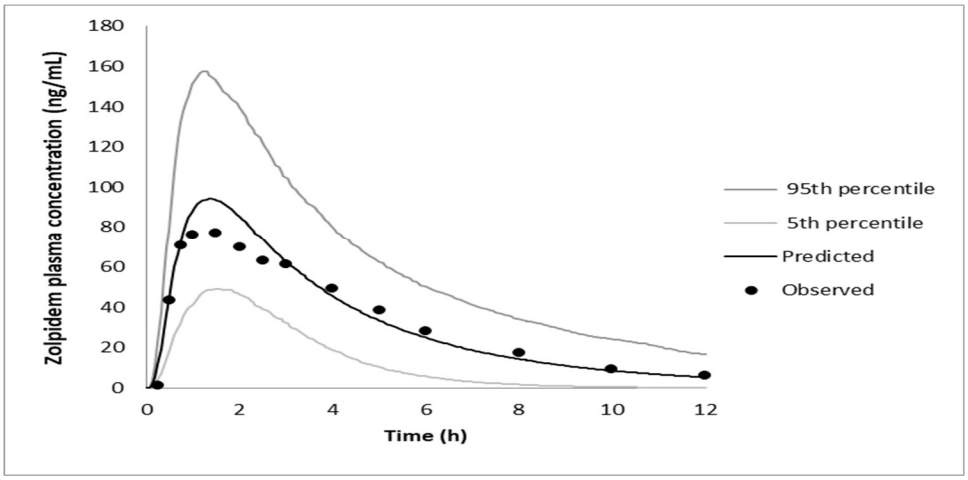


Figure 13. Simulated (mean, 5th percentile and 95th percentile) and observed mean plasma zolpidem concentrations after zolpidem administration in the fasted state. Reproduced with permission from Paraiso et al. (2019).

Table 8. Pharmacokinetic parameters of zolpidem for the food effect study with the corresponding simulation results using in vitro data input from biorelevant dissolution tests simulating the fasted state.

Pharmacokinetic parameters	**Simulated – FaSSGF and FaSSIF-V2 media**	**Observed – fasted**	**% difference simulated - observed**	**AFE/AAFE**
C_{max} (ng/mL)	94.2	100.1 (42)	-5.88	
AUC_{0-12} (ng.h/mL)	404.7	398.9 (56)	1.45	
T_{max} (h)	1.35	1.36	0.73	
AFE	-			1.01
AAFE	-			1.64

Table 8 summarizes the calculated PK parameters (T_{max}, C_{max} and AUC), along with AFE and AAFE values. The results show that dissolution in FaSSGF and FaSSIF-V2 provided an appropriate simulation of the *in vivo* behavior of zolpidem formulation in the fasted state: the observed mean plasma profile and the PK parameters (T_{max}, C_{max} and AUC) lie well within the profiles simulated on the basis of the biorelevant dissolution experiments, taking into account the variability in physiology in this population. Furthermore, the AFE and AAFE values lie well under 2.

3. 3.2.1.2 – Parameter sensitivity analysis (PSA) to determine the effect of gastric emptying time (GET) in the zolpidem PK profile.

To explore whether the gastric emptying time (GET) has an impact on the plasma profile of zolpidem when is administered in the fasted state, a parameter sensitivity analysis (PSA) was conducted. The GET values were allowed to float over the range of 0.1–2 h, which corresponds relatively to the duration of gastric motility cycle in the fasted state. The results are shown in Figure 14.

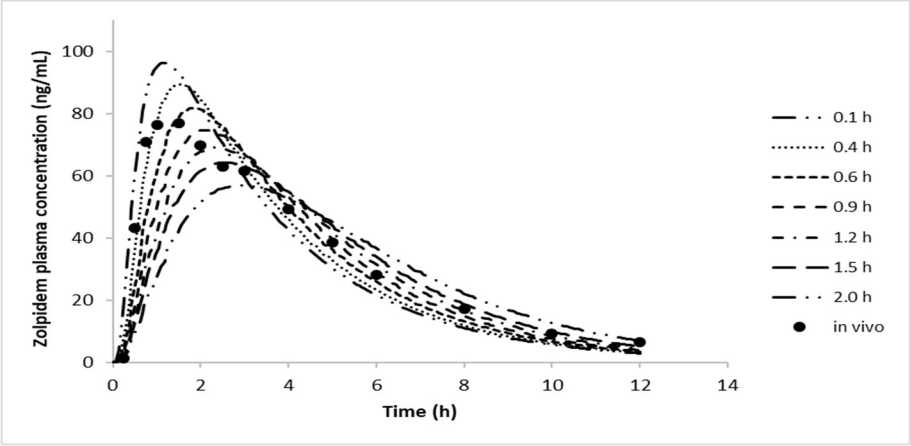


Figure 14. Zolpidem pharmacokinetic profile, applying PSA on GET in the fasted state. Reproduced with permission from Paraiso et al. (2019).

The results show that at longer gastric emptying times, the C_{max} and AUC are reduced and T_{max} is prolonged. The outcomes are in line with the general performance of BCS class I drugs with short elimination half-lives administered as immediate release dosage forms. After the simulations, it was observed that when the GET was set to values of 0.6 or 0.9, the values of C_{max} came close to the observed in the clinical study, however, for T_{max} the value was best simulated when the GET was 0.1h. These simulations suggest that, although GET has a large influence on the plasma profile, factors other than GET may also play a role in zolpidem absorption. A comparison of the range of concentrations reached over this large variation in gastric emptying with the 5^{th} and 95^{th} percentile curves is shown in Figure 13. The results, which reflect the overall variability in the physiological parameters of the subject population, also

suggest that inter-individual variations in additional aspects of the GI physiology may also influence the food effect.

3.3.3 - Biorelevant dissolution to build the PBBM model for zolpidem in the fed state

In order to simulate dissolution conditions in the postprandial stomach, Lipofundin® MCT 20, was added to the media in various concentrations and the pH was also adjusted to reflect the changes of stomach pH after food intake.

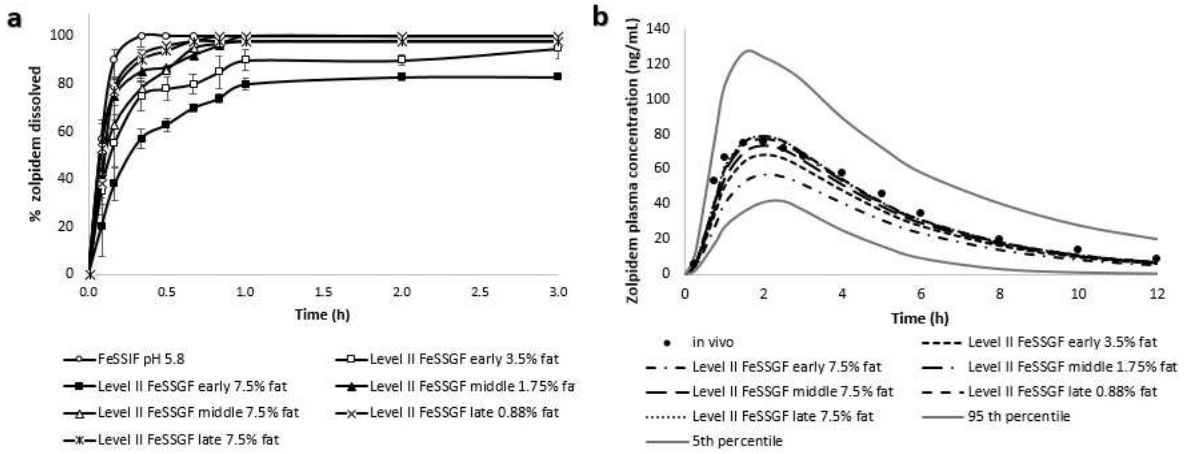


Figure 15. Mean (± standard deviation) dissolution profiles of 10 mg immediate release Stilnox® tablets in biorelevant Level II dissolution media representing the fed state to explore the effect of fat and pH on drug release, using USP apparatus II. Simulated and observed plasma concentration profile of zolpidem using different biorelevant dissolution profiles. The 5th and 95th percentiles are for the simulations with $FeSSGF_{middle.}$ Reproduced with permission from Paraiso et al. (2019).

To investigate the influence of fat concentration on zolpidem release from the tablets, further tests were realized for all three FeSSGF versions at a higher (7.5%) fat content. Figure 15 depicts the dissolution profiles of Stilnox® tablets in the media simulating the gastric environment in the different levels of FaSSGFs, with and without increasing the fat amount to 7.5%.

Of three different FeSSGF versions, the dissolution of zolpidem was slowest in $FeSSGF_{early}$ (pH 6.4 and 3.5% fat). Increasing the fat amount to 7.5% in this version of FeSSGF slowed the dissolution rate even more. The dissolution rate of zolpidem from the Stilnox® tablets was highest in $FeSSGF_{late}$ (pH 3.0 and 0.88% fat) and increasing the fat content from

0.88% to 7.5% in this medium made little or no difference to the rate of dissolution in this case. The results for $FeSSGF_{middle}$ fell between those in the early and late media.

3.3.3.1 – Simulation of the observed data using the fed state PBBM model

3.3.3.1.1 - Simulation using variations in the level of pH and fat content

In order to simulate the plasma profile after food ingestion, the same structural model as used for predicting the fasted state was utilized. All parameters (Table 6) were kept constant to simulate the plasma profiles observed in the fed state, except that 1) the dissolution data obtained under conditions representing the fed state were applied, and 2) the default values for the Simcyp healthy male population in the fed state were implemented.

Input of the data obtained from dissolution test in the early and late version of FeSSGF into the PBBM model (Figure 15b) resulted in a generally good fit of the observed mean fed state plasma profile. The only exception was that the results from the early FeSSGF medium at the higher fat content of 7.5%, resulted in a simulation which was lower than the other simulated profiles. The pharmacokinetic parameters (C_{max}, AUC and T_{max}) from the simulations were used to calculate the AFE and AAFE. The values are presented in the following table.

Table 9. Pharmacokinetic parameters of zolpidem from the bioavailability and food effect studies with the corresponding simulation results using in vitro data input from experimental conditions to simulate the effect of pH and high fat dissolution experiments.

Media	Pharmacokinetic parameters	Parameter value predicted from fed state media	Observed		% difference observed – predicted (fed state)	Food effect (Simulated fed vs. observed fasted data)
			Fed	Fasted		
$FeSSGF_{early}$ pH 6.4 3.5% fat	C_{max} (ng/mL)	68.1	75.7 (43)	100.1 (42)	-10.04	0.68
	AUC_{0-12} (ng.h/mL)	361.0	344.0 (52)	398.9 (56)	4.94	0.90
	T_{max} (h)	2.0	2.16 (72)	1.36 (66)	-7.40	1.47
	AFE	-	-	-	0.85	-
	AAFE	-	-	-	1.45	-
$FeSSGF_{middle}$ pH 5.0 1.75% fat	C_{max} (ng/mL)	77.7	75.7 (43)	100.1 (42)	2.64	0.78
	AUC_{0-12} (ng.h/mL)	408.8	344.0 (52)	398.9 (56)	18.84	1.02
	T_{max} (h)	1.99	2.16 (72)	1.36 (66)	-7.87	1.46
	AFE	-	-	-	0.98	-
	AAFE	-	-	-	1.34	-
$FeSSGF_{late}$ pH 3.0 0.88% fat	C_{max} (ng/mL)	78.7	75.7 (43)	100.1 (42)	3.96	0.79
	AUC_{0-12} (ng.h/mL)	413.0	344.0 (52)	398.9 (56)	20.05	1.03
	T_{max} (h)	1.95	2.16 (72)	1.36 (66)	-9.72	1.43
	AFE	-	-	-	0.98	-
	AAFE	-	-	-	1.33	-
$FeSSGF_{early}$ pH 6.4 7.5% fat	C_{max} (ng/mL)	57.1	75.7 (43)	100.1 (42)	-24.57	0.57
	AUC_{0-12} (ng.h/mL)	304.7	344.0 (52)	398.9 (56)	-11.42	0.76
	T_{max} (h)	2.05	2.16 (72)	1.36 (66)	-5.09	1.51
	AFE	-	-	-	0.71	-
	AAFE	-	-	-	1.67	-
$FeSSGF_{middle}$ pH 5.0 7.5% fat	C_{max} (ng/mL)	73.5	75.7 (43)	100.1 (42)	-2.9	0.73
	AUC_{0-12} (ng.h/mL)	387.7	344.0 (52)	398.9 (56)	12.7	0.97
	T_{max} (h)	2.00	2.16 (72)	1.36 (66)	-7.4	1.47
	AFE	-	-	-	0.92	-
	AAFE	-	-	-	1.38	-
$FeSSGF_{late}$ pH 3.0 7.5% fat	C_{max} (ng/mL)	79.4	75.7 (43)	100.1 (42)	4.89	0.79
	AUC_{0-12} (ng.h/mL)	416.6	344.0 (52)	398.9 (56)	21.10	1.04
	T_{max}(h)	1.93	2.16 (72)	1.36 (66)	-10.64	1.42
	AFE	-	-	-	0.99	-
	AAFE	-	-	-	1.33	-

The AAFE values for all media were lower than 2, indicating acceptable simulation of the *in vivo* results. However, the best simulations in terms of AFE and AAFE were obtained with $FeSSGF_{middle}$ and $FeSSGF_{late.}$ In the media simulating a high fat (7.5%) in the stomach, the AFE values were slightly to moderately below 1.0, indicating that the observed profile was under-predicted. The effect was most pronounced for $FeSSGF_{early}$. In this medium, the AFE at 3.5% fat (standard value) was 0.85 and at 7.5% fat it was 0.71, and thus further from the ideal value of 1.0.

