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Application of Design of Experiments (DoE) to Dissolution Method Development in Pharmaceutical Industry

Renato Cesar de Souza*

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Adviser: Prof. Dr. Edenir Rodrigues Pereira Filho
*Company: Eurofarma

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Assinaturas dos membros da comissão examinadora que avaliou e aprovou a Defesa de Dissertação de Mestrado do candidato Renato Cesar de Souza, realizada em 28/04/2017:

Prof. Dr. Edenir Rodrigues Pereira Filho
UFSCar

Prof. Dr. Rodrigo César da Silva
EUROFARMA

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UFSCar

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List of Acronyms

ADME	Absorption, Distribution, Metabolism and Excretion
ANOVA	Analysis of Variance
ANVISA	Agência Nacional de Vigilância Sanitária
AUC	Area under the Curve
BCS	Biopharmaceutical Calssification System
BE	Bioequivalence
CAT	Compartmental Absorption and Transit model
CL	Clearence
Cmax	Maximun concentration
CR	Contolled Release
DoE	Design of experiment
ER	Extended release
FCC	Face Centered Cubic
FDA	Food and Drug Administration
FMEA	Failure Mode and effect Analysis
HIV	Human Immunodeficiency Virus
IVIVC	*In vivoIn vitro* correlation
k_a	Absorption constant
k_{el}	Elimination constatn
LoF	Lack of Fit
OFaT	One Factor at time
pAUC	Partial Area under the Curve
RPN	Risk Priority Number
RS	Reference standard
USP	United States Pharmacopeia
V_d	Distribution Volume

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RESUMO

APLICAÇÃO DE DELINEAMENTO DE EXPERIMENTOS (DOE) PARA DESENVOLVIMENTO DE MÉTODO DE DISSOLUÇÃO NA INDUSTRIA FARAMCÊUTICA

O objetivo desse trabalho foi aplicar uma metodologia de delineamento de experimentos no desenvolvimento de um método de dissolução durante a fase inicial de desenvolvimento de um produto genérico na indústria farmacêutica. O produto modelo utilizado foi o Zolpidem CR, que contém 12,5 mg de hemitartarato de zolpidem por comprimido. Antes de aplicar a metodologia de delineamento de experimentos, fez-se uma Análise de Modos de Falhas e seus Efeitos (*FMEA*) para escolher as variáveis mais importantes. Quatro variáveis foram consideradas as mais importantes para afetar a taxa de liberação do produto, sendo elas, aparato, velocidade de rotação, volume e pH do meio de dissolução. As variáveis selecionadas foram avaliadas pelo Planejamento Fatorial Completo com o objetivo de identificar os principais fatores que afetam a dissolução do produto. Os fatores mais críticos, pH do meio de dissolução velocidade de rotação e volume de meio foram então utilizados para construir um modelo de superfície de resposta, a fim de modelar e todas as variáveis e compreender suas interações. O objetivo nessa fase inicial de desenvolvimento do produto foi alcançar uma dissolução *in vitro* que seja semelhante a dissolução *in vivo*, portanto todas as respostas foram baseadas na taxa de absorção *in vivo* do medicamento referência previamente descrito na literatura. Os perfis médios de concentração plasmática de Zolpidem em relação ao tempo foram deconvoluidos pelo método de Wagner Nelson e a curva deconvoluida foi modelada pelo modelo Logistico. Os parâmetros da equação foram utilizados como resposta de referência. As respostas monitoradas foram aporcentagem dissolvida em 0,25 e4,0h e os parâmetros α, β e R^2 do modelo logístico. A confiabilidade do modelo e a significância dos efeitos estudados foram avaliados pela análise de variância (ANOVA). Os métodos de desejabilidade e sobreposição de superfície de resposta foram utilizados para determinar a melhor condição de dissolução. A condição de dissolução de aparato cesto com velocidade de rotação de 50 rpm e meio de HCl 0,01 M (pH 2,0) e volume de 500 mL mostrou-se satisfatória e atendeu os critérios de desejabilidade estabelecidos. O delineamento de experimentos mostrou ser uma metodologia mais eficiente comparada ao método

tradicional de um fator por vez (OFaT), reduzindo o número de experimentos, número de amostras e tempo de desenvolvimento do método. A compreensão dos fatores que afetam a taxa de liberação do produto e as conclusões obtidas se mostraram significativamente superiores ao método tradicional.

ABSTRACT

APPLICATION OF DESIGN OF EXPERIMENTS (DOE) TO DISSOLUTION METHOD DEVELOPMENT IN PHARMACEUTICAL INDUSTRY

The aim of this work is to apply design of experiment methodologyin the development of a dissolution method during the early phase ofdevelopment of a generic product in pharmaceutical industry. The model drug used during the development was the biphasic release product Zolpidem CR, which contains 12.5 mg of Zolpidem Hemitartrate per tablet. Previous to experimental design, a Failure Mode and Effect Analysis (FMEA)was performed to choose the most important variables.Four variables were considered the most importants to affect product release rate, which wereapparatus, rotation speed, volume and pH of the dissolution media. The variables selected were analysed by the full factorial design with the objective to identify the principal factors. The most critical factors were then used to construct the response surface model in order to model all variables and understand its interaction. The objective at this phase of product development was to reach an*in vitro* dissolution similar to the one *in vivo*, therefore all the responses were based on the *in vivo* absorption rate of the reference product obtained from literature. The mean Zolpidem plasma concentration-time profiles was deconvoluted by Wagner Nelson method and the deconvoluted curve was modeled to Logistic model. The parameters of the equation wereused as reference response. The responses monitored were the percentage dissolved in 0.25, 4.0 h, and the α ,β and R^2 parameters of logistic equation. The model reliability and significance of the factors studied was evaluated by the analysis of variance (ANOVA). Two methods were used to determine the best dissolution condition, the first one was the Desirability Function and the second was the response surface sobreposition. The dissolution condition of basket with a rotation speed of 50 rpm and dissolution media of HCl 0,01 M (pH 2,0) with a volume of 500 ml was choosen as the condition that satisfied the desirability criterias stablished at the beginning of the experiment. The design of experiment methodology proved to be a more efficient methodology comparade to the traditional one factor at time (OFAT) method, reducing the number of experiments, number of samples and time to method development. The comprehension of the factors that affect release rate of the product and conclusion showed to be significantly superior to the traditional method.

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INTRODUCTION

1. - INTRODUCTION

The rate at which a drug is released from a dosage form and goes into solution is important for absorption kinetics. Therefore, when the absorption of a drug is dissolution limited, tests related todissolution evaluation finds application in pharmaceutical industry as a tool for development, usually appliedto guide formulation development and to select an appropriate dosage for *in vivo* testing (KRÄMER; GRADY; GAJENDRAN, 2005). The creative use of dissolution technique can speed up the formulation development, enabling prompt identification of potential problems in drug release rate (JOSHI et al., 2008).

Development of dissolution methodhave been carried outby one-factor-at-a-time (OFaT) experiments, where one factor or variable is investigated, while the others are kept fixed (CZIROM, 1999).This strategyis inefficient and may lead to false optimun conditions (ANTONY; CHOU; GHOSH, 2003).

The design of experiment (DoE) is the most effective way to plan and execute the experiments, in order to determine the effect of two or more factors on the response once this chnique requires minimumexperimentation and time, thus providing to be far more efficient and cost effective than conventional methods of product development.

Therefore, the current study aimed to apply the design of experiment methodology to the development of a dissolution method, evaluating*in vitro* factors that affect the dissolution rate and model this factors in a way to choose the best dissolution condition that could be correlated the *in vivo* data. The comercial product used was Stilnox® CR 12.5 mg of Zolpidem Tartrate from Sanofi-Aventis.

Zolpidem tartrate Extended Release (ER) formulation consists of a coated two-layer tablet. One layer releases its drug content immediately and the other layer allows a slower release of the additional drug content. This product is indicated for the treatment of insomnia characterized by difficulties with sleep onset and/or sleep maintenance (SANOFI-AVENTIS, 2012).

The development of dissolution method for this product is an interesting way to evaluate two different release mechanisms in a single dosage form.

GOALS

2. - GOALS

The main goal was to evaluate the applicability of DOE in dissolution method studies during an early phase of a generic drug product development.

It was expected to prove the advantages of the use of the DOE methodology instead of OFaT method, considering the economy of time, samples and the best capacity of understanding *in vitro* drug release factors that affect dissolution rate of a product.

LITERATURE REVIEW

3. –LITERATURE REVIEW

3.1. – Therapeutic Equivalence

Two drug products are considered therapeutic equivalents if they have the same efficiency and safety effects after administration at the same molar dose. Such effects are assessed by bioequivalence, pharmacodynamics, clinical trials and / or *in vitro* studies (BRASIL, 2007a). In summary, it means that two drug products are considered equivalents if their therapeutic response are equal.

In the development of pharmaceutical drugs, it is clear that success of therapeutic effect or equivalence of the drug is not solely due to the effect of the drug molecule itself. The therapeutic response of a drug administered through gastrointestinal route depends on a lot of properties that can be summarized as(1) the pharmaceutical phase, in which a drug molecule is administered to the body; (2) the pharmacokinetic phase, during which the drug circulates in the body to its target receptor; and (3) the pharmacodynamics phase, in which the drug molecule interacts with the target receptor (TURNER, 2007). A general scheme describing this dynamic relationship is illustrated in FIGURE 1.

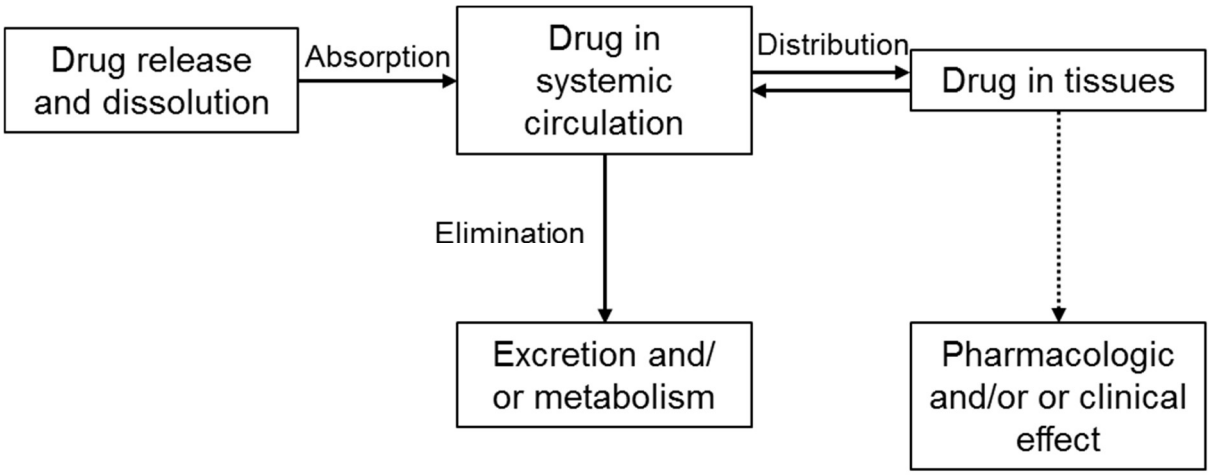


FIGURE1.Scheme demonstrating the dynamic relationship between the drug, the drug product, and the pharmacologic effect.This figure was adapted from SHARGEL; YU, 2017.

3.1.1 Pharmaceutical Phase

The pharmaceutic phase is the first phase of drug action. As the orally administered pharmaceutical dosage form passes through the human gastro-intestinal (GI) tract, drug should be released from the dosage form and be available in solution at or near the optimal site for drug absorption to occur (GUPTA; ROBINSON, 1992). For some drugs the absorption step is so fast that the rate at which the drug enters the bloodstream is almost totally dependent on the rate at which the drug dissolves. The absorption of such drugs is named dissolution limited (STRANDGARDEN et al., 1999; LEVY and HOLLISTER, 1965). Therefore, the rate at which the drug is released from a dosage form and goes into solution is important for the kinetics of drug absorption.

In vivo dissolution rate is affected by two factors: (1) the physicochemical properties of drug and (2) drug product processing and formulation factors. The Nernst and Bruner equation (Eq. 1) published in 1904, which is a modification of Noyes-Whitney equation, can be used to identify the important factors that affect drug dissolution kinetic.

$$\frac{dC}{dt} = \frac{DS}{Vh}(Cs - C) \qquad (1)$$

where dC/dt is the dissolution rate, D is the diffusion coefficient, h the thickness of the difusion layer, V is the volume of the dissolution medium, S is the surface area available for dissolution, Cs is the saturation solubility of the drug and C is the amount of dissolved drug (DOKOUMETZIDIS; MACHERAS, 2006). There are many physicochemical and physiological factors that can have a great influence on the factors in Eq. (1) and therefore on the dissolution rate.

The crystaline form of a drug is an example of how this physicochemical factor affect the saturation solubility term (Cs) of the equation 1. Many drugs crystallize through different forms, each one having a own energy and thereby differing in physicochemical properties such as melting point, solubility, heat of fusion, density, refractive index (HÖRTER; DRESSMAN, 2001). Elquidra et al. (2004) investigated the effect of polymorphism on *in vitro-in vivo* properties of Carbamezapine. Three different polymorphs and a dihydrate of carbamezapine were obtained and the conventional tablets of these crystalline forms at the dose of 200

mg were prepared. The authors observed that the dissolution rate was significantly changed by the polymorphic forms of Carbamezapine. In the same way, Atorvastatin is unstable and the hydroxy acid form is converted to lactone form that is 15 times less soluble than the hydroxil acid form (KERC, 2006). This instability of atorvastatin calcium leading to poor solubility (0.1mg/mL) was the main cause for low bioavailability of the drug after oral administration as the absolute bioavailability of atorvastatin calcium is only 14% (KHAN; DEHGHAN, 2011).