The early FeSSGF medium, which reflects an initial elevation in the gastric pH induced by food intake, may have prevented the dosage form from completely disintegrating. Another ramification of a high pH early after meal ingestion for weak bases like zolpidem (pKa 6.06) would be a reduction in the solubility and therefore dissolution rate, although this effect is expected to be less important for highly soluble weak bases like zolpidem. Either or both of these factors could have contributed to prediction of slower absorption and hence a reduction in C_{max} in the fed state simulations.

3.3.3.3 - Biorelevant dissolution to investigate effect of HPMC and pH on release of the drug

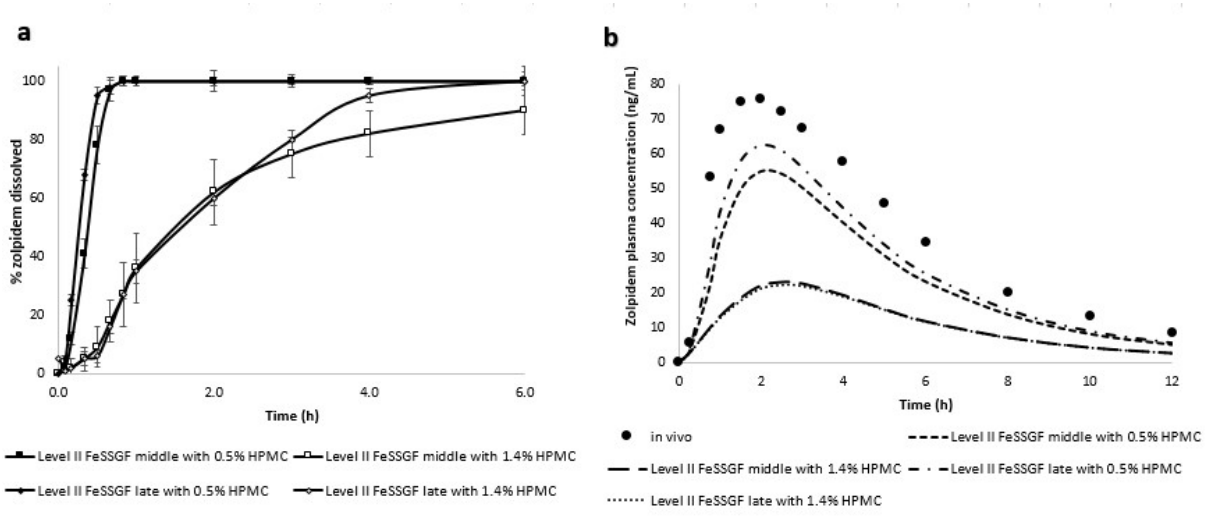


Figure 16. a- Mean dissolution profiles of 10 mg immediate release Stilnox® tablets in biorelevant Level II dissolution media in the fed state to explore the effect of pH and viscosity on drug release, using USP apparatus II. b-Simulated and observed plasma concentration profile of zolpidem using various dissolution profiles. Reproduced with permission from Paraiso et al. (2019).

Figure 16a depicts the results of the dissolution experiments after increasing the viscosity of the media using 0.5% or 1.4% HPMC. Due to the high viscosity of the medium with 1.4% HPMC slowing down the rate of release, it was necessary to increase the duration of sampling from 3 to 6 hours and even then it was not possible to reach a drug release of 100%. It may be assumed that poor wetting and reduced hydrodynamic shear rate in viscous media contributed to the slow and incomplete dissolution of the drug.

3.3.3.3.1 - Simulations using variations in viscosity

The results from the dissolution studies simulating the increase in viscosity of the stomach contents were integrated in the PBBM model to obtain the simulated mean plasma concentration profile of zolpidem, as shown in Figure 16b. From the simulations, the pharmacokinetic parameters C_{max}, T_{max} and AUC were calculated and these are presented together with the AFE and AAFE values in the following Table.

Table 10. Pharmacokinetic parameters of zolpidem for the food effect studies with the corresponding simulation results using in vitro data input from experimental conditions simulating the effect of pH and high viscosity (HPMC) on release from Stilnox® 10 mg immediate release tablets.

Media	Pharmacokinetic parameters	Parameter value predicted from fed state media	Observed		% difference observed – predicted (fed state)	Food effect (Simulated fed vs. observed fasted data)
			Fed	Fasted		
FeSSGF $_{middle}$ pH 5.0 0.5 % HPMC	Cmax (ng/mL)	23.0	75.7 (43)	100.1 (42)	-69.62	0.23
	AUC_{0-12} (ng.h/mL)	135.0	344.0(52)	398.9 (56)	-60.75	0.33
	T_{max} (h)	2.6	2.16 (72)	1.36 (66)	20.37	1.91
	AFE	-	-	-	0.66	-
	AAFE	-	-	-	1.47	-
FeSSGF $_{late}$ pH 3.0 0.5 % HPMC	C_{max} (ng/mL)	62.6	75.7 (43)	100.1 (42)	-17.30	0.62
	AUC_{0-12} (ng.h/mL)	331.6	344.0 (52)	398.9 (56)	-3.60	0.83
	T_{max} (h)	2.12	2.16 (72)	1.36 (66)	-1.85	1.56
	AFE	-	-	-	0.75	-
	AAFE	-	-	-	1.35	-
FeSSGF $_{middle}$ pH 5.0 1.4 % HPMC	C_{max} (ng/mL)	54.9	75.7 (43)	100.1 (42)	-27.48	0.55
	AUC_{0-12} (ng.h/mL)	293.0	344.0 (52)	398.9 (56)	-14.82	0.73
	T_{max} (h)	2.18	2.16 (72)	1.36 (66)	0.92	1.60
	AFE	-	-	-	0.31	-
	AAFE	-	-	-	2.26	-
FeSSGF $_{late}$ pH 3.0 1.4 % HPMC	C_{max} (ng/mL)	22.2	75.7 (43)	100.1 (42)	-70.67	0.22
	AUC_{0-12} (ng.h/mL)	132.4	344.0 (52)	398.9 (56)	-61.51	0.33
	T_{max} (h)	2.65	2.16 (72)	1.36 (66)	22.68	1.94
	AFE	-	-	-	0.21	-
	AAFE	-	-	-	2.49	-

In all media simulating the increase of viscosity with 0.5% and 1.4% of HPMC the AFE values were less than 1.0, indicating that these media under-predict the observed mean plasma profile (Figure 16b). Further, the under-prediction of the pharmacokinetic parameters was more pronounced for the media in which 1.4% HPMC was used. In the media with highest viscosity (1.4% HPMC), the values obtained from AFE for $FeSSGF_{middle}$ and $FeSSGF_{late}$ were respectively 0.31 and 0.21, very far from the ideal value of 1.0, and values of AAFE were higher than 2 and therefore not considered acceptable. The AAFE values for 0.5% HMPC media were below 2 and thus would be considered acceptable, but with AFE values of 0.66 for $FeSSGF_{middle}$ and 0.75 for

$FeSSGF_{late}$, media with 0.5% HPMC appear to be less suitable for simulating the *in vivo* behaviour than the standard biorelevant media. Further, the $FeSSGF_{late}$ dissolution results predicted a mean maximum concentration plasma below the 5th percentile value obtained in the $FeSSGF_{middle}$ simulations and far lower than the observed mean C_{max} of just over 75ng/ml. Taken together, these values indicate that, for zolpidem immediate release tablets, the high viscosity media do not reflect the dissolution process *in vivo* adequately.

3.3.4 Parameter sensitivity analysis (PSA) to investigate the effect of gastric emptying time (GET) in the fed state zolpidem PK profile.

As for the fasted state, to investigate the influence of the GET on the plasma profile of zolpidem in the fed state, a parameter sensitivity analysis (PSA) was conducted. In the fed state, the GET values were floated over the range 0.1 to 4 h. The longer GET in the fed compared to the fasted state was based on literature data (Koziolek et al., 2014, 2013). The PSA of GET for the fed state simulation is shown in Figure 17.

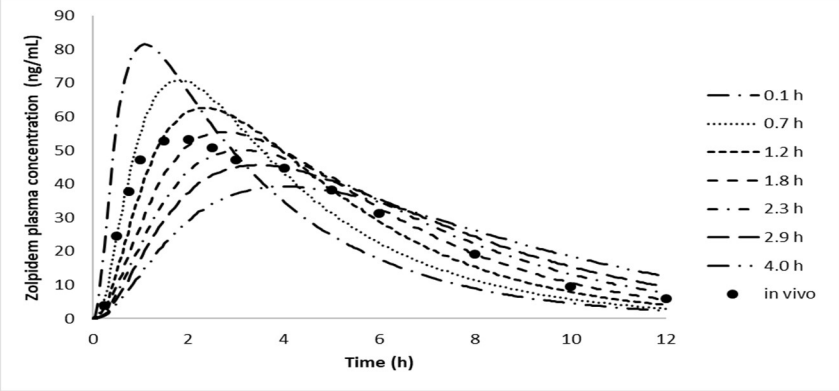


Figure 17. PSA of the zolpidem pharmacokinetic profile for GET in the fed state. Reproduced with permission from Paraiso et al. (2019).

As for the fasted state, the longer gastric emptying time, the lower the calculated C_{max} and AUC and the longer the T_{max}. Due to the larger range of GET values applied, the changes in the pharmacokinetic parameters appear more pronounced than for the fasted state. The best fit for T_{max} was found at 0.7 h while the best fit for C_{max} was at 1.8 h. As for the fasted state, it appears that other mechanisms are also involved to some extent in determining the variation in plasma profiles.

3.3.5 – Survey of clinical studies in the literature on the negative food effect for zolpidem immediate release tablets.

In the commercial label of Stilnox® 10 mg IR tablets and in previous publications, zolpidem is described as having a negative food effect (Andreas et al., 2017; David J. Greenblatt et al., 2013; Paraiso et al., 2019). In Figure 11, which shows the mean plasma profiles from the food effect study, it is visually possible to observe the negative food effect.

To better understand the food effect, a literature search was conducted to locate publications of zolpidem IR clinical trials that had been conducted under fasted or fed conditions to compare their pharmacokinetic parameters (C_{max} T_{max} and AUC). A total 13 studies were located, 9 of which had been conducted in the fasted state and 4 in the fed state. In Figure 18 the values of C_{max} (18a), AUC (18b) and T_{max} (18c) for these trials are showed.

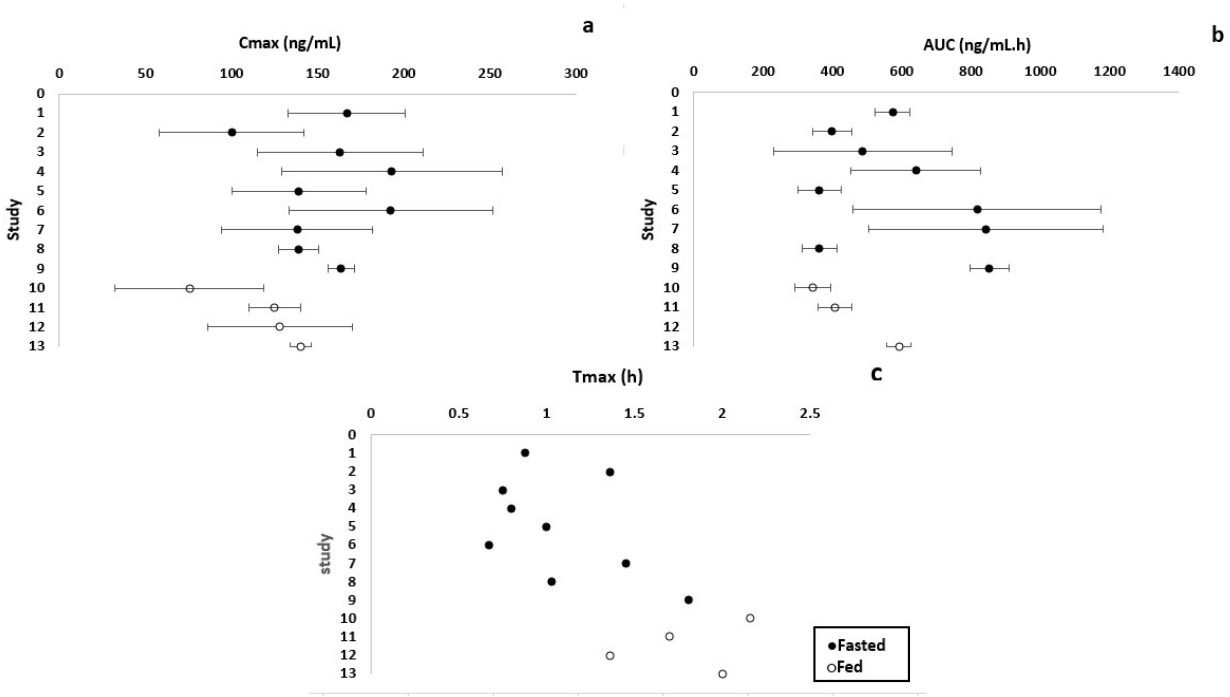


Figure 18. Studies comparing the pharmacokinetic parameters of zolpidem when administered in the fasted and fed states. (a) C_{max}, (b) AUC and (c) Tmax values. Closed dots (●) represent studies in the fasted state and open dots (○) in the fed state. In figure 13 (a) and (b) the values are presented as the mean and SD, in figure 13 (c) only by the average values. Reproduced with permission from Paraiso et al., 2020.

Studies listed in Figure 18

1 Weinling et al., 2006. Study in male healthy volunteers.
2 Stilnox® – FDA label food effect study, 1992. Fasted state arm (Male healthy volunteers).
3 Zolpidem - Public Assessment Report Netherlands, 2010. Study in male healthy volunteers.
4 Park et. al., 2011. Study in male healthy volunteers.
5 Stilnox® – FDA - Label food effect study 1992. Fasted arm (male and female healthy volunteers).
6 Public Assessment Report Netherland - Zolpidem Tartraat Aurobindo, 2018. Study in male healthy volunteers.
7 Galitz et al., 2015. Study with male and female healthy volunteers.
8 Durand et al., 1992. Study in male healthy volunteers.
9 Allard et al., 1998. Study in female healthy volunteers.
10 Stilnox® - FDA - Label food effect study 1992. Fed state arm (Male healthy volunteers, compare with Study 2)
11 Greenblatt et al., 1998. Study in male healthy volunteers.
12 Greenblatt et al., 2000. Study in male and female healthy volunteers.
13 Greenblatt et al., 2006. Study in male and female healthy volunteers.

In these studies, the average (mean) values for C_{max} and AUC in the fed state tended to be lower than in the fasted state, while the T_{max} was mostly prolonged in the fed state compared to the fasted state. However, there is some overlap in the pharmacokinetic parameters between the fed and fasted states.

3.4 – Discussion

3.4.1 - Simulation using variations in the level of pH and fat content

The presence of food in the gastrointestinal tract can change the oral bioavailability of drugs mainly via changes in the rate and/or extent of absorption, pre-systemic metabolism, and systemic drug clearance (Porter CJH, Charman WN, 2001; Olanof, et all 1986; Gupta, 1990; Humberstone, 1995).

Normally the gastric pH is elevated after food ingestion and the amount of lipids in the stomach increases (Chaman, et. al, 1997). This increasing fat content in the gastric environment may have significant impact on dissolution and solubility, mainly for drugs belonging to BCS II and IV, leading to an increase in the bioavailability of oral formulations. The influence of ingested lipids on drug absorption can be due to different mechanisms. During lipid digestion, colloidal structures are produced and the drug may traffic between these structures and aqueous medium. This process can have an impact on drug solubility and dissolution (Fulden Buyukozturk, 2013). Furthermore, the presence of lipids in the GI tract increases the time for the gastric emptying, and thus potentially the rate and extent of drug absorption. On the one hand, since zolpidem is highly soluble, the higher fat content is unlikely to affect its solubility. On the other hand, the change in the gastric emptying pattern is expected to reduce at least the rate of absorption of zolpidem, due to its short half-life.

The elevation of the pH in the stomach after food ingestion may have impact in the dissolution and absorption of weakly acidic and basic drugs. Due to the low gastric pH in the fasted state, the dissolution rate of weak bases is typically greater in gastric fluids than in the intestine, whereas for weak acids there is minimum dissolution in the stomach but as the undissolved API is transported from the stomach to the intestine, the drug will start to dissolve at the higher intestinal pH (Charman, 1997). Since zolpidem is a weak base (pKa 6.06), the increase in the pH after food ingestion may lead to a reduction in the solubility and therefore dissolution rate of the drug formulation (Paraiso, et. al., 2019). This may explain the lower exposure in the simulations with early FeSSGF medium, although this effect is expected to be less important for highly soluble weak bases like zolpidem.

A further effect of meal intake is to increase the viscosity of the gastric contents. This can lead to formation of a "film layer" on the surface of the tablet, resulting in a decrease in the rate of penetration of the medium into the tablet and impeding the wetting and disintegration of the tablet *in vivo* (Koziolek et al., 2013; Levy and Jusko, 1965; Parojči´c et al., 2008; Radwan et al., 2017; Reppas et al., 1991). Developing a dissolution medium which is capable of mimicking the increase in the viscosity of the gastrointestinal contents and its effects on dosage form disintegration is therefore of great importance. Due to factors such as the amount of food ingested, fluids ingested with the meal as well as the rate of saliva and gastric juice secretion, the composition and viscosity of chyme is highly variable and thus difficult to model *in vitro* (Dikeman et al., 2006; Koziolek et al., 2013; Marciani et al., 2000; Mudie et al., 2010; Takahashi and Sakata, 2002). Various authors have proposed dissolution media to simulate the increase in the viscosity of gastric content using soup or various gelling agents such as guar gum, potato granules, HPMC and hydroxyl- ethylcellulose to adjust the meal viscosity (Parojči´c et al., 2008; Radwan et al., 2017, 2012; Reppas et al., 1991). The large range of viscosities implemented in these studies demonstrate that it is difficult to identify a specific viscosity value that can be recommended for biorelevant media. Although the HPMC-media applied in the zolpidem studies certainly provide a good qualitative description of the food effect, further work needs to be done in this area to arrive at a more quantitatively predictive dissolution method.

Since the immediate release dosage form of zolpidem is used for sleep induction, the food effect study was conducted overnight and in the supine position. It is important to mention that the pattern of gastric emptying is changed during sleep. On the one hand, there is a profound inhibitory effect on GI motility pattern during sleep. Several studies have demonstrated the suppression of both gastric and colonic motilities (Davis et al., 1992; Goo et al., 1987; Haase et al., 2015; Ziessman et al., 2009). On the other hand, the delay in the gastric emptying due to the supine position is controversial. Some studies have shown no significant effect of body position in the gastric emptying time (Mojaverian P, 1988; Steingoetter et al., 2006; Treier et al., 2006), whereas others have shown differences in GET due to changes in body position. (Horowitz M et al., 1993; Hunt JN, Knox MT, Ogisnki A, 1965; Moore JG et al, 1988).

The delay in gastric emptying time provoked by the circadian cycle, together with the ingestion of food with high caloric content may be responsible at least partially for the decrease in the rate (C_{max}) and extent (AUC) of absorption of zolpidem. Although the administration of

zolpidem tablets in the fasted state would be preferable, both for optimization of efficacy as well as assurance of safety, most people do not like to go to bed on an empty stomach.

3.4.2 - Studies regarding the negative food effect of zolpidem

The effect of food in the drug absorption may be associated with the drug physicochemical characteristics, the formulation or the physiology of the human gastrointestinal tract. Physiological characteristics in the gastrointestinal tract e.g. gastric emptying time, release of bile salts and enzymes, gastrointestinal pH and splanchnic blood flow, are usually highly variable. Changes in these parameters after food intake may alter the luminal metabolism of the drug, the interaction the drug or dosage form with the gastrointestinal fluids. This complex combination of factors may lead to alterations in the *in vivo* dissolution from the drug product, degradation of the drug and/or its absorption and thus a positive or negative food effect.

The dissolution experiments simulating fasted and fed states had showed that an increase in the fat content of the biorelevant media had only a modest effect on the dissolution of zolpidem and in consequence in the PK parameters, however, the longer gastric emptying time associated with a higher fat content could slow gastric emptying and thus indirectly affect the absorption profile. Similarly, when Andreas and co-workers (2017) tested the modified release formulation of zolpidem, they concluded that the negative food effect of zolpidem is associated with the delay in the gastric emptying time of the formulation (Andreas et al., 2017).

In the zolpidem studies shown in Figure 11 from the food effect study, the variability of C_{max} and AUC was high in both the fasted and fed arms of the clinical studies. These results are consistent with the fact that gastric emptying can vary widely among individuals, with the phase of the motility cycle in the fasted state, and between the fed and fasted states. Since zolpidem is highly soluble and permeable and has a short elimination half-life, the limiting step for absorption for this drug may well be the gastric emptying time (Amidon et al., 1995; Andreas et al., 2017; Colo et al., 2015). In general drugs with a short half-life, the C_{max} becomes more sensitive to differences in gastric emptying and this sensitivity is intensified when solubility and permeability are not rate-limiting to absorption. Thus, the PK parameters of zolpidem, a BCS Class I substance, are particularly prone to intra and inter-individual variability in the gastric emptying time (Andreas et al., 2017; Markopoulos et al., 2015a; Wagner et al., 2012). Although

data from the literature combine to suggest that the zolpidem IR formulation has a negative food effect, the high variability in T_{max}, C_{max} and AUC, the variability in the mean values from trial to trial and the paucity of trials in the fed state do not allow a firm conclusion to be reached. To confirm the negative effect of zolpidem, larger crossover study in healthy men and women would be necessary.

3.4.3 The administration of zolpidem should be fasted or fed state?

The human circadian cycle can be different for each individual, therefore, the eating habits and bedtime can be specific for each person. Nevertheless, in general practice nutritionists and doctors instruct the patients to wait at least two to three hours between the last meal of the day and bedtime. This interval additionally allows for better food digestion, preventing problems like heartburn at night and even insomnia (de Castro, 2004; De Zwaan et al., 2003; Schenck, 2006; Zwaan et al., 2006). Since zolpidem is used to sleep induction, it seems likely that most patients would take the product in the fed state. The marginal negative food effect could potentially decrease the effectiveness of the drug, while the delay in T_{max} may incur a greater risk of residual sedation when compared with the fasting state. These considerations are in line with the product label, which indicates that for optimal effect Stilnox® tablets should not be taken with or immediately after a meal.

3.5 - Conclusions

These studies demonstrated that PBBM modelling, the combination of biorelevant dissolution testing with PBPK modeling, is a useful way forward to characterize the *in vivo* behavior of zolpidem after administering Stilnox® 10 mg immediate release tablets as well as to attain a more mechanistic understanding of the influence of food in the process of zolpidem absorption. This approach can be applied to other drugs and contribute significantly to the drug development process, saving time and reducing the costs associated with bringing new drug products to the market.

Additional experiments to evaluate the influence of increasing the viscosity of the media to simulate fed state conditions failed to provide a quantitative description of the *in vivo* behaviour of zolpidem. Results of experiments that evaluated the effect of high amounts of fat on dissolution in the fed stomach suggested that there is no direct relationship between the increasing the amount of fat in the stomach and the release of zolpidem over most of the postprandial period. However, this higher fat content may lead to a lower C_{max} by reducing the gastric emptying rate. Indeed, the gastric emptying rate appears to be the most important factor determining the onset action and peak exposure of zolpidem, although other factors may also play a role.