The surface area is usually affected by the particle size of a drug. The dissolution rate is directly proportional to the surface area of the drug, which in turns increases with decreasing particle size. The effect of particle size on the dissolution behaviours of poorly soluble drugs was examined by Chu et al. (2012). The dissolution rate of hydrochlorothiazide, aceclofenac and ibuprofen were evaluated with different particle size for each drug. The authors observed that the specific surface area increased with decreasing particle size of the drug, resulting in an increase in dissolution rate.

The diffusion coefficient, D, which is in part related to solvent viscosity, will decrease with increasing solvent viscosity, and decreasing dissolution rate. Some types of stirring agitation during dissolution will decrease the stagnant layer (h) by removing solute molecules faster from the particle surface, increasing dissolution rate.

Most of the factors discussed until now are related to physicochemical properties of drug molecule, but factors related to drug product formulation and process are as important as the physicochemical factors. Some dosage forms are formulated to modulate the drug input (i.e., dissolution or absorption) in the intestinal tract to achieve a predefined plasma profile. Common modes of drug release include delayed release (e.g., using an enteric coating), size-specific or timed release (e.g., for colonic delivery), extended release (e.g., zero-order, first order), or programmed release (e.g., pulsatile) (QIU; ZHOU, 2011). Omeprazol and Pantoprazol are examples of labile molecules in acidic pH of stomach which are formulated with a gastro-resistent coating to prevent its degradation and release the drug in a less acidic enviroment (JUNGNICKEL, 2000).

In some cases, the interaction of excipients in formulation may change release rate. The influence of spray dried lactose, microcrystaline cellulose and partially pregelatinized starch on drug release from hydroxypropylmethylcellulose

matrices was studied by Levina and Rajabi-Siahboomi (2004). It could be observed that using lactose or microcrystaline cellulose in the formulation resulted in faster drug release profiles, while partially pregelatinizes maize starch contributed to retardation of drug release.

Similarly to the physical features of the drug, many physiological parameters can also play na important role determining the dissolution rate. The solubility of the drug is not only a function of its crystalinity and lipophilicity, but also depends on the medium into which it must dissolve. In the gastrointestinal tract, surfactants, pH, buffer capacity, and food components can all play a role in determining the local solubility of the drug. Fosamprenavir, a drug approved for the treatment of human immunodeficiency virus (HIV), exhibits pH-dependent solubility, with maximal solubility pH at 3.3, and reduced solubility at higher pH values. The effect of antacids and ranitidine on plasma pharmacokinetics after the administration of Fosamprenavir showed a reduction on drug absorption, caused by the lower solubility of the drug at higher pH (FORD et al., 2004). The same effect was observed by YEH et al (1998) on the pharmacokinetic of Indinavir, a weak base with pH dependent solubility.

The boundary layer thickness is dependent on the hydrodynamics, which can be interpreted in terms of gastrointestinal physiology, as the mixing patterns and flow rates in the gastrointestinal tract.

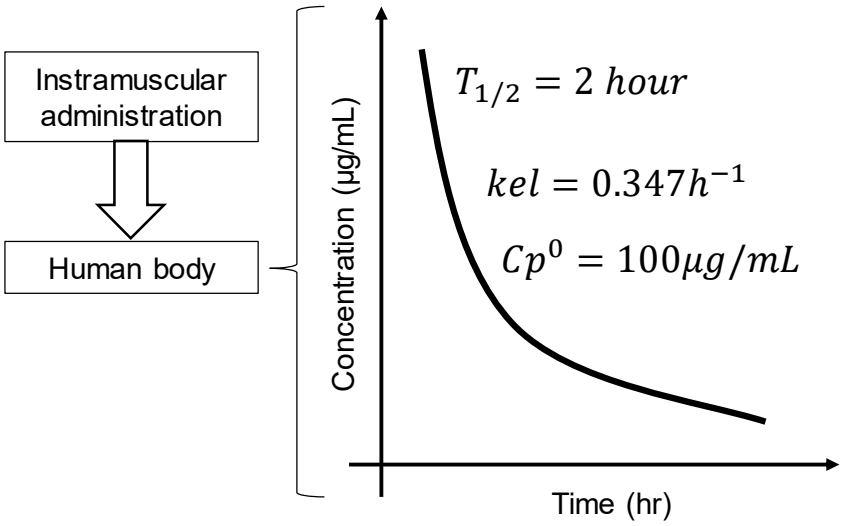
The concentration of the drug already in solution, C, has an influence on the driving force for dissolution, which results from the difference between the solubility and the concentration in the solution. Highly permeable drugs will be quicly absorbed and therefore will stay at lower concentrations in solution, thus maintaining a maximal driving force for dissolution. Therefore, the permeability of the gut wall to the drug can also indirectly affect dissolution rate of the drug (HÖRTER; DRESSMAN, 2001).

3.1.2 The Pharmacokinetic and Pharmacodynamic Phase

The pharmacokinetic phase describes the time course and position of a drug in the body, based on its absorption, distribution, metabolism and elimination (RATAIN; PLUNKETT, 2017). It is the measure of the rate (kinetics) of absorption, distribution, metabolism and excretion (ADME).

Drug effect is often related to its concentration at the site of action, but the measurement of drug at these sites is not practical. Changes in the plasma drug concentration reflect changes in drug concentration at the site of action, thus, it's possible to measure the amount or the concentration of drugs in blood, urines or other fluids or tissues at different times after the administration. With this data, much information can be obtained on drug absorption, path of drug molecules in blood, tissues and finally on the drug elimination (SPRUIL et al., 2009; URSO; BLARDI; GIORGI, 2002).

When the drug concentration is continuously measured in the plasma and its concentration is plotted against time, a graph known as plasma concentration/time curve is obtained. This graph is very useful, since it's possible to obtain information related to distribution volume (Vd), clearance (CL), elimination half-life ($T_{1/2}$), elimination constant (k_{el}) and area under the curve (AUC), in cases where the drug is orally administraded, it's possible to obtain the absorption constant (k_a), absorption half-life (t), maximum concentration (Cmax) and time necessary to reach maximum concentration (T_{max}) (Figures2 and 3).



$$Vd = \frac{Dose}{Co}$$

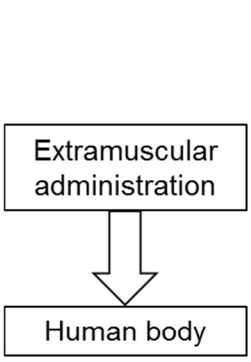
$$CL = kel.Vd$$

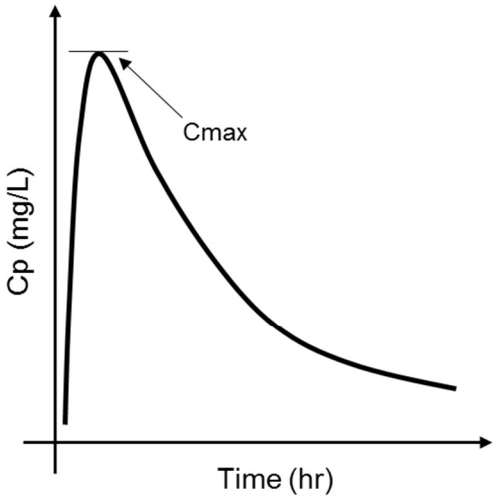
$$T_{1/2\beta} = \frac{0.693}{kel}$$

$$AUC_{0-inf} = \frac{Co}{kel}$$

$$C(t) = Co.e^{-kel.t}$$

FIGURE2. Main equations used for determination of pharmacokinetic parameters after intramuscular administration. Vd= distribution volume; CL- clearance, T1/2= elimination half-life; AUC= Area under the curve. Adapted from Campos (2008).





$$Vd = \frac{F \cdot Dose}{AUC.kel}$$

$$CL = \frac{F \cdot Dose}{AUC}$$

$$T_{1/2\beta} = \frac{0.693}{kel}$$

$$AUC_{0-inf} = \text{AUC}$$

$$AUC_{0-inf} = \frac{\text{ka} \cdot F \cdot Dose}{Vd(ka - kel)} \cdot \left(e^{-kel \cdot t} - e^{-ka \cdot t}\right)$$

FIGURE 3.Main equations used for determination of pharmacokinetic parameters after extramuscular administration. Vd= distribution volume; CL- clearance, T1/2= elimination half-life; AUC= Area under the curve.Adapted from Campos (2008).

To simplify the body process, mathematical principles are applied to describe pharmacokinetic data. A model of body is selected, usually the compartmental model. Compartmental models are categorized by the number of compartments needed to describe the drug's behaviour in the body and can be one-compartment, two-compartment, and multicompartment models. However, when the goal is only the analysis of Cmax and AUC, a non compartmental model can be applied. This model is suggested to the analysis of data obtained from bioequivalence study (BRASIL, 2006).

Pharmacodynamic is defined as a measure of the time course of pharmacological response to the presence of a given drug. Since it's the final step responsible by the terapheutic response of a drug, changes in pharmaceutical or pharmacokinetic phase may modifypharmacological response.

3.2 Generic Drug Products

A generic drug is identical to a brand name drug in dosage form, safety, strength, route of administration, quality, performance, characteristics and intended use (BRASIL, 1999).

In order to prove that a generic drug is identical to a brand-name drug it's necessary to perform the bioequivalence test (BRASIL, 2007). In a bioequivalence trial, systemic drug levels are measured following administration of both the brand-name and generic drug and bioequivalence is declared when (1) there is no significant difference between the generic drug and the reference one in the rate and (2) extent to which the active ingredient becomes available when administered at the same dose under similar conditions and in an appropriately designed study. Two drug products are considered bioequivalent if the entire 90% confidence interval (CI) of key pharmacokinetic parameters, such as peak concentration (Cmax) and area under the curve (AUC) lie between 80% and 125% of the value (BRASIL, 2006). An example of this criteria evaluation is represented on Figure 4.

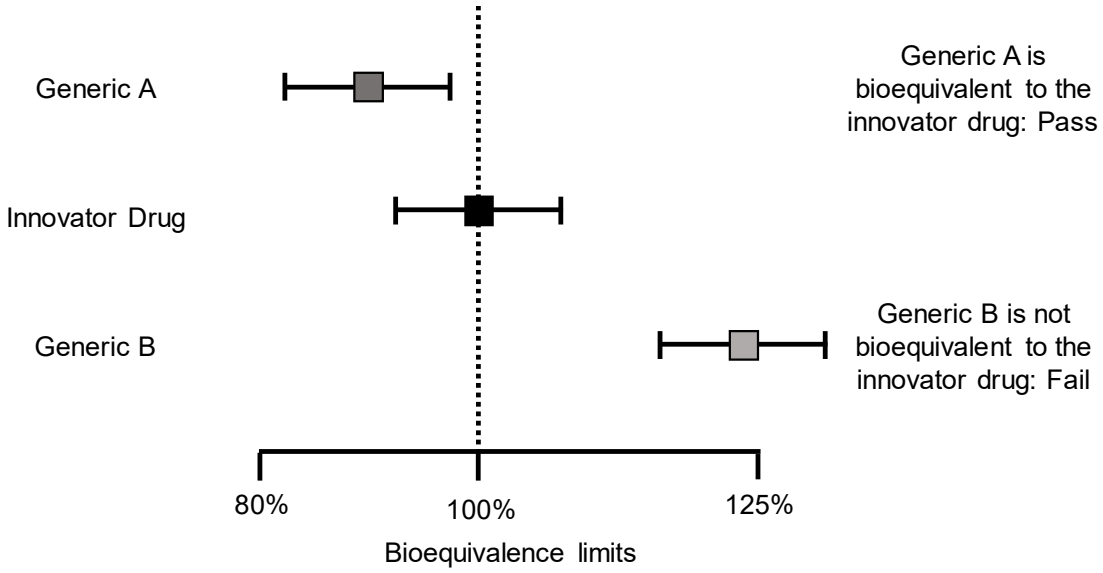


FIGURE 4.Example of evaluation of bioequivalnece criteria for two gereneric drugs. The generic A is bioequivalent to innovator product since 90% confidence interval lie whithin 80% and 125%. The generic B is not bioequivalent to inovator product.

The pharmaceutical industry interested in to develop a generic product need to develop a formulation that deliver the main active ingredient at the same rate and extend of the brand-name product, in order to have the same effectiveness.

Once that many factors related to formulation or physicochemical properties of a drug may affect these pharmacokinetic parameters, the development of a generic product is not a simple task, because even if the reference drug product is known qualitatively and quantitatively, there are several factors that can affect dissolution rate of the product that must be evaluated (VIVIAN, 2017).

Ever since dissolution is known to have a significant effect on bioavailability and clinical performance, and employining the bioequivalence test in a routine basis is not possible due to certain obvious reasons, the compreension and evaluation of *in vitro* dissolution has become one of the most important tests in drug product development and manufacturing as well as in regulatory assessment of drug product quality (LEE; RAW; YU, 2008).

3.3 The Biopharmaceutical Classification System

The biopharmaceutical classification system (BCS) was developed by Amidon et al (1995) and is used by pharmaceutical industries to help in the development of a new formulation (MEYER et al., 1992) and requirement of biowaiver.