Finally, a review of clinical studies from the open literature casts some doubt on the negative food effect for this drug and to settle this question, a crossover study in men and women would need to be conducted.

CHAPTER 4

The use of PBBM/PD to establish clinically relevant dissolution specifications for zolpidem immediate release tablets.

4.1 - Introduction

Zolpidem is an imidazopyridine, non-benzodiazepine hypnotic agent which has been shown to be effective in inducing and maintaining sleep in adults and is one of the most frequently prescribed hypnotics in the world (Norman et al., 2017; Salvà and Costa, 1995).

The main factors which contribute to its success are the rapid absorption of the drug after oral administration of the tablet, which facilitates a rapid onset of action (in this case induction of sleep), and its short elimination half-life (2.4h), which leads to fewer "hangover" effects compared with other options (e.g. benzodiazepines) used for sleep induction (de Haas et al., 2010; Vermeeren, 2004).

In general, dissolution tests of oral drug products are performed in simple buffer media that are described in the pharmacopeia. These tests are often used in the pharmaceutical development of drug products containing highly soluble drugs (those in BCS I and III) to guide the formulation process and in quality control testing for batch release and stability studies (Tsume et al., 2018). For poorly soluble drugs, BCS II and IV biorelevant dissolution media, which simulate the composition of the fluids in the gastrointestinal environment, are often more appropriate for pharmaceutical development studies (Fotaki and Vertzoni, 2010a; Jantratid et al., 2009; Klein, 2010; Markopoulos et al., 2015a). Biorelevant media have also been applied to set clinically relevant dissolution specifications (CRDS) and to establish a dissolution "safe space" for drug formulations, a process which is characterized by linking the *in vitro* dissolution with the *in vivo* performance of drug formulation (Hermans et al., 2017; Paul A. Dickinson et al., 2008; Tsume et al., 2018). However, establishing this relationship between dissolution performance and *in vivo* results is not always straightforward.

Recent advances in physiologically based pharmacokinetic (PBPK) modeling platforms together with improvement of *in vitro* tests has facilitated the translation of *in vitro* dissolution data into predictions of the *in vivo* plasma profile of drugs to set the CRDS (Hermans et al., 2017; Tsume et al., 2018). Using this approach, there is a possibility to integrate dissolution data into the PBBM model and couple it with the PD model to establish a truly clinically driven "safe space" for the dissolution of the drug formulation. In a first application, Cristofoletti and Dressman applied PBPK-PD to understand the extent to which dissolution drives the clinical response to ibuprofen, a BCS Class II drug (Cristofoletti and Dressman, 2014). In the current

study, the PBBM/PD approach was applied to set the CRDS for zolpidem immediate release tablets.

4.2 – Materials and Methods

4.2.2 - Biorelevant Dissolution Media

The composition of biorelevant media, $FeSSGF_{middle}$ Level II, and in FeSSIF-V2 Level II (pH 5.8), and the set-up of the experiments is described in Chapter 3.2.3. The two media were selected to align the *in vitro* conditions to the clinical trial of the pharmacodynamics of Stilnox® tablets, in which they were ingested after a low fat breakfast (Greenblatt et al., 2006).

4.2.3 – Clinical Study

In the clinical studies to compare the pharmcodynamics and pharmacokinetics of zolpidem immediate and modified release tablets realized by Greenblatt and co-workers (2006), zolpidem immediate release tablets were administered to healthy volunteers (men and women) two hours after eating a light breakfast (Greenblatt et al., 2006). To best match with these trial conditions, the simulation in Simcyp® was run in a "healthy volunteer" cohort with the same gender ratio and age as the study cohort, in which the gastrointestinal physiology was adapted to represent conditions after ingestion of a low fat meal.

4.2.4 - PBBM model

The PBBM part of the PBBM/PD model was based on the model described in Chapter 3, again using the Simcyp® Simulator (V18.1; Certara, Sheffield, UK). The drug parameters inputted into the PBBM/PD model are shown in Table 11. The unbound fraction in plasma was kept at 8%, as reported by Durand et al. (1992) (Durand et al., 1992). Values for the volume of distribution found in the literature were 0.54L/kg (Durand et al., 1992) and 0.68 L/kg (Chetty et al., 2014). In the PBBM model used in the current chapter, a value of 0.54L/kg was adopted for the volume of distribution to provide the best fit. To describe zolpidem metabolism and elimination, the values from enzymatic clearance were initially used to fit the data, as in the model described in Chapter 3. But as the resulting simulation did not adequately fit the observed results in the clinical studies relevant to the question being asked in this work, the oral clearance

was applied instead. The results using the different approaches should, in principle, be the same. However, zolpidem is recognized as a high variable drug with significant variations in drug exposure and pharmacokinetics parameters, depending on the clinical study. This variability is depicted clearly in the pharmacokinetic parameters shown in figure 18. From those results it is obvious that the there is no "one size fits all" PBPK model that will simulate all studies published in the literature.

4.2.4.1 - Verification of the PBBM model using an internal PK data set

The parameters and conditions of the trials depicted in Table 11 were used to build the PBBM/PD model. An internal verification of the PBBM/PD model adopting the pharmacokinetic and pharmacodynamic *in vivo* data from Greenblatt and co-workers (2006) was conducted (Greenblatt et al., 2006). Since no other studies using similar trial conditions (men and women, light breakfast) could be found in the literature, it was not possible to use external data to validate the model.

Table 11. Parameters used to implement the zolpidem PBBM model (Simcyp Simulator®V18.1).

Parameters	Value	Reference/Comments
Molecular weight (g/mol)	307.39	Salvà and Costa, 1995
Log P	2.42	Salvà and Costa, 1995
Compound type	Monoprotic base	Salvà and Costa, 1995
pKa	6.16	Salvà and Costa, 1995
Absorption (low fat fed model)	ADAM model	
Dissolution level II $FeSSGF_{middle}$	Dissolution profile	Paraiso et al, 2019
Dissolution level II FeSSIF-V2	Dissolution profile	Paraiso et al, 2019
P_{eff}	6.5×10^{-4} cm/s	Andreas et al., 2017b
Absorption (fasted model)	ADAM model	
Dissolution level II FaSSGF	Dissolution profile	Paraiso et al, 2019
Dissolution level II FaSSIF-V2	Dissolution profile	Paraiso et al, 2019
P_{eff}	6.5×10^{-4} cm/s	Andreas et al., 2017b
Distribution	Minimal PBPK model	
Main binding protein	albumin	Salvà and Costa, 1995
Vss (L/kg)	0.54	Durand et al., 1992
Fu gut	0.035	predicted using Simcyp®
Blood to plasma coefficient (B:P)	0.76	Salvà and Costa, 1995
Fu plasma	0.08	Durand et al., 1992
Elimination		
Oral clearance (L/h)	16.56	Greenblatt et al., 2006a
CLR (renal clearance) (L/h)	0.18	Simcyp® default
PD model	Power function	
Exponent (m)	1.17	Estimated form Greenblatt et al., 2006a
Slope (α)	21.2	Estimated from Greenblatt et al., 2006a
E_0	0	
Baseline type	Additive	
Trial condition		
Formulation option in the software	Immediate release (IR) with interpolation of the observed *in vitro* dissolution data	Default setting in Simcyp®
Population modeling	Virtual population trials were conducted using 700 (70x10) male and female healthy volunteers, age ranged 18-45 years for each study.	Greenblatt et al., 2006a

4.2.5 - PD model

4.2.5.1 – Percentage of β-EEG amplitude variation

Normally the effect of hypnotic drugs is evaluated measuring objective and subjective parameters after the drug administration. Observed rated sedation, self-rated sedation and the

digit symbol substitution test (DSST) are considered subjective, while variations in α, β and γ brain waves are considered objective (Cysneiros et al., 2007; de Haas et al., 2010; Drover et al., 2000; Greenblatt et al., 2006, 1998). Since the % change in the β-EEG amplitude is the objective parameter most often used to describe the hypnotic effects of zolpidem, this parameter was chosen to build the PD model.

Results for % change in the β-EEG were taken from a published PK/PD study that was used to compare the pharmacokinetics and pharmacodynamics of zolpidem IR with the modified release (MR) formulation. This study was conducted in 70 healthy volunteers (Greenblatt et al., 2006), who were administered a light meal before ingesting the tablet. Since the time to reach the maximum drug concentration in plasma (T_{max}) and the time to reach the maximum pharmacological response (TR_{max}), together with maximum plasma concentration (C_{max}) and the maximal response (R_{max}), showed an almost linear correlation, with no hysteresis (Figure 19), the authors concluded that the % change in the β-EEG profile after treatment with zolpidem would be well described by a single exponential equation:

$$y = \alpha x^m \qquad \text{Equation 6}$$

For this reason, the power function model (the default model in the software) was used to develop the PD model in Simcyp®. The power function model is represented by the following equation:

$$R1 = E_0 + \alpha \cdot (PKInput)^m \qquad \text{Equation 7}$$

where R1 is the pharmacological response, E_0 is the initial % change in the β EEG wave (in our model E_0=0), α is a linear coefficient, $PKInput$ is the plasma profile concentration entered for time t, and *m* is exponential coefficient of the equation.

The *in vivo* data obtained from Greenblatt and co-workers study were used to estimate the α and m parameters of the model (Greenblatt et al., 2006).

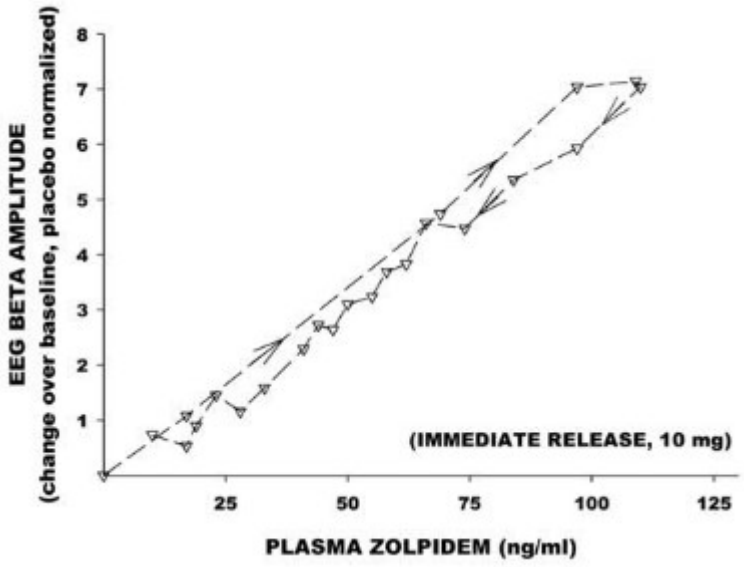


Figure 19. Relationship between mean plasma zolpidem concentration and mean changes in the percentage electroencephalographic (EEG) of β brain waves at corresponding times. Arrows indicate the direction of increasing time. Reproduced with permission from Greenblatt and co-workers (2006).

4.2.6 – PBPK/PD Simulations

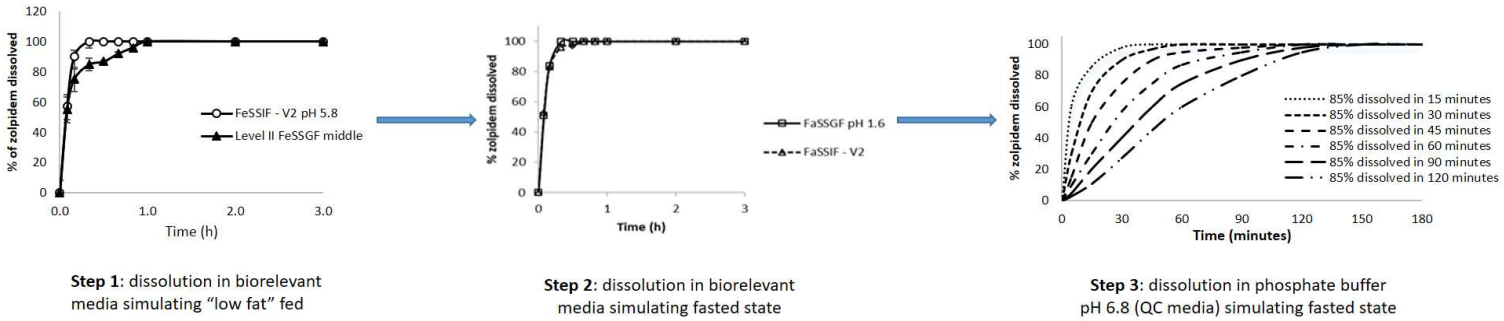
Simcyp Simulator® version 18.1 was used to predict the plasma profile and the % change in the β wave by zolpidem. To run the simulation trials, the virtual population was selected to closely match the population recruited by Greenblatt and co-workers with regard to age range and gender ratio (Greenblatt et al., 2006). The virtual trials simulating the "low fat" fed state were performed on the basis of administering zolpidem IR tablets with 250 mL of water 2 hours after a light breakfast. Starting with the intake of the drug, the clinical trial was conducted over 12 h. The following simulations were performed:

1. To evaluate the hypnotic effect of zolpidem using the % change in the β-waves, a virtual trial was conducted enrolling 70 adults aging 19–45 years with proportion of 46% females receiving a single oral dose of 10 mg zolpidem administered as the IR tablet. To internally verify this simulation, the predicted data were compared with the observed data. As no other publication with a similar trial design could be located in the open literature, no external validation was possible.
2. To identify the crucial parameters in the PBBM/PD model, a sensitivity analysis was performed. The effect of two different input variables on the PK and PD responses of

zolpidem was evaluated using the 'one-at-a-time' variation approach. Gastric emptying time (GET) (with values ranging from 0.4 to 2.0 h) and effective permeability (P_{eff}) (with values ranging from 4 to 8 $x10^{-4}$ cm/s) were the two variables tested.

3. To set the clinically relevant dissolution specification for zolpidem IR tablets, the model developed for zolpidem administered after a low fat meal was extrapolated to the fasted state, because the quality control dissolution studies are performed under conditions simulating the fasted state. In order to simulate the plasma profile and the % change in β-EEG amplitude in the fasted state, the same structural model as used for the low fat fed state was applied. All parameters (Table 11) were kept constant, with the exceptions that the dissolution under conditions representing the fasted state and the default values in Simcyp® for the healthy population in the fasted state were applied. After the simulation in the fasted condition was generated, the plasma profile was verified with external data from Weinling and co-workers (2006) (Weinling et al., 2006b). That study had been conducted in 24 healthy male volunteers, age range 18-45 years, under fasting conditions. The volunteers received 10 mg zolpidem tartrate IR tablets in a cross-over design. The pharmacodynamic profile could not be validated as no PD studies in the fasted state have been reported in the open literature. After constructing and verifying this scenario internally, different dissolution rates (ranging from 85% dissolved in 15 min to 85% dissolved in 120 min) in a fasted state intestinal medium were entered into the model to generate the PK and PD responses of zolpidem under these conditions. These simulations were conducted in 10 virtual trials, each enrolling 70 adults aging 18–40 years and receiving a dose of 10 mg of zolpidem IR tablets on an empty stomach.

4.2.7 Approach for imputing the dissolution data into ADAM model to build the PBBM/PD model



Step 1: dissolution in biorelevant media simulating "low fat" fed

Step 2: dissolution in biorelevant media simulating fasted state

Step 3: dissolution in phosphate buffer pH 6.8 (QC media) simulating fasted state

Figure 20. Dissolution profiles inputted into ADAM model.

Description of the steps shown in Figure 20:

Step 1 – To build the PBPK/PD model, dissolution profiles simulating low fat dissolution media (stomach: level II $FeSSGF_{middle}$ and small intestine: FeSSIF-V2) were inputted into the ADAM model. This approach was applied as results for PK/PD data for zolpidem IR tablets are only available in studies where the tablet was administered after a light breakfast meal.

Step 2 – In this stage the "low fat" fed PBPK/PD model was extrapolated to the fasted state. To do this extrapolation, dissolution data obtained from studies performed with biorelevant media simulating the fasted state (stomach: FaSSGF and small intestine: FaSSIF V2) were inputted into the ADAM model. The obtained model was verified with data from Weinling and co-workers (2006) (Weinling et al., 2006b). It should be noted that, due to the high solubility of zolpidem at pH 6.8, the dissolution behavior in the biorelevant and the quality control dissolution test at intestinal pH can be assumed to be the same.

Step 3 – To waive *in vivo* studies, the regulatory agencies accept dissolution experiments in compendial media (QC) pH 1.2; 4.5 and 6.8. Therefore, to use the model to set the clinical relevant dissolution specification of zolpidem IR tablets, dissolution profiles simulating different dissolution rates in dissolution media pH 6.8 were imputed into the PBBM/PD model obtained in Step 2.

4.2.8 PBBM/PD model strategy

A schematic of the steps used to create the models is shown in Figure 21.

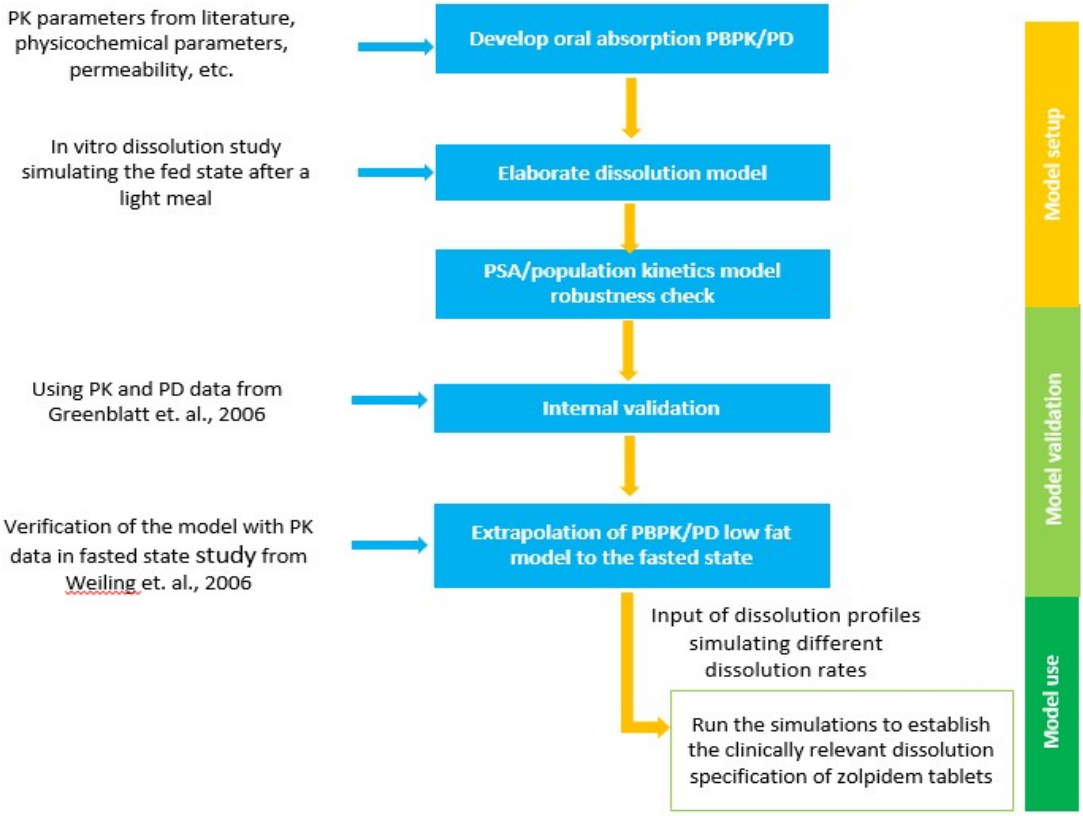


Figure 21. PBBM/PD model strategy. Reproduced with permission from (Paraiso et al., 2020).

4.2.9 - Data Presentation and Statistical Analysis

As for the studies on the food effect, the simulated plasma profiles obtained with Simcyp® software ("predicted") were compared with *in vivo* data ("observed") using the average fold error (AFE) and absolute average fold error (AAFE) (Obach et al., 1997; Poulin and Theil, 2009).

4.3 - Results

4.3.1 - Development and verification of the PBBM/PD model of zolpidem

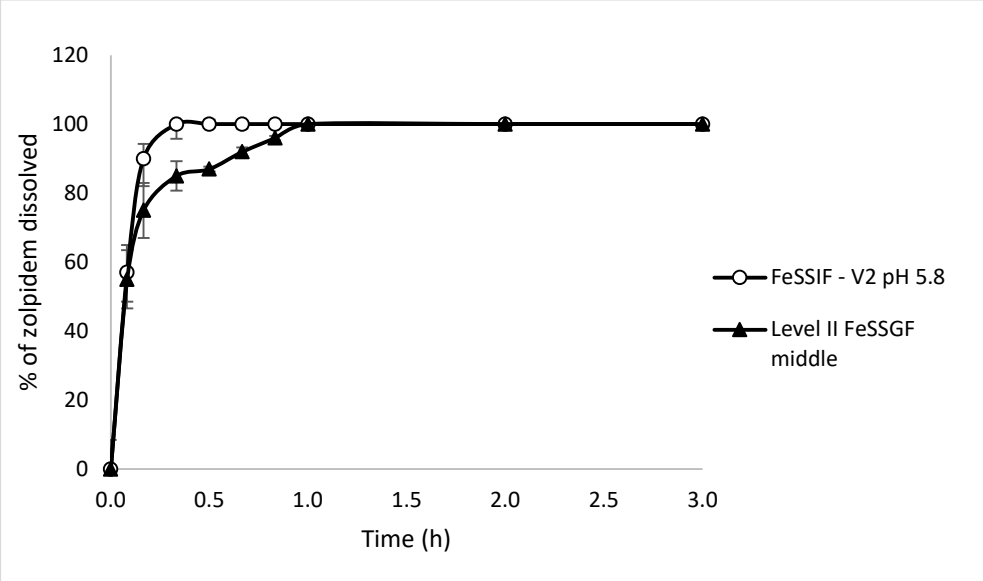


Figure 22. Mean dissolution profiles of 10 mg immediate release Stilnox® tablets in biorelevant Level II dissolution media simulating the low fed state using USP apparatus 2. Reproduced with permission from Paraiso et al. (2019).

Figure 22 depicts the dissolution profiles of 10 mg Stilnox® immediate release tablets in biorelevant media simulating the gastric ($FeSSGF_{middle}$) and upper intestinal (FeSSIF-V2) environment after ingesting a low fat meal. This figure shows that there were only small differences in the rate and extent of dissolution comparing the gastric (lower) and intestine environment (higher).

In order to simulate the "low fat" fed state plasma and pharmacodynamic profile after ingestion of zolpidem IR, the dissolution profiles simulating the gastric and small intestine were inputted into the PBBM/PD model in Simcyp® together with drug properties and pharmacokinetics parameters listed in Table 11.

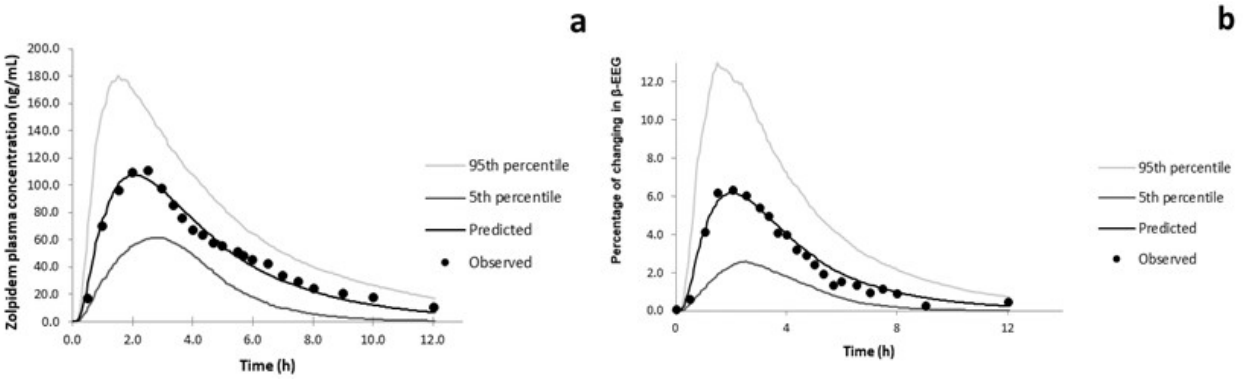


Figure 23. (a) Mean observed (●) and model predicted PK profile (solid line) for health adults after administration of zolpidem IR tablets in the "low fed state". (b) Mean observed (●) and model predicted PD profile (solid line) after administration of zolpidem IR tablets over time. Reproduced with permission from (Paraiso et al., 2020).

Figure 23a shows the predicted and observed mean zolpidem plasma profile and 23b mean % change in the β-waves profiles together with the predicted 5th and 95th percentiles around the means.