According to BCS a substance is classified on the basis of its aqueous solubility and intestinal permeability into four classes i.e., high solubility/high permeability (Class I), low solubility/high permeability (Class II), high solubility/low permeability (Class III), low solubility/low permeability (Class IV).This classification is represented on Figure 5.

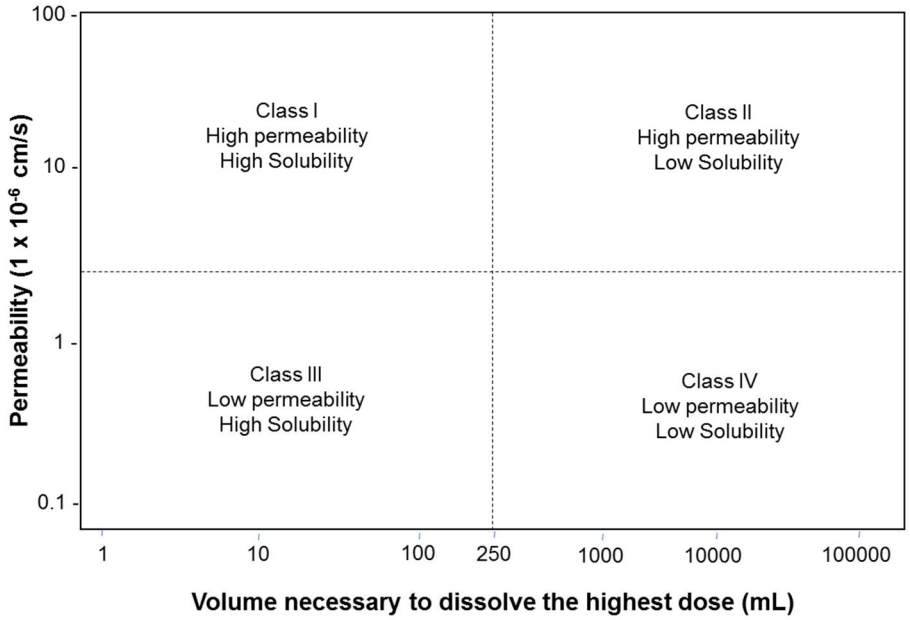


FIGURE 5. The Biopharmaceutical Classification System scientific framework.

Drugs are considered highly soluble when the highest dose strength of the drug substance is soluble in less than 250 mL water over a pH range of 1-6.8 and considered highly permeable when the extent of absorption in humans is determined to be greater than 90% of the administered dose.

According to AMIDON et al., 1995 the four drugs classes described in Figure 5have the following characteristics:

Class I: Drugs classified as class I has a good permeability. Since it also has a good solubility, the limiting factor for its absorption is the gastric emptying rate and no correlation with dissolution rate is expected. This suggest that a dissolution specification for immediate release (IR) dosage forms of perhaps 85% dissolved in less than 15 min may insure bioequivalence.

Class II: In this case, drug dissolution *in vivo* is the rate controlling step in drug absorption, since drug permeability is high and solubility is low. The compreension and evaluation of *in vitro* dissolution rate is important tool that can be used to obtain a *in vitroin vivo* correlation.

Class III: For this class of drugs, permeability is the rate controlling step in drug absorption. Therefore, it's important that the *in vivo* dissolution rate to be high as the expected for class I drugs. Both the rate and extent of drug absorption may be highly variable for this class of drugs, but if dissolution is fast i.e. 85% dissolved in less than 15 min, this variation will be due to the variable gastrointestinal transit rather than dosage form.

Class IV: This class of drugs present significant problems for effective oral delivery (AMIDON et al., 1995).

This classification system is very useful to pharmaceutical industry and, when strategically deployed, it can save time and resources during generic drug development (COOK; ADDICKS; WU, 2008).

3.4 Pharmaceutical Dissolution Testing

Since *in vivo* dissolution rate may become a limitating step on drug absorption and action, it's important to pharmaceutical industry evaluate the dissolution rate of a product *in vitro* to try to stablish a correlation of this data with *in vivo* behavior. Dissolution testing is an*in vitro* method that characterizes how an active pharmaceutical ingredient (API) is extracted out of a solid dosage form. It can indicate the efficiency of *in vivo* dissolution but DoEs not provide any information about drug substance absorption.

The specific dissolution technique employed is determined by the dosage form characteristics and the intended route of administration. For solid dosage forms, industry standard dissolution testing methodologies are the United States Pharmacopeia (USP) Apparatus 1 (basket), USP Apparatus 2 (paddle), USP apparatus 3 (reciprocating cylinder); apparatus 4 (flow-through cell), apparatus 5 (paddle over disk), apparatus 6 (cylinder) and apparatus 7 (reciprocating holder) (UNITED STATES PHARMACOPEIA, 2016). The dissolution testing apparatus is typically constructed so that dissolution testing may be performed on sampling units of six tablets or capsules simultaneously. The choice of the dissolution apparatus should be considered during the development of the dissolution methods, since it can affect the results and the duration of the test. The type of dosage form under investigation is the primary consideration in apparatus selection.

When compared with the others 5 types, Apparatus 1 and 2, are the most widely used around the world, due to its simplicity and robustennes (KRÄMER; GRADY; GAJENDRAN, 2005). The first official method adopted in 1970 was the rotating basket (Apparatus 1), this apparatus consists of a metalic drive shaft connected to the cylindrical basket. The basket is positioned inside a vessel made of glass or other inert, transparent material (Figure 6). The rotating paddle, although method 2, is actually the most widely used in dissolution testing, the specification for

Apparatus 2 are identical with those for Apparatus 1 except that the paddle is substituted for the rotating basket (Figure 6) (QLA, 2017).

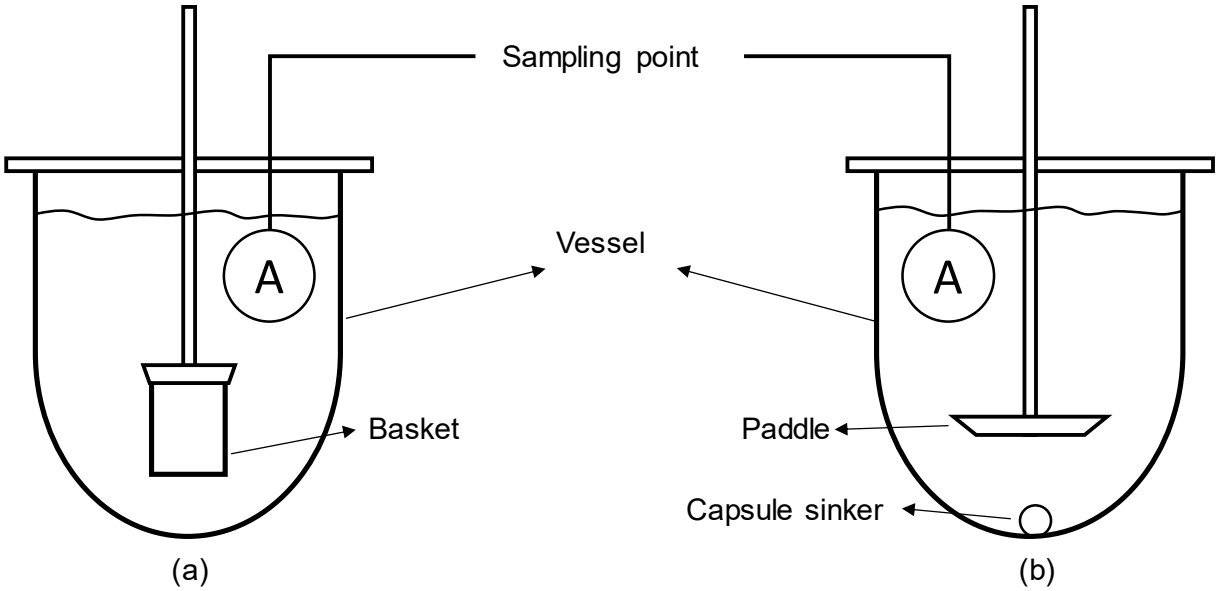


FIGURE 6. Representation of USP Apparatus 1 (a) and 2 (b)

A variety of factors can affect the rate of dissolution considerably as discussed before. Thus the development of a dissolution procedure involves selecting an appropriated dissolution condition in order to simulate the gastrointestinal environment accurately and be applicable to the pharmaceutical dosage form (SIEW, 2016; GHAYAS et al., 2013; KRÄMER; GRADY; GAJENDRAN, 2005). The dissolution media, apparatus type and hydrodynamics (agitation rate) are the most critical factors that must be studied during a dissolution method development in order to develop a procedure with an adequate discriminating power and robustness.

The choice of apparatus is based on knowledge of the formulation design and pratical aspects of dosage form performance in the *in vitro* test system. The basket method(Figure 6a) is routinely used for solid oral dosage forms such as capsule or tablet formulations at an agitation speed of 50-100 rpm, although speeds of up to 150 rpm have been used. The paddle (Figure 6b) method is frequently used for solid oral dosage forms such tablet and capsule formulation at 50 or 75 rpm (UNITED STATES PHARMACOPEIA, 2016).

The choice of a dissoluion medium can be very challenging. The dissolution medium must meet regulatory requirements in a global environment, balance discriminating ability with robustness, and lead to development of an appropriate specification (MARTIN; GRAY, 2011). Dissolution rate-limited absorption

implies that there is no build-up of drug concentration in the gastrointestinal fluids, i.e., the fluids function as a perfect sink. Unless this condition is embodied in the design of the *in vitro* test, *in vitro* results will bear little relationship to *in vivo* observations (GIBALDI; FELDMAN, 1967). Therefore, when developing a dissolution procedure, one goal is to have sink conditions, which are defined as having a volume of medium at least three times the volume required to form a saturated solution of drug substance (UNITED STATES PHARMACOPEIA, 2016).

The media typically used in dissolution studies include acidic solutions, buffers, surfactants, and surfactants with acid buffers (UNITED STATES PHARMACOPEIA, 2016). Media with bile salts and other relevant physiologically based ingredients, sometimes called biorrelevant media, can be used in regulatory tests, but typically are used as research tools or for *in vitro-in vivo* correlations studies.

Other factors need to be taken into account during a dissolution method development, such as the need for sinker in cases of buoyancy of dossage form during the test with apparatus 2, deaeration of the dissolution medium, because air bubbles can act as a barrier to the dissolution process and change the dissolution rate (UNITED STATES PHARMACOPEIA, 2016).

Dissolution testing finds application as a tool in drug development, providing control of the manufacturing process, for batch release, as a mean of identifying potential bioavailability problems and to assess the need for further bioequivalence studies relative to scale-up and post –approval changes and to signal possible bioinequivalence of formulations. It is used to guide formulation development and to select an appropriate formulation for *in vivo* testing. With respect to quality assurance and control, almost all solid oral dosage forms require dissolution testing as a quality control parameter before a drug product is introduced and/or released into the market. Dissolution profile comparison has additionaly been used extensively in assessing product equivalence, especially when pos-approval changes are made(ANAND et al., 2011).

It’s an important test for pharmaceutical industry that involves many variables that need to be evaluated during the development phase in order to obtain a dissolution condition relevant to product evaluation(ANAND et al., 2011).

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3.4.1 The Dissolution Profile Curve

The dissolution value of an active ingredient is measured at various predetermined time intervals to perform a dissolution profile analysis. For immediate-release dosage forms, the duration of the dissolution procedure is typically 30-60 min (UNITED STATES PHARMACOPEIA, 2016) and for extended release it depends on dosage form release characteristic. Figure 7 represents the *in vitro* dissolution profilles of propanolol hydrochloride from different release rates formulations of a extended-release tablet (CHENG et al., 2014).

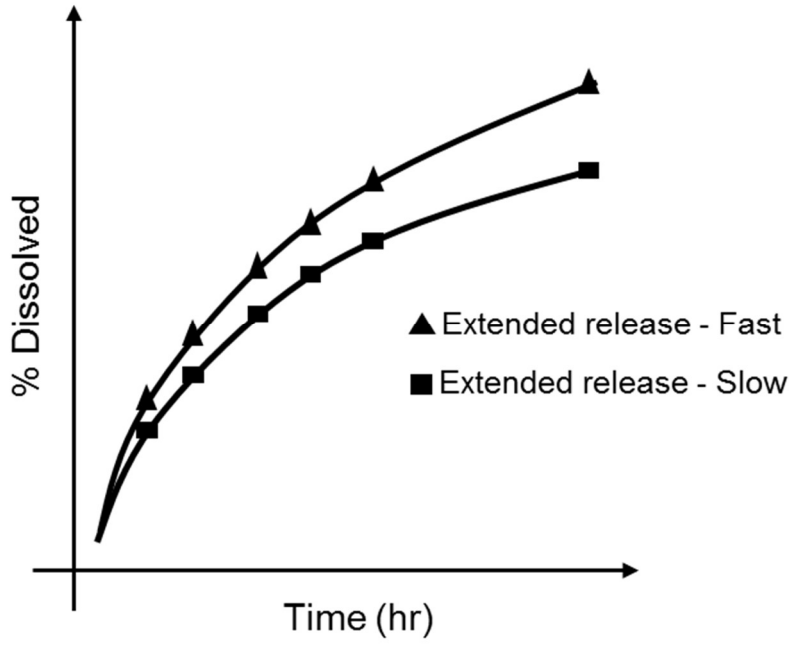

FIGURE 7.*In vitro* dissolution profiles of propanolol hydrochloride from different release rates formulations extended-release tablet (Addapted from CHENG et al., 2014).