Table 12. Pharmacokinetic parameters obtained in the clinical trial of zolpidem with the outcomes from simulation using in vitro data inputted from biorelevant dissolution tests simulating the fed state after a low fat meal.

PK parameter	Simulated	Observed	% difference	AFE/AAFE
C_{max} (ng/mL)	107.47	108.85	-1.26	-
AUC_{0-12} (ng.h/mL)	577.23	565.78	-1.98	-
T_{max} (h)	2.00	2.02	0.02	-
AFE	-	-	-	1.07
AAFE	-	-	-	1.10

Table 12 summarizes the calculated PK parameters (T_{max}, C_{max} and AUC), along with AFE and AAFE values which compares the simulated and observed values. The results demonstrate that dissolution profiles obtained with $FeSSGF_{middle}$ and FeSSIF-V2 combined with the PBBM model were able to predict the *in vivo* behavior of zolpidem IR tablets. The AFE for the simulation was 1.07 and the AAFE was 1.10, very close to 1.0, showing that the developed model predicted the plasma profile of zolpidem accurately.

Table 13. Zolpidem pharmacodynamic parameters obtained in the study with the corresponding simulation results using in vitro data input from biorelevant dissolution tests simulating the low fat fed state.

PD parameter	Simulated	Observed	% difference	AFE/AAFE
R_{max}	6.22	6.35	-2.05	
$AUCR_{0-12}$ (ng.h/mL)	30.08	30.01	-0.7	
TR_{max} (h)	2.12	2.0	0.12	
AFE	-	-	-	1.11
AAFE	-	-	-	1.13

Table 13 depicts the simulated and observed PD parameters for zolpidem. As for the PK parameters, the simulated PD parameters (R_{max} and AUCR and TR_{max}) were very close to those achieved in the *in vivo* study. The AFE was 1.11 and the AAFE was 1.13, indicating that the developed model was able to closely predict the pharmacodynamic as well as the pharmacokinetic profile of the drug.

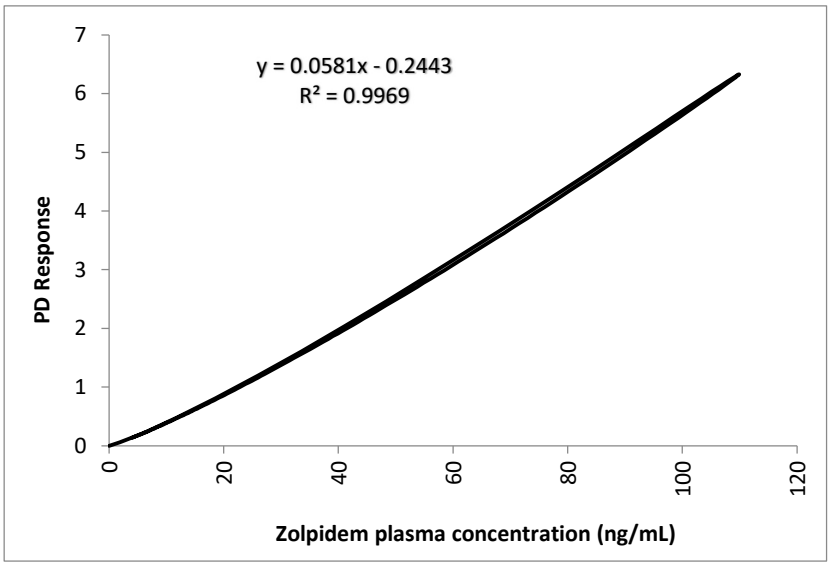


Figure 24. Relationship between mean values of plasma concentration versus PD response of zolpidem. Reproduced with permission from Greenblatt and co-workers (2006).

Figure 24 depicts the relationship between plasma concentration and pharmacodynamic response for zolpidem. The relationship between PK and PD has r^2 of 0.9969, indicating the excellent correlation between the plasma concentration of the drug and the PD response.

4.3.1.1 - Parameter sensitivity analysis (PSA) to determine the effect of gastric emptying time (GET) and effective permeability (P_{eff} x10^{-4}) on the zolpidem PK profile.

Subsequent to developing and internally verifying the PBBM/PD for zolpidem in low fat fed state, a parameter sensitivity analysis (PSA) was conducted to observe which parameters could be critical to predict the plasma profile and or PD response for zolpidem. Since the pharmacodynamic response is practically linearly related to the pharmacokinetics, the pharmacodynamic profiles are not shown.

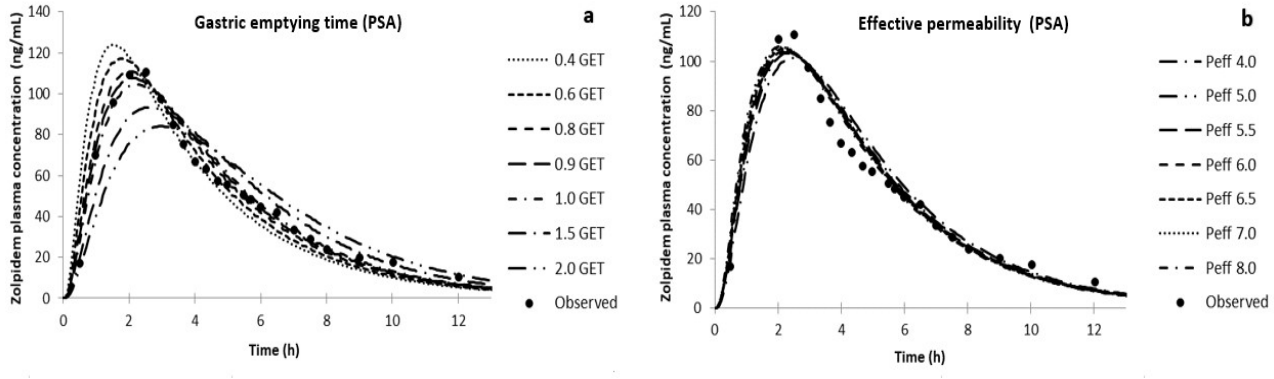


Figure 25. Zolpidem pharmacokinetic profile applying PSA: (a) on GET and (b) on P_{eff} in the fed state after a low fat meal. Reproduced with permission from (Paraiso et al., 2020).

Figure 25a depicts how the plasma profile of zolpidem changes regarding of gastric emptying. Sensitivity analysis demonstrated that at longer gastric emptying times the *in vivo* C_{max} and AUC were underpredicted and the T_{max} was longer. The simulations came closest to the observed mean C_{max} when a GET between 0.8 and 1.0 h was applied, and the T_{max} was best simulated by a GET of 0.9 h. These results suggest that GET plays a major role in zolpidem absorption and thus onset of action of this drug. By contrast, P_{eff} (Figure 25b) did not change the PK profiles significantly over the range tested.

4.3.2 - Dissolution profile to generate the clinical relevance of dissolution for zolpidem

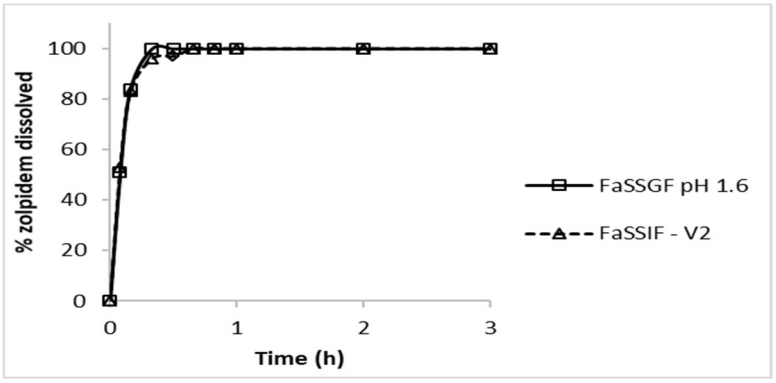


Figure 26. Mean dissolution profiles of 10 mg immediate release Stilnox® tablets in biorelevant Level II dissolution media simulating the fasted state using USP apparatus II. Error bars lie within the symbols in all cases. Reproduced with permission from (Paraiso et al., 2020).

Figure 26 depicts the dissolution profile of 10 mg Stilnox® immediate release tablets in biorelevant media simulating the gastric (FaSSGF) and upper intestinal (FaSSIF-V2) environment in the fasted state. This figure shows that the rate and extent of dissolution between the simulated gastric and small intestine environments are almost the same.

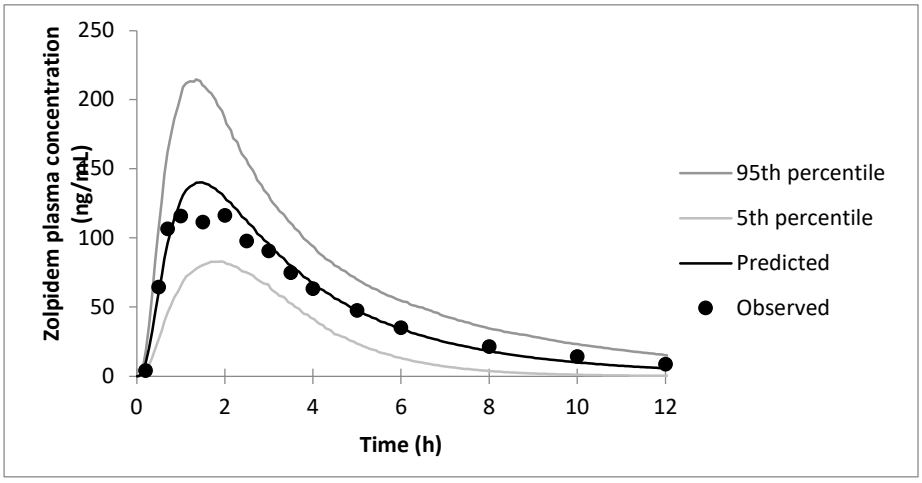


Figure 27. Simulated and observed mean plasma zolpidem concentrations after simulation of zolpidem administration in the fasted state. Reproduced with permission from (Paraiso et al., 2020).

After the development and the internal verification that the PBBM/PD model to represent the fed state after a low fat meal, this model was extrapolated to the fasted state to set the clinically relevant dissolution specification of zolpidem IR tablets, as described in section 4.2.7. Figure 27 depicts the simulated plasma profile together with the *in vivo* data obtained from Weinling and co-workers (2006) (Weinling et al., 2006b).

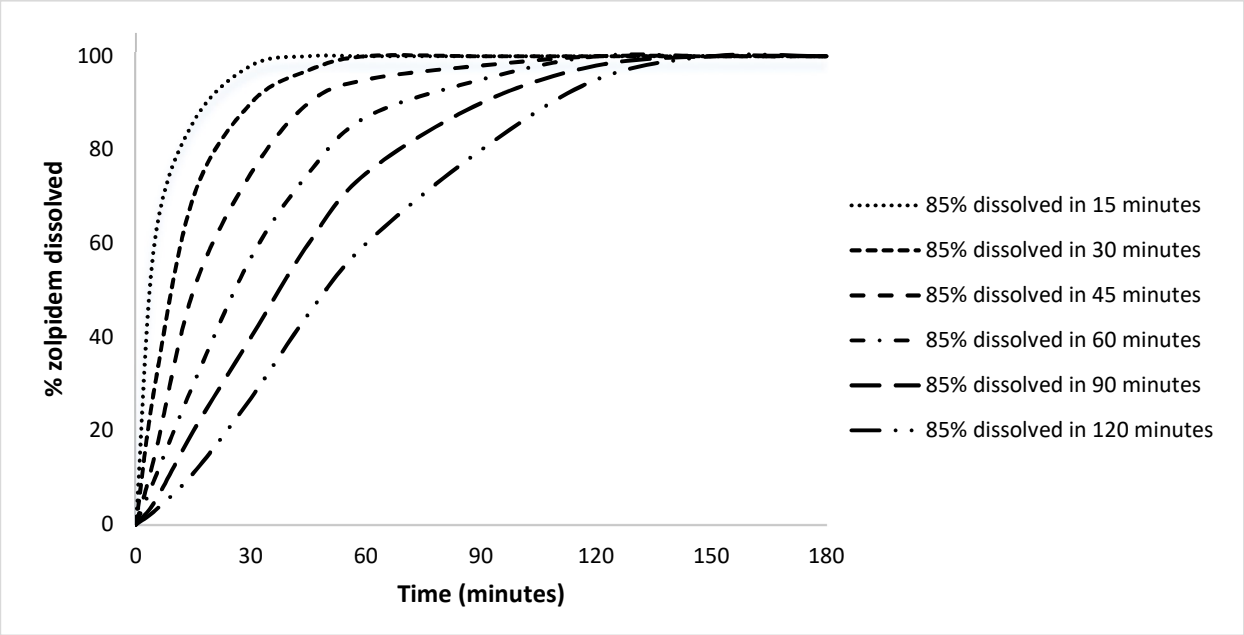


Figure 28. Simulated dissolution profile in pH 6.8 to determine the clinical relevant dissolution specification. Reproduced with permission from (Paraiso et al., 2020).

Due to zolpidem's highly soluble characteristics, the clinical relevant dissolution specification for zolpidem was evaluated by simulating different dissolution rates at pH 6.8 (Figure 28), ranging from 85% dissolved in 15 min to 85% dissolved in 120 min. These simulated fasted state dissolution profiles were then entered as the small intestinal dissolution profile in the ADAM module. The results for the PK and PD responses of zolpidem using the Simcyp® model outputs are demonstrated in Figure 29.

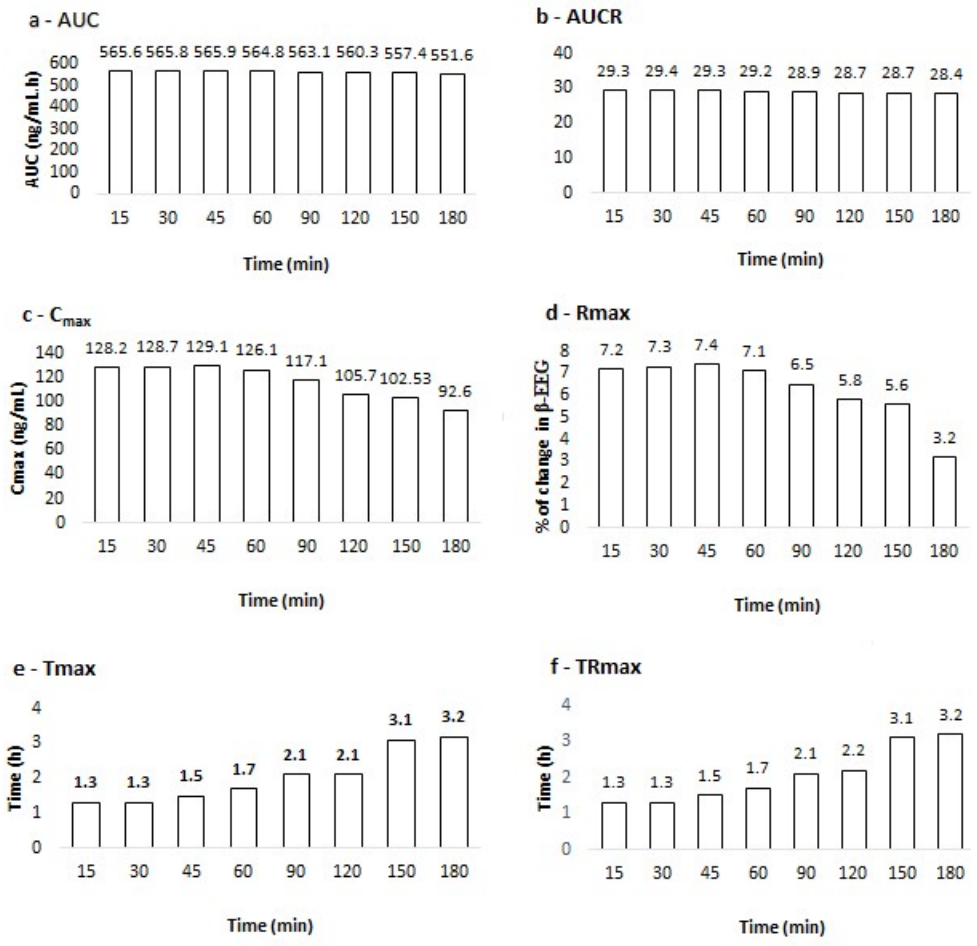


Figure 29. Predicted dose-response after imputing the simulated dissolution profile into the PBBM/PD model of zolpidem. a, c and e represent the pharmacokinetic parameters and b, d and f the pharmacodynamic parameters. Reproduced with permission from (Paraiso et al., 2020).

Figure 29 shows the pharmacokinetic and pharmacodynamic parameters values obtained after inputting the simulated dissolution profile into the PBBM/PD model in fasted state for zolpidem. On the one hand the results suggest that different dissolution rates would affect the magnitude of $AUC_{0–12h}$, AUCR, C_{max}, and R_{max} values by less than 10% when $Q \geq 85\%$ in 15, 30 or 45 min. On the other hand, when slowly dissolving tablets, with $Q \geq 85\%$ in 60 min were simulated, the T_{max} and TR_{max} were delayed to 1.7 h, which translates into an expected delay in the onset of hypnotic effect compared with the response elicited by simulating the case of very rapidly dissolving tablets, $Q \geq 85\%$ in 15 minutes. Therefore, the results suggest that only in the case of tablets with a slow dissolution of $Q \geq 85\%$ in 60 minutes or more would lead to clinically significant changes in the time at which the maximum PK and PD responses are observed.

4.4 – Discussion

4.4.1 - Clinically relevant dissolution specifications for zolpidem IR products

The clinically relevant dissolution specification is set by establishing the relationship between *in vitro* dissolution experiments (quality control or biorelevant) and *in vivo* studies. Although there is large variability in the gastrointestinal environment among humans and it is thus challenges to establish relationships between *in vitro* and *in vivo* data, biorelevant dissolution coupled with PBPK modeling platforms provides an important way forward to establishing such relationships (Hermans et al., 2017; Markopoulos et al., 2015a; Tsume et al., 2018). Drugs that belong to BCS class I and III immediate release drug products with fast/very rapid dissolution are considered low risk products, for which there is generally no need to establish an *in vitro/in vivo* correlation (IVIVC) (Hermans et al., 2017; Polli et al., 2008; Tsume et al., 2018). But although zolpidem is a BCS class I, it has a short half-life of elimination, which most probably contributes to its classification as a highly variable drug. Further, for hypnotic drugs like zolpidem, a rapid onset of action is clinically highly desirable. Therefore, for this drug in particular, it is important to set clinically relevant dissolution specifications based on the relationship between *in vitro* dissolution and clinical response.

Although standard IVIVC approaches recommended by the regulatory agencies and are often used to set the CRDS, zolpidem IR tablets are not amenable to classical Level A IVIVC. For this reason, we used the alternative approach consisting of PBBM model coupled with the PD response. This approach has the further advantage of linking the CRDS all the way to the PD response, whereas classical IVIVC stops at correlating the dissolution with the PK parameters. Dissolution media that can be used to set clinically relevant dissolution specification for drug products can be anywhere between Levels 0 and 3 in the classification according to Markopoulous, Andreas et al. (Markopoulos et al., 2015a).

Since zolpidem belongs to BCS class I and its dissolution is not affected by the gastrointestinal pH, theoretical dissolution curves at pH 6.8 (Level 0) with different dissolution rates (Figure 28) were implemented into the PBBM/PD model to generate the corresponding plasma and PD profiles of this drug. As shown in the simulation results (Figure 29), it appears that the PK and PD parameters would only be significantly impacted if the formulation has

disintegration / dissolution issues corresponding to Q ≥ 85% being reached after more than 45 minutes.

Guidance from EMA, FDA and ICH depicts the possibility of a BCS-based biowaiver for drugs that belong to BCS Classes I and III, for which solubility and dissolution rate it is not the limiting step for the drug absorption (EMA, 2014; FDA, 2017; ICH, 2018). With regard to dissolution performance, this waiving of *in vivo* studies approach is possible when the drug product dissolves rapidly for Class I drugs like zolpidem, releasing 85% of the drug content within 30 minutes in compendial media at pH 1.2, pH 4.5, and pH 6.8.(EMA, 2014; FDA, 2017; ICH, 2018). Comparing these limits to the PK/PD model results obtained in this study for zolpidem IR tablets, it suggests that the regulatory agencies could be more flexible with regard to the biowaiver criteria when zolpidem IR tablets are evaluated. According to the results obtained in our simulations, as long as 85% of the drug is released in 45 minutes or less, the impact on the PK and PD profiles of zolpidem would be minimal (<10%).

Although the number of publications using dissolution data in conjunction with PBPK modeling and simulation has significantly increased in the last years, attempts to extend simulations through to clinical relevance by linking the results to a PD model have been few. Cristofoletti and Dressman (2014) used a PBBM/PD model to demonstrate that changes in pharmacokinetic parameters do not produce a proportional change in the antipyretic and anti-inflammatory activity of the drug, such that even if two drug products are not bioequivalent with each other, they may still be therapeutically equivalent. This situation arises when the drug (in this case ibuprofen) has a flat dose-response relationship in the usual dosing range (Cristofoletti and Dressman, 2014). More recently, AbbVie used a PBBM modelling approach to broaden the dissolution criteria for Orilissa® (elagolix) tablets. According to the original specifications, two commercial batches failed to meet the dissolution acceptance criteria. But using the absorption modeling approach which was implemented from the DMPK model submitted to the FDA, the company was able to demonstrate to the regulatory agency, that even when the drug product releases the drug more slowly than the specifications require, there is no clinically significant effect on the exposure of the drug (FDA Quality Assessment for Elagolix Tablets, 2018). In a further example of using PBBM to set CRDS, Astra Zeneca proposed specifications for the particle size and dissolution rate of lesinurade from Zurampic® tablets that would ensure

appropriate clinical performance of each batch, by running virtual bioequivalence trials in the Gastroplus® PBPK platform (Pepin et al., 2016).

Even though there are just few examples which absorption PBPK modeling has been submitted to the regulatory agencies, with the improvement of *in vitro* experiments and software platforms this approach will be utilized more and more to describe the pre-absorptive and post-absorptive of drug formulation. Besides its application to setting CRDS, PBBM modeling may be useful to understand the crucial factors for the drug formulation performance in humans, information which can be used to guide the development of *in vitro* dissolution tests (Hermans et al., 2017).

4.5 - Conclusions

With the continuing evolution of PBPK platforms together with *in vitro* experiments, absorption physiologically based pharmacokinetics coupled with pharmacodynamics model is appearing as an alternative methodology for development of IVIVC relationships. The integration of *in vitro* quality control or biorelevant dissolution experiments with *in silico* methods such as PBBM or PBBM/PD is a promising tool to set clinically relevant dissolution specifications and thus link the pharmaceutical development to the patient.

In this work, clinically relevant dissolution specifications for zolpidem immediate release products were set, showing that as long as drug release can meet a specification of Q ≥ 85% in 45 minutes or less, the product will have acceptable pharmacodynamic characteristics. This result additionally suggests that the BCS-based biowaiver specifications (Q ≥ 85% in 30 minutes plus f2>50 or Q ≥ 85% in 15 minutes) could be more flexible for zolpidem IR products and potentially for other which belongs to BCS Class I drugs with short half-lives of elimination.

5 - Concluding Remarks

Computational oral absorption models, in particular PBBM models, provide a powerful tool for researchers and pharmaceutical scientists in drug discovery and formulation development, as they mimic and can describe the physiologically processes relevant to the oral absorption. PBBM models provide *in vivo* context to *in vitro* data experiments and allow for a dynamic understanding of *in vivo* drug disposition that is not typically provided by data from standard *in vitro* assays. Investigations using these models permit informed decision-making, especially regarding to formulation strategies in drug development. PBBM models, but can also be used to investigate and provide insight into mechanisms responsible for complex phenomena such as food effect in drug absorption. Although there are obviously still some gaps regarding the *in silico* construction of the gastrointestinal environment, ongoing research in the area of oral drug absorption (e.g. the UNGAP, AGE-POP and InPharma projects) will increase knowledge and enable improvement of these models.

PBBM can nowadays provide an alternative approach to the development of *in vitro–in vivo* correlations. The case studies presented in this thesis demonstrate how PBBM can address a mechanistic understanding of the negative food effect and be used to set clinically relevant dissolution specification for zolpidem immediate release tablets. In both cases, we demonstrated the importance of integrating drug properties with physiological variables to mechanistically understand and observe the impact of these parameters on oral drug absorption.

Various complex physiological processes are initiated upon food consumption, which can enhance or reduce a drug's dissolution, solubility, and permeability and thus lead to changes in drug absorption. With improvements in modeling and simulation software and design of *in vitro* studies, PBBM modeling of food effects may eventually serve as a surrogate for clinical food effect studies for new doses and formulations or drugs. Furthermore, the application of these models may be even more critical in case of compounds where execution of clinical studies in healthy volunteers would be difficult (e.g., oncology drugs).