On several occasions, the objective of the dissolution test is compare the dissolution curves between the test lot, i.e. a generic product in development phase, and the standard lot, i.e. the reference product. The dissolution profille comparison may be carried out using the methods based on analysis of variance (ANOVA), model independet and model dependent methods (YUKSEL; KAMK; BAYKARA, 2000).

A wide variety of mathematical models have been developed to fit the drug release data, most of which are presented as nonlinear equations (ZHANG et al., 2010; COSTA; LOBO, 2000). Some of these equations are shown in Table 1. The quantitative interpretation of the values obtained in dissolution assays and understanding of sample release mechanism is easier using mathematical equations (COSTA; LOBO, 2000; RAMTEKE et al., 2014).

TABLE 1. Mathematical models used to describe drug dissolution curves (COSTA; LOBO, 2000.

Model	Equation
Zero order	$Q_t = Q_0 + K_0 t$
Fisrt order	$ln\ Q_t = ln\ Q_0 + K_1 t$
Second order	$Q_t/Q_\infty = (Q_\infty - Q_t)K_2 t$
Hixson-Crowell	$Q_0^{1/3} - Q_t^{1/3} = K_s t$
Weibull	$\log\left[-\ln\left(1-\left(\frac{Q_t}{Q_\infty}\right)\right)\right] = b \times log\ t - \log a$
Higuchi	$Q_t = K_H\sqrt{t}$
Baker-Lonsdale	$\left(\frac{3}{2}\right)\left[1-\left(1-\left(\frac{Q_t}{Q_\infty}\right)\right)^{\frac{2}{3}}\right] - \left(\frac{Q_t}{Q_\infty}\right) = Kt$
Korsmeyer-Peppas	$\frac{Q_t}{Q_\infty} = K_k t^n$
Quadratic	$Q = 100(K_1 t^2 + K_2 t)$
Gompertz	$Q_t = Ae^{-e-K(t-y)}$
Hopfenberg	$\frac{Q_t}{Q_\infty} = 1 - [1 - \frac{k_0 t}{C_0 a_0}]^n$

3.1.1 *In vivo-In vitro* correlation

From biopharmaceutical standpoint the correlation may be referred to the relationship between appropriate *in vitro* release characteristics and *in vivo* bioavailability parameters. *In vivo-in vitro* correlation (IVIVC) is a predictive mathematical model describing the relationship between *in vitro* property of a dosage form and a relevant *in vivo* response. Generally, *in vitro* property is the rate or extent

of drug dissolution, or release while the *in vivo* response is the plasma drug concentration, or amount of drug absorbed (EMAMI, 2006).

Based on the ability of the correlation to reflect the complete plasma drug level-time profile, five correlation levels have been defined in the IVIVC FDA guidance (EMAMI, 2006). Level A correlation represents a point-to point relationship between *in vitro* dissolution rate and *in vivo* input rate of the drug from the dosage form. The percent of the drug absorbed may be calculated by means of model dependent techniques such as Wagner-Nelson procedure or Loo-Rielgeman method. This level is considered as the highest category of correlation, and in case of a level A correlation, an *in vitro* dissolution curve can serve as a surrogate for *in vivo* performance. Thus, change in manufacturing site, method manufacture, raw material supplies, minor formulation modification, and even product strength using the same formulation can be justified without the need for additional human studies(EMAMI, 2006). Figure 8 demonstratesan example of an *in vivoin vitro* correlation level A.

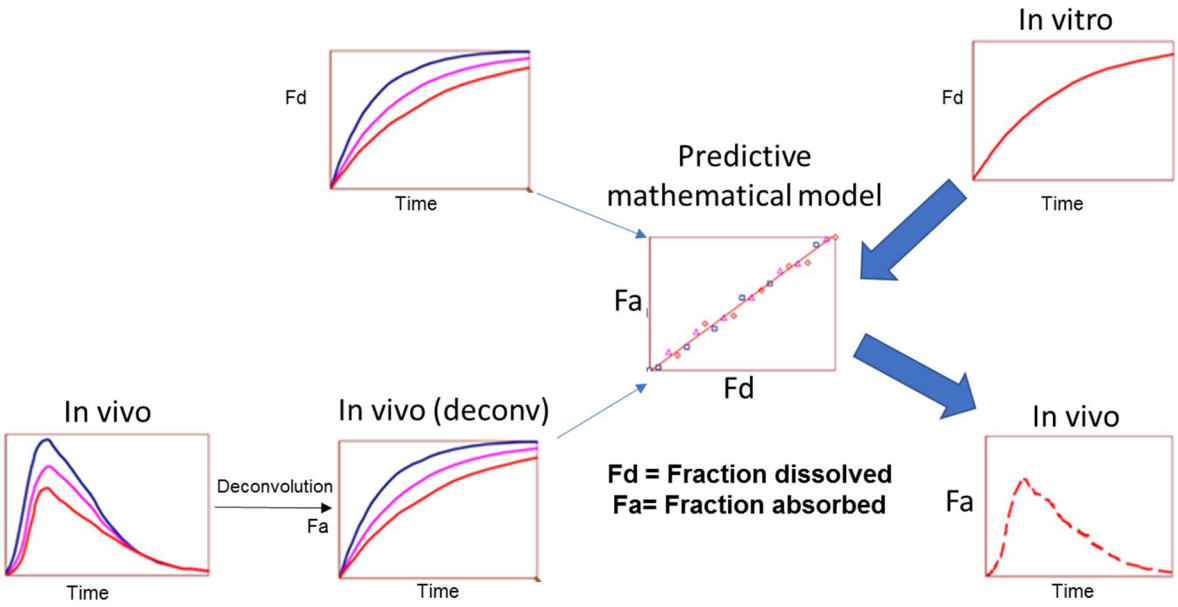

FIGURE 8.Example of an *in vivoin vitro* correlation level A.

3.1.2 Design of Experiment as a tool for dissolution Method Development

Development of dissolution method is manly performed by changing the levels of each variable separately at a time. This methodology known has One Factor at Time (OFAT) is based on a large number of experiments and often relies merely on the experience of the analyst (KINCL; VREčER; VEBER, 2004).

Desing of experiment (DOE) is known in literature as an efficient tool for planning experiments where the data obtained can be analyzed to yield valid and objective conclusion with the fewest number of experiments (MYERS et al., 2009)

DOE has been shown to be a fundamental tool for pharmaceutical industry, used with great efficiency for drug product development (HWANG; KOWALSKI, 2005; CHUNYK; SPRIGGS, 2015) and analytical method developments (REID et al., 2013). This tool makes possible to reach a reduction on the time to develop bioequivalent generic from 2 years to 4 months using DoE (VERGO PHARMA RESEARCH LABORATORIES, 2017).

Previously results has proven to be an interesting tool for dissolution method development either. Kincl, S.Turk and Vrecer (2005) applied experimental design methodology in the development and optimization of a drug release method tosodium diclofenac prolonged release tablets. The authors considered this methodology a very economic way for extracting the maximun amount of complex information, significant experimental time saving factors and moreover, saves in material used for analyses and personal costs. Throught this work, It was possible to understand the effect of rotation speed, pH of the dissolution media and relative ionic strenght on dissolution rate. Choosing a rotation speed of 80 rpm, the authors demystified traditional thinking of limiting rotation speed to 50, 75, and 100 rpm or volumes of 500, 900, and 1000 mL.

In the same way, a dissolution method for oxcarbazepine capsules, using mixed-level factorial design was developed by Polonini et al. (2011). Evaluating factors as stirring speed, dissolution medium and apparatus, a dissolution condition of Apparatus 2 with a rotation speed of 80 rpm and dissolution medium of sodium lauryl sulfate 1% m/v aqueous solution was obtained, and the method was considered useful as a quality control methodology of the drug product.

A simple, fast and robust method for dissolution test was developed for Omeprazole DDR capsules using DOE. The method could be used for routine quality control purpose (MANRANJAN; YADAV; JOGIA, 2014).

A design of experiment was used to screen nine critical variables from dissolution Apparatus 2 using USP Prednisone Reference Standard (RS) Tablets. The effect of the variables was different depending on the response evaluated, considering the average percent dissolved results, the deaeration of the dissolution medium, vessel type and rotation speed were statistically significant, and for standard

deviation it was possible to detect the influence of five variables and their interactions (EATON et al., 2007). This study shown how important is to carry out a dissolution test in an calibrated equipament.

The design of experiment may be useful to evaluate the robustness of a dissolution method. Blommfield e Butler (2000) observed that, for the dissolution of atovaquone using USP Apparatus 4, at 15 min, the concentration of sodium hydroxide in the dissolution media, peristaltic pump speed and flow rate were assessed as statistically significant. For a sample time of 30 min or above, the factors evaluated was not considered significant, therefore, the method could be considered as robust to changes in all the main parameters evaluated. Due to non robustness at 15 min, those factors started to be routinely controlled in the method.

When working with more than one batch, the experimental design can be applied to reduce variation from experimental runs, allowing to detect the difference between run variation and identifying any systematic errors resulting from differences between vessels (LEWIS; STEVENS, 1987).

3.2 Zolpidem Hemitartrate

Zolpidem is sold under the brand names Ambien, Ambien CR, Stilnox, and Sblinox is a prescription medication used for the treatment of insomnia, as well as some brain disorders. It works quickly (usually within 15 minutes) and has a short half-life (two to three hours). Zolpidem has not adequately demonstraded effectiviness in maintaining sleep (unless delivered in a controlled –release form); however, it is effective in initiating sleep (PUBCHEM, 2017).

The free base of zolpidem is insoluble in water, zolpidem tartrate is slightly soluble in water (23 g/L at 20ºC), pratically insoluble in dichloromethane, sparingly soluble in methanol. Figure 9 presents the chemical structure of zolpidem hemitartrate.

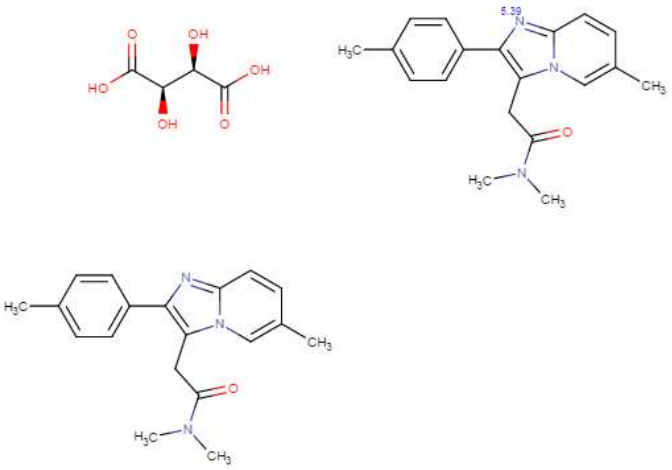

FIGURE 9. Chemical structure of Zolpidem Hemitartrate (CHEMICALIZE, 2017).

Zolpidem is a weak base with a single ionization constant of pKa= 5.39and a molecular weight of 764.35 Da (CHEMICALIZE, 2017). The species distribution in function of pH are represented in Figure 10.

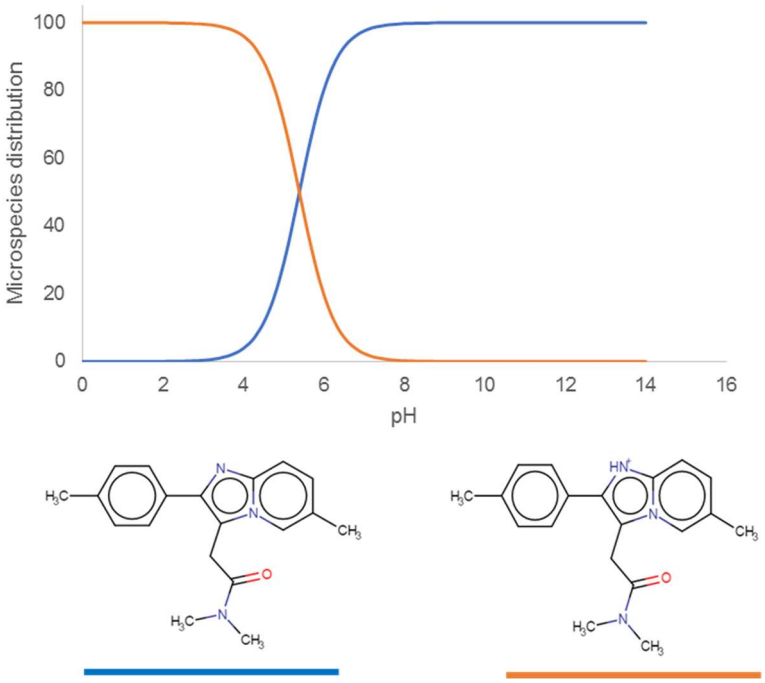

FIGURE 10.Especies distribution of Zolpidem in function of pH. The blue line represents the unionized form and the red line represents the ionizide one (CHEMICALIZE, 2017).

Zolpidem ispresented in Brazilian market as sublinguial tablet 5.0 mg (PATZ SL - EMS Sigma Pharma), immediate release coated tablets 10mg (STILNOX

- Sanofi-Aventis) and controlled release bilayer tablet of 6.25 and 12.5 mg (STILNOX CR - Sanofi-Aventis).