In the fourth chapter we have demonstrated the establishment of the link between biopredictive *in vitro* dissolution testing (QC or biorelevant method) PBBM coupled with PD modeling opens the opportunity to set truly clinically relevant specifications for drug release.

This approach can be extended to other drugs regardless of its classification according to the BCS.

With the increased adoption of PBBM, we expect that best practices in development and verification of these models will be established that can eventually inform a regulatory guidance. Therefore, the application of Physiologically Based Biopharmaceutical Modelling is an area with great potential to streamline late-stage drug development and impact on regulatory approval procedures.

6 - Deutsche Zusammenfassung

Nach der peroralen Einnahme von einem Arzneimittel muss der Arzneistoff aus der Darreichungsform freigesetzt und aufgenommen werden, bevor er über den größen Blutkreislauf in die verschiedenen Organe und Gewebe im Körper verteilt werden kann. Bestenfalls wird durch diese Verteilung eine Konzentration des Arzneistoffs am Wirkort erreicht, die den gewünschten therapeutischen Effekt ausübt. In der Vergangenheit wurde die klassische Pharmakokinetik verwendet, um den Verlauf der Arzneistoffkonzentration im Blutkreislauf zu beschreiben. In den letzten 20 Jahren hat die physiologisch basierte pharmakokinetische (PBPK) Modellierung der klassischen Pharmakokinetik für diesen Zweck langsam überholt, denn die PBPK ermöglicht eine genauere Beschreibung der verschiedenen Erreignisse, die die Aufnahme, Verteilung, Metabolismus und Ausscheidung des Arzneistoffes in und aus dem Körper beeinflussen. In jüngerer Zeit begannen manche Forschungsgruppen sogar die PBPK mit der Pharmakodynamik zu kuppeln, um eine komplette Beschreibung der Erreignisse von der Einnahme bis zur Wirksamkeitsprofile zu erreichen.

Physiologisch basierte biopharmazeutische Modelle (PBBM), die Freisetzungsdaten mit PBPK-Modellen kombinieren, bieten die Möglichkeit, die Wechselwirkung unter verschiedenen Aspekten des Arzneistoffs, der Formulierung und der Physiologie zu untersuchen. Dadurch können sie die Entwicklung von peroralen Darreichungsformen sehr gut unterstützen. Mithilfe Algorithmen zur Beschreibung der verschiedenen Prozessen im Körper können solche PBBM-Modelle die physiologisch relevanten Schritte in der peroralen Resorption simulieren. Dadurch bereitet PBBM Modellierung einen *in vivo* Kontext der *in vitro* Daten und folglich ein dynamisches Verständnis des Einflusses der *in vitro* Freisetzung auf die *in vivo* Disposition des Arzneistoffes, welches allein mit den *in vitro* Daten nicht möglich ist. PBBM Modelle bereiten viele Möglichkeiten, einschließlich können sie die Entwicklung von Formulierungstrategien für Arzneistoffe, die Erklärung von Food-Effekten und die Setzung von Spezifikationen für Arzneimitteln unterstützen.

Gegenstand dieser Dissertation war zu ermitteln, welche Rolle die verschiedenen physikochemischen Eigenschaften, die GI-Physiologie, die post-absorptive Pharmakokinetik und

die Pharmakodynamik in das Wirksamkeitsprofil von Zolpidem spielen. Zolpidem ist ein Arzneistoff, der als Schlafmittel bzw. Einschlafmittel eingesetzt wird. Daher ist der Verlauf der Zolpidemkonzentration am Wirkort essentiell für eine erfolgreiche Therapie – der Eintritt einer wirksamen Konzentration muss schnell genug sein, darf aber nicht zulange andauern um einen "Hangover-Effekt" zu vermeiden.

Zolpidem ist eines der meist verschriebenen Schlafmittel weltweit. Interessanterweise zeigt es einen negativen "Food-Effekt", das heißt, die Einnahme nach einer Mahlzeit (typischerweise für Schlafmittel wie Zolpidem nach dem Abendessen) führt zu einer verlangsamten Steigerung der Konzentration im Blutkreislauf und in vielen Probanden zu einer niedrigeren gesamten Aufnahme als die Einnahme in nüchternem Zustand. Ein weiterer Gegenstand der Dissertation war die Ermittlung der Gründe für den negative Food-Effekt von Zolpidem. Die Darreichungsform von Zolpidem, die für diesen Zweck ausgewählt wurde, waren die schnell freisetzende Tabletten von Zolpidem (Marktname Stilnox®).

Im ersten Schritt wurden Stilnox® Tabletten unter biorelevanten Bedingungen freigesetzt. Diese *in vitro* Untersuchungen wurden entworfen, um die gastrointestinalen Flüssigkeiten vor und nach dem Essen nachzuahmen. Die Freisetzung von Zolpidem aus den kommerziellen Tabletten wurde zuerst in standard biorelevanten Medien untersucht. Um die Fluide im Magen zu vertreten wurde FaSSGF verwendet, um den präprandialen Zustand zu simulieren, während FeSSGF in drei Versionen (gerade nach der Mahlzeit; während der Hauptverdauungsphase der Mahlzeit im Magen und spat in der Verdauung der Mahlzeit) verwendet wurde, um den postprandialen Zustand zu simulieren. Um die Fluide im Dünndarm zu vertreten wurde FaSSIF-V2 (präprandial) und FeSSIF-V2 (postprandial) verwendet.

Zusammen mit den physicochemischen Eigenschaften und pharmakokinetischen Parametern für die Verteilung, Metabolismus und Ausscheidung nach i.v. Verarbreichung aus der offener Literatur wurden die Freisetzungsprofile verwendet, um ein PBPK-Modell für Zolpidem nach Einnahme im prä- und postprandialen Zustand zu bilden. Die simulierten Plasmaprofile wurden dann mit Literaturdaten für die Pharmakokinetik verglichen, um die Genauigkeit des Modells zu testen.

Um weiterhin das Modell zu verifizieren, wurden Daten von einer Studie genommen, die von Sanofi-Aventis durchgeführt aber nie in der Literatur veröffentlicht wurde. Die klinische Studie wurde in 30 gesunden männlichen Probanden in zwei Phasen durchgeführt. In der ersten Phase wurden den Probanden jeweils eine Stilnox® 10 mg Tablette zusammen mit 240 mL Wasser im nüchternen Zustand verabreicht. Nach einer Woche wurde die zweite Phase durchgeführt, in der den Probanden eine Stilnox® 10 mg Tablette zusammen mit 240 mL Wasser verabreicht wurde, dieses mal nach Einnahme einer Mahlzeit. In beiden Phasen wurden Plasmaproben entnommen, um das pharmakokinetische Profil von Zolpidem zu bestimmen. Von Sanofi-Aventis wurden die durchschnittlichen Plasmaspiegelprofile sowie die Zusammenfassungs-Statistik vorgelegt. Mit dem PBPK-Modell wurden die Ergebnisse dieser Studie nach Verabreichung in den prä- und postprandialen Zuständen adäquat simuliert.

Als nächster Schritt wurden weitere *in vitro* Experimente durchgeführt, um mögliche Gründe für den negativen Food-Effekt von Zolpidem zu ermitteln: die erhöhte Viskosität der Magenfluide im postprandialen Zustand, der Fettgehalt der Mahlzeit und die Verlangsamung der Magenentleerung nach Einnahme einer Mahlzeit. Um den Effekt der Viskositätserhöhung auf die Freisetzung von Zolpidem aus Stilnox® 10 mg Tabletten zu evaluieren, wurde Hydroxypropylmethylcellulose (HPMC) in Konzentrationen von 0,5% und 1,4% dem postprandialen Freisetzungsmedium zugesetzt. Jedoch war die Freisetzung so deutlich verlangsamt, dass den simulierten Plasmaspiegelprofilen die tatsächlichen Plasmaprofilen grob unterschätzten.

In einer weiteren Serie von *in vitro* Experimenten wurde der Einfluss des Fettgehalts auf die Freisetzung in postprandialen Freisetzungsmedien untersucht. Hierfür wurden Konzentrationen von Lipiden von bis zu 7% den Medien zugesetzt. Nach Eingabe der verschiedenen Freisetzungsprofile in das PBPK-Modell, konnte kein signifikanter Unterschied in den Simulationen gesehen werden. Diese Ergebnisse führen zur Schlußfolgerung, dass der Fettgehalt keinen direkten Einfluss auf die Pharmakokinetik von Zolpidem hat. Allerdings ist es bekannt, dass mit erhöhtem Fettgehalt die Magenentleerungsrate entschleunigt wird, welches zu einer niedrigeren maximalen Konzentration des Arzneistoffs im Plasma führt.

Aus diesem Grund wurde eine Parameter-Sensitivitäts-Analyse (PSA) für die Magenentleerungsrate im Modell durchgeführt. Die PSA zeigte eine ausgeprägte Sensitivität des Plasmaspiegels von Zolpidem auf die Magenentleerungsrate in Bezug auf Rate und Ausmaß der Resorption. Insgesamt zeigten die Ergebnisse, dass durch eine Kombination von biorelevanten *in vitro* Experimenten und PBPK-Modellierung die Gründe für den Food-Effekt erklärt werden können. Dieser Ansatz kann natürlich auch verwendet werden, um die Gründe für den Food-Effekt für andere Arzneistoffen zu identifizieren.

Als letzter Gegenstand der Dissertation wurde ein PBPK/PD Modell für Zolpidem generiert, um klinisch relevante Spezifikationen für die Freisetzung von Zolpidem aus kommerziellen Tabletten zu setzen. Dafür wurden die *in vitro* Freisetzungsprofile von Zolpidem aus Stilnox® Tabletten, die für den Food-Effekt-Ermittlung aufgesetzte PBPK-Modell für Zolpidem und quantitative Ergebnisse for die Pharmakodynamik von Zolpidem, basierend auf der %-Änderung in ß-EEG-Amplitude, aus der offenen Literatur eingesetzt. Da Daten für die Pharmakodynamik nur nach Verabreichung einer "leichten" Mahlzeit etabliert wurden, musste das daraus entstehenden PD-Modell auf den nüchternen Zustand extrapoliert werden. Die PK-Simulationen mit diesem Modell wurden mit externen Daten aus der Literatur verifiziert.

Infolgedessen wurden im PBPK-PD Modell verschiedene Freisetzungsprofile (von 85% in 15 Minuten bis zu 85% in 120 Minuten freigesetzt) verwendet, um die PK und PD von Zolpidem unter diesen Freisetzungs-Bedingungen zu simulieren. Die Simulationen zeigten, dass klinisch relevante Änderungen im Zeitpunkt der maximalen PK und PD nur dann resultieren würden, wenn die Tabletten Zolpidem sehr langsam freisetzen ($Q \geq 85\%$ in ≥ 60 Minuten). Verglichen mit den Voraussetzungen für eine *in vitro* Prüfung der Bioäquivalenz (das so-genannte BCS-basierte Biowaiver), $Q \geq 85\%$ in ≤ 30 Minuten, ist diese Spezifikation weniger streng – ohne die Wirksamkeit in Frage zu setzen. Sinnvoll ist daher, nicht nur die Berücksichtigung der Pharmacodynamik in der Setzung von Freisetzungs-Spezifikationen sondern auch in der Interpretation der Freisetzungs-Ergebnisse in Rahmen der Bioäquivalenz.

In dieser Dissertation wurden Ergebnisse von *in vitro* Experimenten mit *in silico* Modellen gekuppelt, um das Verhalten von Zolpidem Tabletten *in vivo* zu beschreiben. Weiterhin konnte der prinzipielle Grund für den negative Food-Effekt von Zolpidem identifiziert werden. Drittens wurden *in vitro* Freisetzungsdaten und das PBPK-Modell mit einem PD Modell

kombiniert, um klinisch relevante Freisetzungs-Spezifikationen für Stilnox® Tabletten zu setzen. Das Verhältnis zwischen Wirkstofffreigabe aus dem Arzneimittel und der Wirksamkeit eines Arzneistoffs wurde in dieser Dissertation zum ersten Mal anhand biorelevanten Medien und PBPK-PD-Modellen etabliert. Dieser Ansatz zu klinisch relevanten Freigabe-Spezifikationen kann zukünftig auch von anderen Arbeitskreisen in der pharmazeutischen Industrie und den Zulassungsbehörden umgesetzt werden.

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