3.2.1 Pharmacokinetic of Zolpidem Hemitartrate

Zolpidem is 92% bound to plasma proteins, absorbe readly from the gastrointestinal tract with a first-pass metabolism which results in a absolute bio-availability of about 70% (FODA; ALI, 2012). Zolpidem CR is rapidly absorbed after oral administration and reaches a peak median concentration in 1.5 hours (range, 0.5-3.5 houras). The C_{max} of Zolpidem CR was 82% of the immediate release product. Administration of Zolpidem with food decreased the peak concentration by 30% and the area under the plasma concentration curve (AUC) by 23%. The median tmax was prolongued from 2 to 4 hours with food and the product labeling indicates that ingestion with food may delay the hypnotic effects of Zolpidem CR (KIRKWOOD; NEILL; BREDEN, 2007).

Zolpidem pharmacokinetics are unchanged during multiple-dose treatment and are not significantly influenced by gender. There are no significant differences in the pharmacokinetic parameters between various racial groups (SALVÁ; COSTA, 1995).

3.2.2 Pharmaceutical Studies of Zolpidem Hemitartrate

Zolpidem hemitartrate is a high solubility drug according to the biopharmaceutical classification system. The solubility at pH 1.2, 4.5 and 6.8 was 48.24, 23.23 and 6.60 mg/mL, respectively (USECHE et al., 2015). The molecule showed a pH dependent solubility, with a lower solubility at the highest pH, which is expected for a weak ionizable base with a pKa at 5.39. The permeability is high when compared with metropolol permeability, therefore, zolpidem is classified as a class I drug according to the biopharmaceutical classification system (USECHE et al., 2015).

Useche et al. (2015) evaluated several *in vitro* dissolution conditions with three zolpidem formulations of immediate release tablets of Zolpidem hemitartrate 10 mg/tablet: the reference (Stilnox), a bioequivalent formulation (BE), and a non-bioequivalent formulation (N-BE). The target of the authors was to develop a discriminatory dissolution method with a correlation with the results obtained *in*

vivo. The dissolution condition reached, which is different from the one described at the USP pharmacopeia, works with a rotation speed of 30 rpm in Apparatus 2 with a dissolution medium of phosphate buffer at pH 6.8. This dissolution condition detected a difference in release rate of the non-bioequivalent formulation .

Food and Drug Administration (FDA) recommends for Zolpidem tartrate extended release tablets the use of partial area under the curve (pAUC) metrics to determine its bioequivalence (FDA,2011). In order to understand the need for pAUC measures and also proper pAUC times, Lionberger et al. (2012) performed modeling and simulation studies using deconvolution techniques, *in vitro*/*in vivo* correlations, and the Compartmental Absorption and Transit (CAT) model to predict pharmacokinetic profiles. The authors observed that the CAT model was the most physiologically consistent approach of the three models tested and the great advantage of using this model is the fact that the pharmacokinetic profiles are predicted from dissolution ones expressed by the Weibull model. The Weibull model is described in equation 2:

$$F(t) = 1 - \left[\frac{-\left(t - t_{lag}\right)^{b}}{a}\right], \qquad (2)$$

where t is time and t_{lag} is the lag time which was considered to be 0. The a parameter has units of time and is related to the dissolution rate with a larger a indicating a slower dissolution. The b parameter is a dimensionless shape parameter with b=1 being an exponential release, b>1 representing an “S” shaped profile and b<1 representing a profile with faster than exponential release(USECHE et al., 2015).

Using their model, authors simulated the pharmacokinetic profiles from all possible combinations of the *a* and *b* parameters and mapped the dissolution region that passes the BE criteria.

This modeling approach can be a very usefull information for dissolution method development during the initial phase of development of a generic product of Zolpidem.

MATERIALS AND METHOD

4 – MATERIALS AND METHOD

The experimental part of this study was carried out in the research and development laboratory of Eurofarma, located in the city of Itapevi in the state of São Paulo.

4.1 – Samples

Stilnox® CR modified release tablets containing 12.5 mg of Zolpidem Hemitartrate were produced by Sanofi-Aventis. It consist of a coated two-layer tablet. One layer releases its drug content immediately and the other layer allows a slower release of the additional drug content.

4.2 – Reagents

Potassium dihydrogen phosphate, sodium hydroxide, glacial acetic acid and Hydrochloridric acid 37%, all analytical grade, were obtained from Merck and Sodium Acetate Anhydrous were purchased from Sigma-Aldrich. Purified water was obtained from Purelab (Elga). Full flow filters 35 µm from Agilent (USA) was used to filter sample solutions and Graduated measuring cylinders (500, 1000 and 2000 mL), volumetric flask (50 and 100 mL) were all provided by Blau Brand (Germany).

4.3 – Instrumentation

For dissolution test a 708-DS Dissolution Apparatus (Agilent technologies), online connected to Cary 60 UV-Vis spectrophotometer from Agilent was used (Figure 12). Furthermore an Analytical balance (Mettler-Toledo XP205) was used.

FIGURE 11.Agilent scanning UV dissolution system with an online Cary 60 –UV-Vis Spectrophotometer.

4.4 – Spectrophotometric Condition

In drug release experiments, the sample solutions were automatically withdrawn at specified time intervals from each dissolution vessel, respectively, and filtered. The absorbances were measured on an on-line connected UV spectrophotometer at 295 nm with a background correction at 460 nm, using 10 mm quartz cells.

4.5 – Standard Preparation

A standard solution of 0.01388 mg/mL of Hemitartrate Zolpidem (Sunpharma; pot: 0.991) was prepared weighting accurately a mass of Zolpidem hemitartrate which was dissolved in the dissolution medium.

4.6 – Dissolution Medium Preparation

All the aqueous media used for drug release tests, phosphate buffer solution pH 6.8, acetate buffer pH 4.4 and hydrochloridric acid 0.01 mol/L were prepared according to the description in the united State Pharmacopeia NF 39 (2016).

4.7 – Softwares

The design of experiment analysis was executed on Design Expert (Version 7.1.2. Stat-Ease Inc., Minneapolis, MN). The dissolution data modeling were made with the add-in DD-Solver for MS-Excel.

4.8 – Experimental Methods

4.8.1 Risk Assesment

In order to organize hierarchically all factors, the Fish-bone diagram was applied. Then, based on previous knowledge and initial experimental data, failure mode and effect analysis (FMEA) method was further applied. In FMEA methodology each variable (potential failure mode) was scored in terms of severity (S), detectability (D) and probability (P). Severity is a measure of the possible consequences of a failure mode affecting on the safety and efficacy of the final product. Detectability defined that a failure mode can be detected and the final parameter, probability is considered as the occurrence probability or the likelihood of a failure. For each risk, S, D, P scores were multiplied to produce a “Risk Priority Number” (RPN), RPN= S x D x P, which represents the overall magnitude of the risk. The S, D and P were ranked of 3 as worst-case, 1 as best-case value and 2 as moderate-case value, and then a maximum RPN of 28 and a minimum RPN of 1 are possible. The RPN threshold was set at 12, and any variable with a RPN 12 or above, was regarded as potential critical factor, which means that potential risk should be evaluated by subsequent studies, since has a potential impact on dissolution rate of the product. Factors with lower RPN can be eliminated from further study.

4.8.2 Deconvolution of Reference Data

The fraction of drug absorbed from the *in vivo* study of the reference product Ambien CR 12.5 mg / tablet was obtained from FDA, 2017. The Wagner-

Nelson method was used to calculate the percentage of Zolpidem dose absorbed (deconvolution), according to the following equation:

$$F_t = \frac{C_t + k_{el} \int_0^t C dt}{k_{el} \int_0^\infty C dt} \qquad (3)$$

where C_t is plasma concentration at time t and $k_{el}$is elimination rate constant(WAGNER; NELSON, 1963).

The *in vivo* dissolution curve obtained was then fitted to first order, Higushi, Weibull and Logistic model to characterize the absorption rate. Since Zolpidem Hemitartrate is a high permeability drug, it was assumed that the absorption rate is linearly proportional to dissolution rate, therefore the information obtained from the deconvoluted data was used to choose the best dissolution condition.

4.8.3 Screening Study (Full Factorial Design)

Based on the risk assessment results, afull factorial design was used to screen significant factors that affect dissolution rate of the product. The variables were evaluated in two levels, low (-1) and high (+1), with three repetitions for each experiment condition.

4.8.4 Optimization Study (Response Surface Methodology)

Relied on the results of full factorial design screening study, response surface methodology was applied in order to achieve de optimal dissolution method condition. Face centered cubic (FCC) design model was selected, with six replicationson the center point. The factor levels were coded for low, medium and high settings, as -1, 0 and +1 respectively, and it was performed three repetitions of each experimental condition.

4.8.5 Statistical Analysis

The results of the full factorial design were analyzed, and the influence of each parameter on the responses was demonstrated in the calculated effect chart.

Were considered significant factors with a confidence interval of effect that do not include the value zero with a confidence level of 95% (p-value = 0.05).

For the FCC design model analysis, the regression equation describes the effects of the variables on the responses in terms of linear, interaction and quadratic. The equation followed as:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1x_2 + b_5x_2x_3 + b_6x_1x_3 + b_7x_1^2 + b_8x_2^2 + b_9x_3^2 + E$$

where$y$ is the selected response, b_0 is the intercept, b_1-b_9 are the regressions coefficients (b_1-b_3 are the linear, b_4-b_6 are the interactions and b_7-b_9 are the quadractic),x_1, x_2 and x_3 are the factors studied and E is an error term. The *p-value* related to the regression coefficient indicated significance of the factors on the response. ANOVA and the coefficient of regression (R^2) were also applied to determine the suitability of the model. In all cases the confidence interval used was 95%.

4.8.6 Response Selection and Desirability

Table 2 summarizes the responses evaluated, the constrains selected, and some remarks to justify theirchoices. The responses were selected considering pharmacokinetic properties obtained from the deconvoluted data described on item 4.8.2, properties of the product Stilnox CR modified release tablets and its principal active ingredient, Zolpidem Hemitartrate.

TABLE 2. Important responses monitored during the experiments and its desirability range.

Response	Low	High	Remarks
% Dissolved 0.25 h (y_1)	>40%	-	Secure the release of at least 85% of the immediate release layer
% Dissolved in 4h (y_2)	>90%	-	Total drug release in 4h
$R^2(y_3)$		Maximize	Adequabilit to Logistic model
α = Alfa (y_4)		Minimize	Closest to the one obtained from the *in vivo* data modeled
β = Beta (y_5)		Maximize	Closest to the one obtained from the *in vivo* data modeled

The sampling times of 0.5, 1.0, 1.5, 2.0, 3.0, 5.0 and 6.0 hours were evaluated and modeled during the reponse surface phase of the experiment.

RESULTS AND DISCUSSION

5 RESULTS AND DISCUSSION

5.1 Risk Assessment and Variables Selection

Risk assessment was intended to identify all the potential high impact factors which was subjected to a DOE study to stablish a method design space. The first step in the risk assessment was to systematically gather up all the possible method factors that could influence product dissolution kinetic. According to the literature data, previous study performed and pre-formulation data, fish-bone diagrams was applied to organize hierarchically these factors. Fish-bone diagram was constructed to identify the potential risks and corresponding causes that could affect product dissolution (Figure 13).

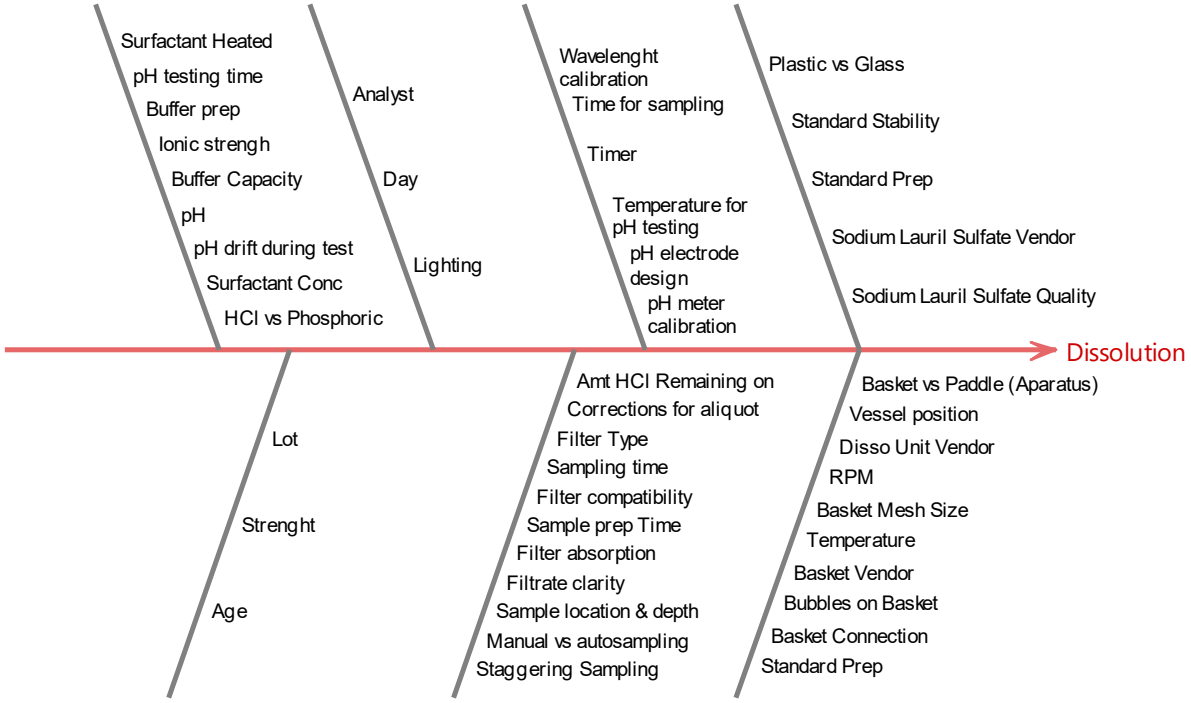

FIGURE 12: Fish-bone diagram illustrating factors that may have impact on dissolution.

The main factors selected on Fish-bone diagram were then evaluated by the Failure Mode and Effect Analysis (FMEA), and variables that could affect *in vivo* performance have generally been scored high. Any factor with a RPN higher than the RPN threshold of 12 was regarded as a potential critical factor while factors with lower RPN was eliminated from futher study (Figure 14).

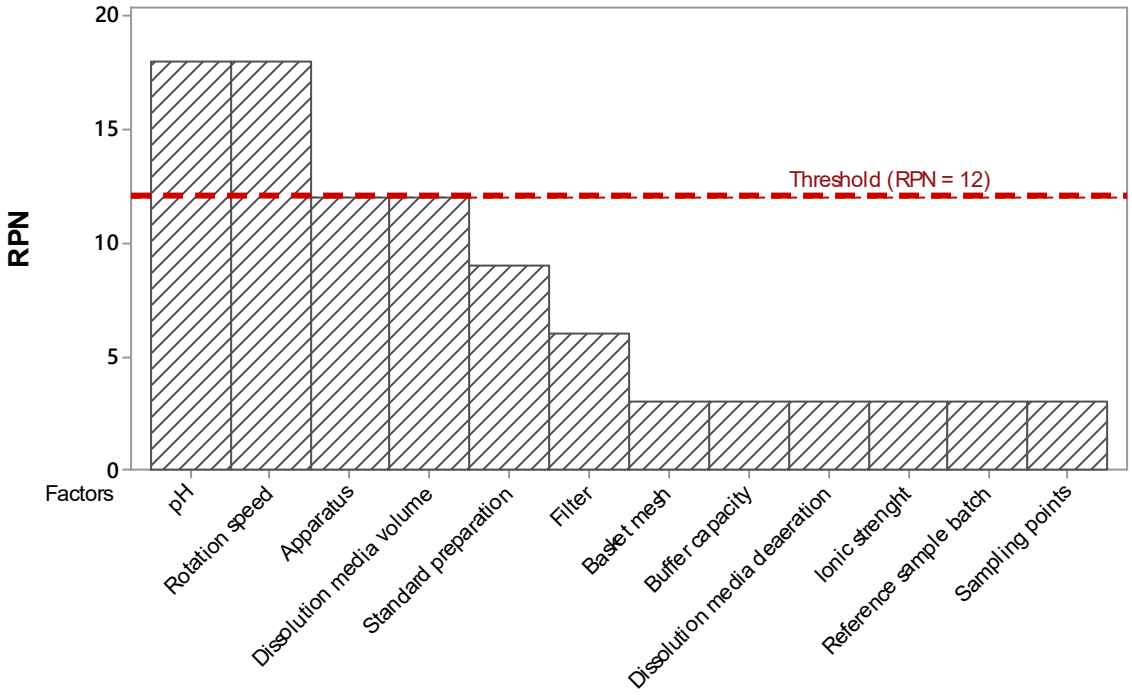

FIGURE 13.Chart showing RPN scores for dissolution method parameters. Parameters that have RPN scores higher than the threshold (RPN=12) were considered for futher experimentation.

Risk analysis study identified four high-risk factors that may have impact from y_1 to y_6 (see Table 2). These factors included:

x_1: Aparattus rotation speed (rpm)
x_2: pH of the dissolution media
x_3: Dissolution media volume (mL)
x_4: Aparattus

5.2 Deconvolution and data modeling

The mean zolpidem plasma concentration-time profiles after single oral administration of the reference product of Zolpidem Tartrate Controled Release (CR) and the deconvoluted cumulative systemic absorption are presented on Figure 15.

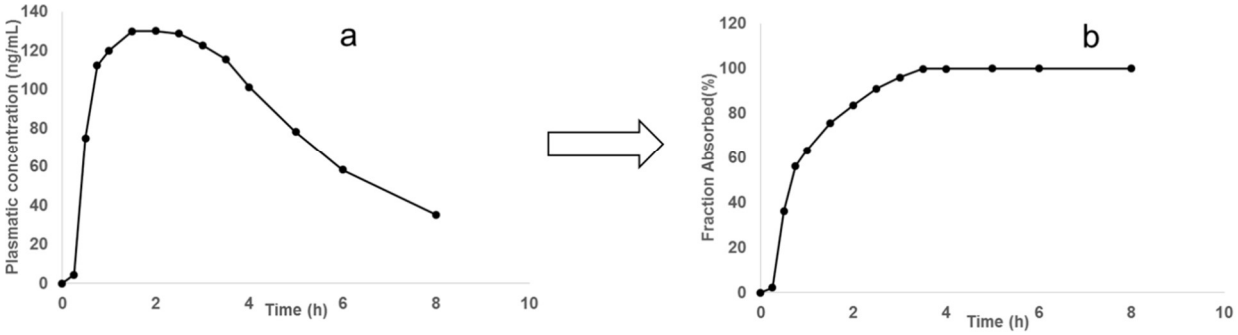

FIGURE 14. a) Mean zolpidem plasma concentration-time profiles obtained after single oral administration of zolpidem CR 12.5 mg (FDA, 2017) b) Deconvoluted data of the mean zolpidem plasma concentration profile.

The results for the fitting of the kinetic model for *in vivo* drug release from the Zolpidem CR tablet are described in Table 3. The correlation coefficient (R^2) was used as an indication of the best fit.

TABLE 3.Modeling of *in vivo* dissolution profile

Model	Equation	Parameters	R^2
Weibull	$F = F_{max} \times \{1 - e^{\left[-\left(\frac{t^{\beta}}{\alpha}\right)\right]}\}$	α = 1.8365 β = 1.1987	0.9080
First Order	$F = 100 \times [1 - e^{(-k_1 \cdot t)}]$	k_1= 1.3519	0.9214
Higuchi	$F = k_H \cdot t^{0.5}$	k_H= 47.2892	0.7846
Logistic	$F = F_{max} \times \frac{e^{\alpha+\beta \cdot \log(t)}}{1 + e^{\alpha+\beta \cdot \log(t)}}$	α = 0.1066 β= 4.2910	0.9710
Gompetz	$F = F_{max} \cdot e^{-\alpha \cdot e^{-\beta \cdot \log(t)}}$	α = 0.2828 β= 5.6344	0.9553

The model choosen to describe the *in vivo* dissolution was the Logistic with a R^2 of 0.971. Figure 16 represents the observed data and the predicted by the model.

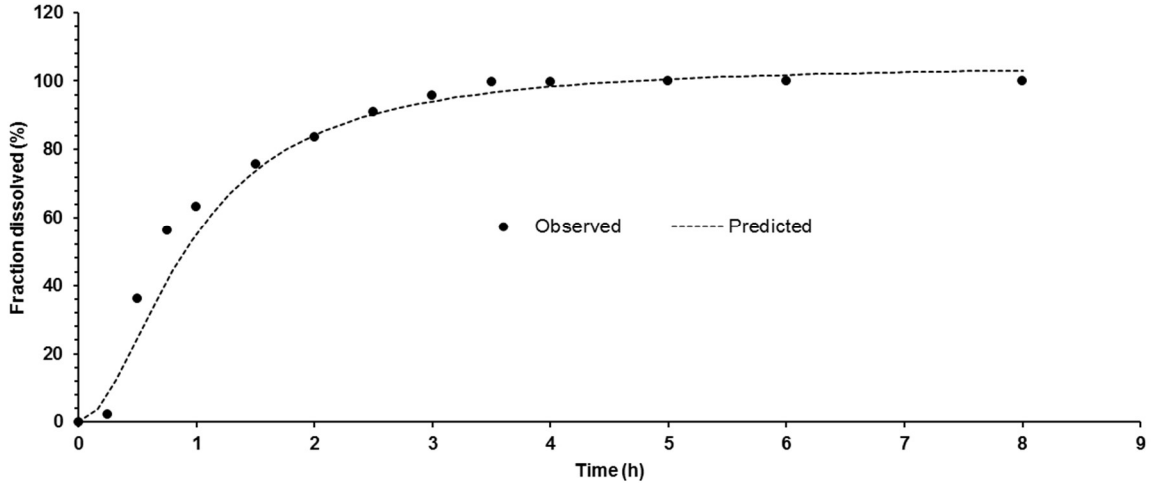

FIGURE 15. Observed data obtained by the deconvolution of the mean zolpidem plasma concentration-time profiles, and the predicted by the Logistic model.

The α parameter of the equation is described as a location or scale parameter, whereas β is described as the acceleration or shape. This information can be used as reference to choose the dissolution condition that provide *in vitro* release that is well described by the Logistic model.

5.3 – Evaluation of Experimental Data

Average results data of all 16 experiments from the full factorial 2^4experiment performed are described on Table 4. Itwas possible to identify a significative difference related to the different combination of factors and levels. The fast release of the immediate release layer of the tablets can be identified at the dissolution time of 0.25 and 0.5h, the following sampling times a sustained release of the second layer from the tablet can be observed.

TABLE 4. Factorial screening design of experiments and their average results (Complete data in Appendix I).

Experiment	Apparatus, x_1	Rotation speed (rpm), x_2	pH, x_3	Volume (mL), x_4	0.25 h y_1	4 h y_2	R^2 y_3	α y_4	β y_5
1	Paddle	100	2.0	900	51.02	87.64	0.969	0.78	1.99
2	Basket	100	2.0	500	51.81	96.03	0.975	0.92	2.25
3	Paddle	100	6.8	500	47.13	80.66	0.951	0.53	1.84
4	Basket	100	6.8	500	49.38	89.78	0.959	0.61	2.03
5	Paddle	50	2.0	900	47.08	86.57	0.957	0.53	2.00
6	Paddle	50	2.0	500	47.07	92.49	0.965	0.63	2.15
7	Basket	50	2.0	900	43.02	90.69	0.975	0.69	2.30
8	Basket	50	6.8	900	37.72	82.08	0.962	0.40	2.19
9	Basket	50	2.0	500	44.38	97.16	0.979	0.65	2.31
10	Paddle	50	6.8	500	42.80	76.63	0.962	0.54	1.89
11	Paddle	100	2.0	500	52.58	89.77	0.970	0.81	1.95
12	Paddle	100	6.8	900	56.64	94.09	0.952	0.72	1.95
13	Basket	50	6.8	500	32.80	84.29	0.964	0.25	2.35
14	Basket	100	2.0	900	52.13	94.99	0.973	0.98	2.26
15	Paddle	50	6.8	900	43.65	80.13	0.962	0.63	1.97
16	Basket	100	6.8	900	54.35	97.88	0.960	0.79	2.19

The results of the full factorial experiment ranged from a low release rate, as observed on experiment 10, which reached a release of 84%after 6 hours of the dissolution test, to a very fast drug release, as experiment 9 which has almost 100% of drug release after 4 hours of the dissolution test (Figure 17).

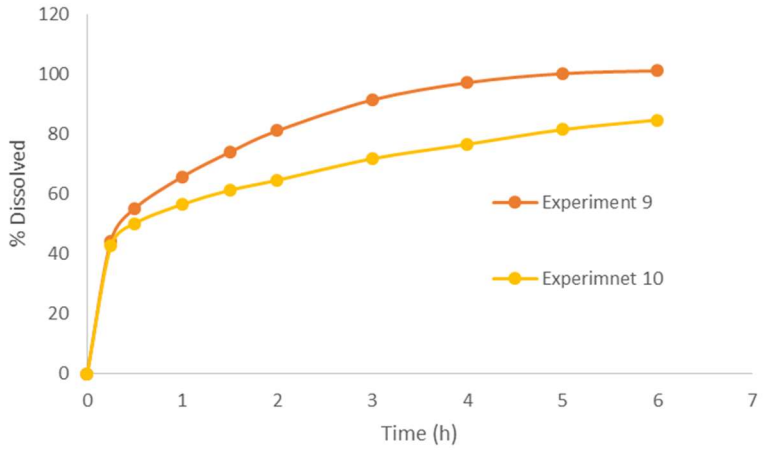

FIGURE 16. Release profile of Zolpidem hemitartrate in accordance with Experiment 9 and 10 of the full factorial experimental design. Experiment 9: basket apparatus with a rotation speed of 50 rpm, dissolution media of HCl 0.01 N (pH 2.0) with a volume of 500 mL. Experiment 10: Paddle apparatus with a rotation speed of 50 rpm and phosphate buffer as dissolution media with a volume of 500 mL.

Difference in release rate of the imediatte layer could be observed either, on Figure 18 it's possible to observe a faster release of the immediatte layer from experiment 14 compared to experiment 13.

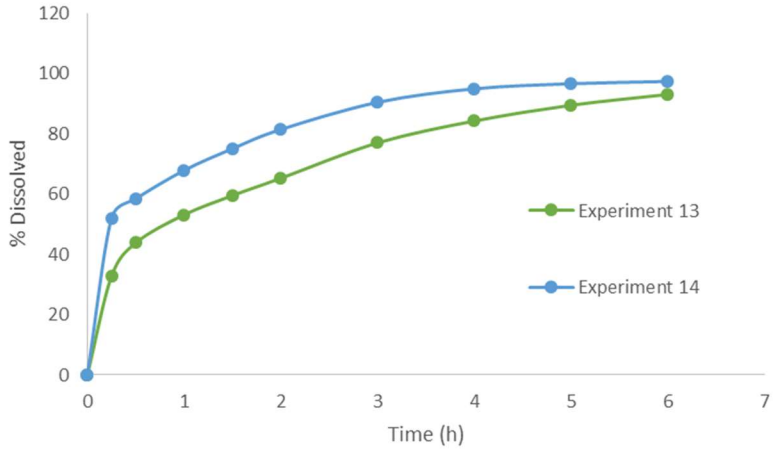

FIGURE 17. Release profile of Zolpidem hemitartrate in accordance with Experiment 13 and 14 of the full factorial experimental design.Experiment 13: basket apparatus

with a rotation speed of 50 rpm, phosphate buffer as dissolution media (pH 6.8) with a volume of 500 mL. Experiment 14: Basket apparatus with a rotation speed of 100 rpm and HCl 0.01 N (pH 2.0) as dissolution media with a volume of 900 mL.

Differences in release model can be observed on Figure 19. While Experiment 3 seems to have a zero-order release rate of the extended release layer, Experiment 2 seems to promote a first-order release

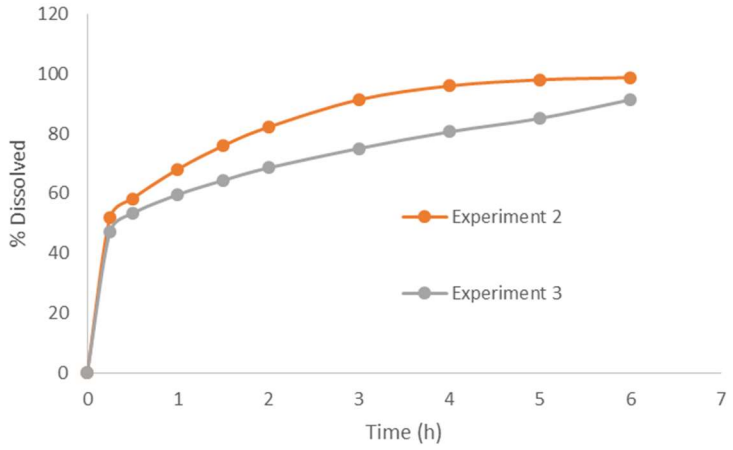

FIGURE 18.Release profile of Zolpidem hemitartrate in accordance with Experiment 2 and 3 of the full factorial experimental design.Experiment 2: basket apparatus with a rotation speed of 100 rpm, HCl 0.01N as dissolution media (pH 2.0) with a volume of 500 mL. Experiment 3: Paddle apparatus with a rotation speed of 100 rpm and phosphate buffer (pH 6.8) as dissolution media with a volume of 500 mL.

An important observation is how the α parameter of the Logistic model for the *in vitro* dissolution test is well correlated with the percentage dissolved at sampling times points of 0.25, 0.50, 1.0, 1.5 and 2 hours. On Figure 20 – 23, it's possible to observe a positive correlation of this parameter with the percentage dissoved at 0.25, 0.50, 1.0 and 2 h time point, therefore, this parameter can be used to describe the factors effect on this dissolution sampling time.

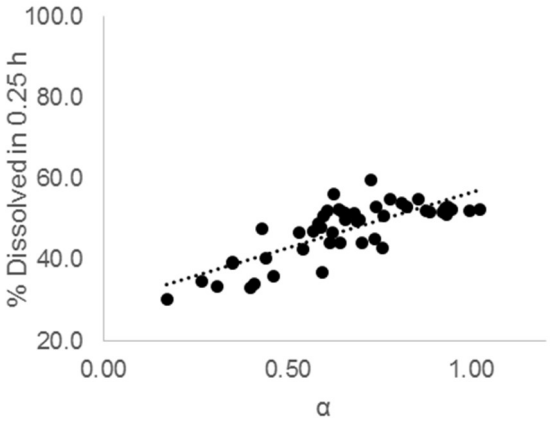

FIGURE 19: Correlation of percentage dissolved at 0.25 h and the α parameter of the logistic model

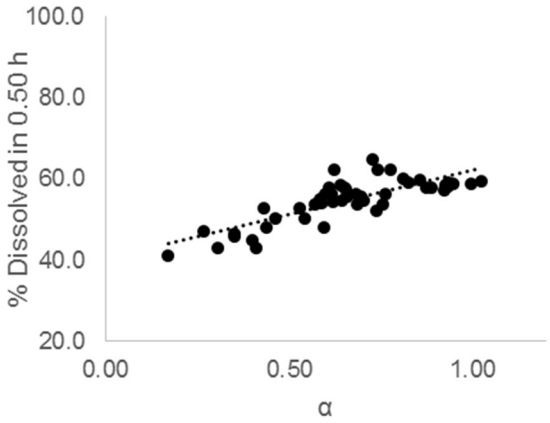

FIGURE 20. Correlation of percentage dissolved at 0.50 h and the α parameter of the logistic model

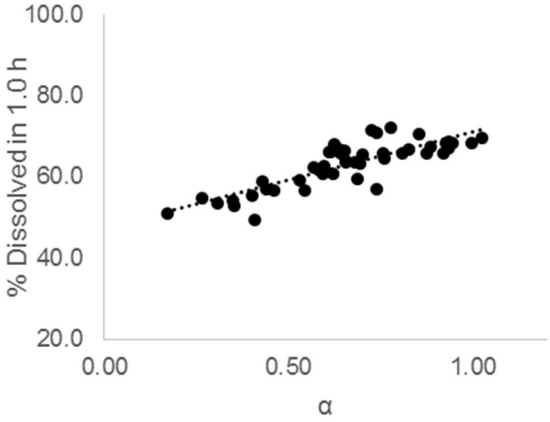

FIGURE 21. Correlation of percentage dissolved at 1.0 h and the α parameter of the logistic model

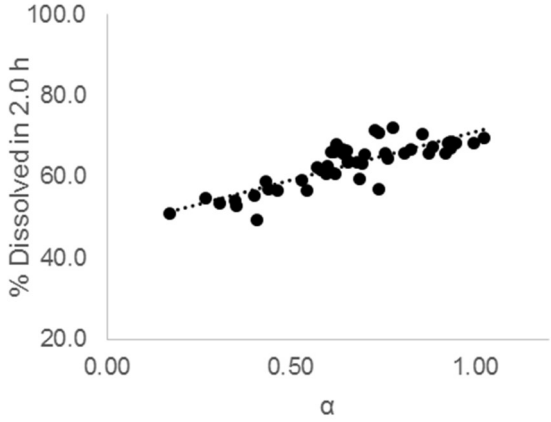

FIGURE 22. Correlation of percentage dissolved at 2.0 h and the α parameter of the logistic model

The differences observed in dissolution rate and pattern of the dissolution curve in each experiment shows how the dissolution condition can affect the release rate of a tablet. Choose the best condition is not an easy task, and understand how the factors affect the release rate is essential to choose an adequate dissolution condition.

5.4 – Results of the Full Factorial Experiment 2^4 – Identification of Important Effects

The full factorial experiment was executed in order to identify the significant variables of the dissolution test that affect product dissolution.

The first evaluation considered all the effects and its interactions for the α parameter of logistic model. The significant effects were selected using a confidence level of 95% and the pareto chart of these effects is represented on Figure 24.

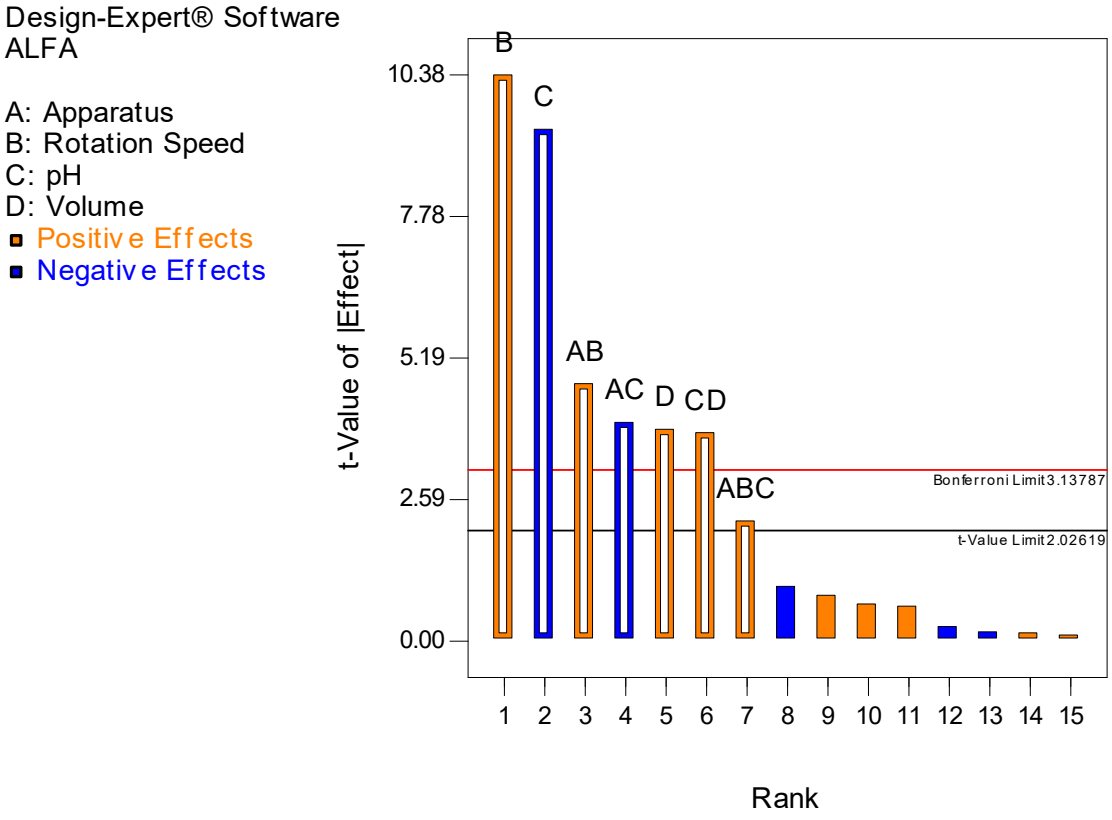

FIGURE 23. Pareto chart showing thet-values of the effects for the α parameter of the Logistic Model for *in vitro* dissolution and it's significance with a confidence level of 95%.

All factors studied were considered significant just as expected with the FMEA. Athough the apparatus was not considered significant, its interaction with rotation speed and pH are important. With an positive effect, the rotation speed is the most important to affect dissolution rate, and the higher the rotation speed, the higher

is the percentage dissolved at the sample times correlated with the α parameter. This was expected and is in concordance with the theory that the higher the hydrodinamics, the higher is the release rate.

It's possible to understand the effect of pH on dissolution rate, rising the pH to 6.8 would reduce the dissolution rate. Considering that Zolpidem is a weak base, it is expected that with pH higher than its pKa, the molecule would be less soluble. Since that drug solubility is an important term on Nernst and Bruner equation, equation 2, the lower release rate would be expected.

Another important aspect observed with this experiment is the secondary and tertiary interactions of factors, observe and understand this interactions would no be possible with an OFAT method.

Since apparatus is a categorical variable, in order to reduce the number of experiments on the response surface phase of the experiment, an evaluation was made to choose the best apparatus to continue with the study. As can be seen on Pareto Chart for the effects on the β term of logistic model (Figure 25), the Apparatus is the most important factor that define the dissolution curve shape.

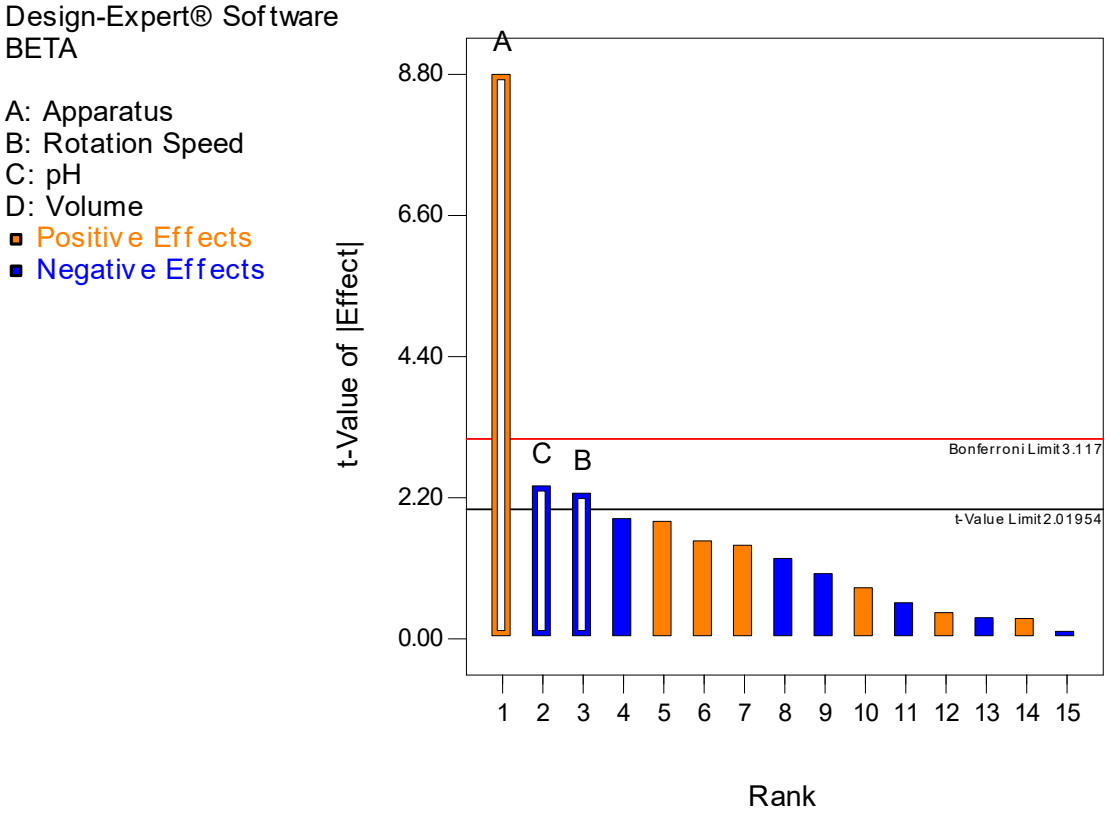

FIGURE 24. Pareto chart showing the effects for the β parameter of the Logistic Model for *in vitro* dissolution and it's significance with a confidence level of 95%.

It's possible to understant the importance of this factor on β parameter observing the effect graphic on Figure 26.

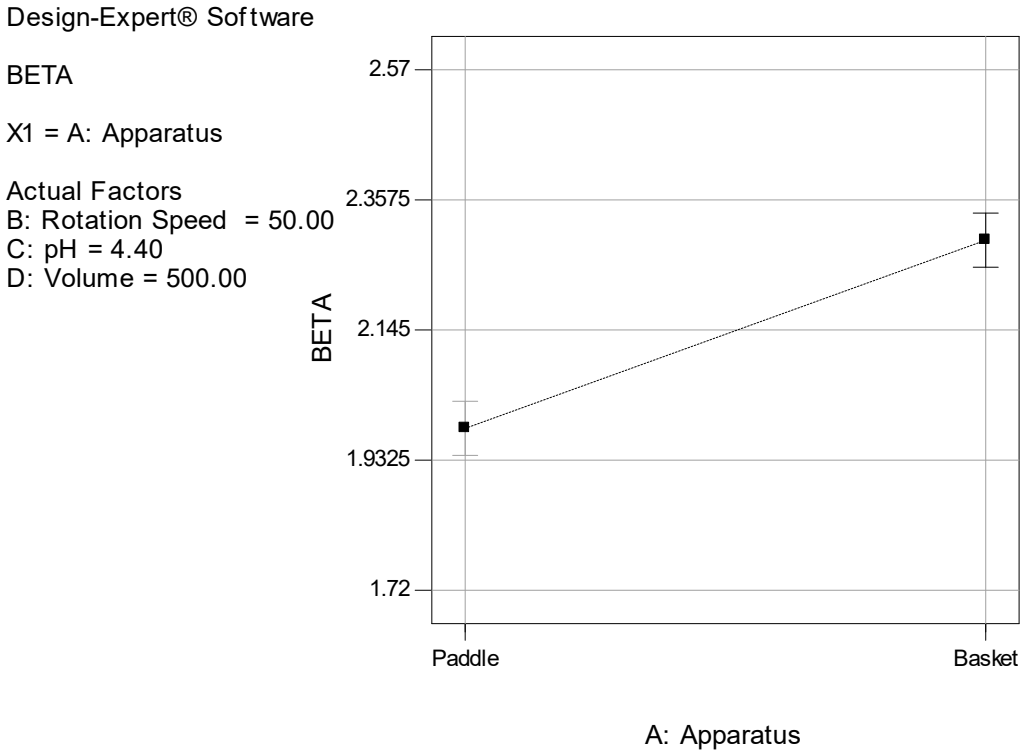

FIGURE 25. Effect chart of apparatus on β parameter of the Logistic model considering a fixed rotation speed of 50 rpm, pH of 2.0 and dissolution media volume of 500 mL.

It's expected an increase in the value of β parameter when the apparatus is changed from paddle to basket. Considering that the objective is to reach an β value closer to the one founded *in vivo* ($\beta_{in\ vivo}$= 4.29), the basket apparatus was considered as the best alternative to get close to this value.

Another advantage of the basket apparatus is its ability to optimize the capacity of the dissolution curve be explained by the logistic model. Working with basket apparatus, it is expected a higher value of the correlation coefficient (R^2) (Figure 27).

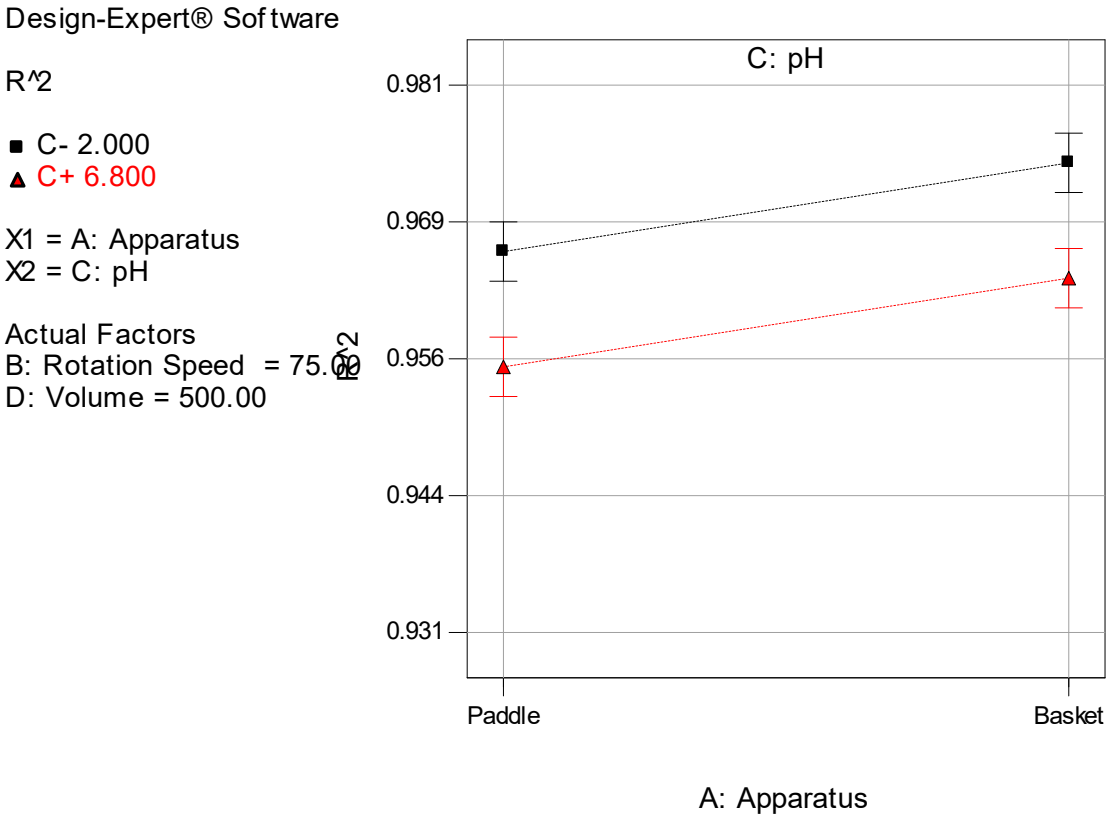

FIGURE 26. Effect chart of apparatus on R^2 considering a fixed rotation speed of 50 rpm, pH of 2.0 and dissolution media volume of 500 mL.

Therefore, basket apparatus was considered the best option to proceed with further experiments.

At this phase of the experiment, it was possible to understand the effects of each factor and its interactions. All the effects observed are in concordance with what was expected. A great advantage until now is the ability to understand how all factors are affecting the release rate of the product. The next phase of the experiment have the objective to not only understand each factor, but to model it, quantify how much these effects affect the responses evaluated, and choose the best dissolution condition.

5.5 – Response Surface Methodology and Quantitative Effect of the Factors

The response surface model was applied to the most important variables obtaining a fine adjustment of the studied variables and propose empirical models that correlate these variables with the responses that have being monitored. Since there are 5 different responses, each being affected differently by the variables under study, it was stablished a compromise model amongthem in order to obtain the best dissolution condition to analize the product.To complete the Face Centered Cube (FCC) desing model, it was performed another 7 experiments including 1 center point with six authentic repetitions, the average results of the FCC experiments which complemented the factorial design are described on Table 5.

The analysis of variance (ANOVA) was applied for estimation of quantitative effects of the factors with a significance level of 95%. Factor effects of the Face Centred Cube (FCC) design model and associated *p-values* for allfive reponses are presented in Table 6 (y1 – y3) and 7 (y4 – y5). A factor was considered to influence the response if the effects significantly differ from zero and the *p-value* is lower than 0.05. A synergic or antagonistic effect is represented by the positive and negative signals, respectively. The model was recalculated after the elimination of coefficients that were considered not significant and the resulted equation coded (Eqs. (4) – (8)) for all fiveresponses y_1, y_2, y_3, y_4, y_5 are presented:

$$y_1 = 49.54 + 5.76x_1 - 1.60x_2 + 1.43x_3 - 3.43x_1^2 + 2.24x_1x_2 \quad (4)$$

$$y_2 = 92.62 + 2.28x_1 - 3.03x_2 + 2.76x_1x_2 + 1.48x_1x_3 + 1.18x_2x_3 \quad (5)$$

$$y_3 = 0.96 - 0.009x_1 + 0.007{x_1}^2 + 0.005{x_3}^2 \quad (6)$$

$$y_4 = 0.76 + 0.14x_1 - 0.15x_2 + 0.066x_3 + 0.031x_1x_2 - 0.076{x_3}^2 \quad (7)$$

$$y_5 = 2.21 - 0.063x_1 - 0.066x_2 + 0.0311x_1^2 - 0.074x_2^2 + 0.038x_1x_3 \quad (8)$$

TABLE 5. Average result of the FCC experimental design (Complete data in Appendix II).

Experimental Design	Rotation Speed (x_1)	pH (x_2)	Volume (x_3)	0.25 h (y_1)	0.5 h	1 h	1.5 h	2 h	3 h	4 h (y_2)	5 h	6 h	R^2 (y_3)	α (y_4)	β (y_5)
Factorial Design n=3	100	2.0	500	51.81	58.31	68.14	76.00	82.21	91.40	96.03	98.07	98.81	0.97	0.92	2.25
	100	6.8	500	49.38	55.24	63.03	69.30	74.63	83.09	89.78	94.53	97.63	0.96	0.61	2.03
	50	2.0	900	43.02	52.35	63.22	70.47	76.29	85.28	90.69	93.62	95.49	0.98	0.69	2.30
	50	6.8	900	37.72	46.34	55.07	60.95	65.96	74.96	82.08	86.84	90.39	0.96	0.40	2.19
	50	2.0	500	44.38	55.20	65.77	73.98	81.12	91.42	97.16	100.20	101.14	0.98	0.65	2.31
	50	6.8	500	32.80	43.85	53.16	59.55	65.30	77.05	84.29	89.53	93.10	0.96	0.25	2.35
	100	2.0	900	52.13	58.46	67.90	75.12	81.48	90.49	94.99	96.67	97.41	0.97	0.98	2.26
	100	6.8	900	54.35	61.44	71.16	77.23	82.28	90.31	97.88	104.78	106.29	0.96	0.79	2.19
Center point n=6	75	4.4	700	48.20	53.87	60.97	67.15	72.22	80.65	87.14	91.39	93.86	0.95	0.78	2.12
CFC n= 3	75	6.8	700	53.28	58.91	66.08	71.92	75.98	83.22	94.66	102.45	104.78	0.93	0.65	2.03
	100	4.4	700	51.67	57.61	66.72	73.88	80.58	89.66	95.05	99.04	100.91	0.96	0.83	2.22
	75	4.4	900	50.30	56.47	65.28	71.84	78.40	87.70	94.01	98.25	99.98	0.96	0.79	2.24
	50	4.4	700	42.55	52.80	61.96	69.15	75.98	85.94	92.73	96.66	98.79	0.96	0.66	2.40
	75	4.4	500	46.69	55.10	64.72	71.81	77.78	87.38	93.85	97.63	101.16	0.97	0.69	2.24
	75	2.0	700	50.88	58.26	67.96	75.22	81.29	90.65	96.19	99.00	101.26	0.97	0.83	2.22

TABLE 6. Factor effects and associated p-values for all responses 1 to 3.

Factor	Response					
	y_1		y_2		y_3	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
x_1	5.76	**<1.00E-3**	2.28	**<1.00E-3**	-2.0E-3	**1.90E-1**
x_2	-1.60	**1.8E-02**	-3.03	**<1.00E-3**	-8.9E-3	**<1.00E-3**
x_3	1.51	**2.80E-2**	0.29	**5.85E-1**	-1.8E-3	**2.41E-1**
x_1x_2	2.24	**3.80E-2**	2.76	**<1.00E-3**	3.3E-4	**8.46E-1**
x_1x_3	0.06	9.38E-1	1.48	1.35E-2	1.4E-4	9.35E-1
x_2x_3	1.21	1.05E-1	1.18	4.45E-2	9.3E-4	5.82E-1
x_1^2	-3.07	**2.33E-2**	-0.55	**5.94E-1**	8.2E-3	**1.00E-2**
x_2^2	1.90	1.51E-1	0.99	3.37E-1	-4.1E-3	1.79E-1
x_3^2	-2.39	7.89E-2	-0.69	5.09E-1	7.1E-3	2.66E-2

TABLE 7. Factor effects and associated p-values for all responses 4 to 5.

Factor	Response			
	y_4		y_5	
	Coefficient	p-value	Coefficient	p-value
x_1	0.140	**<1.00E-3**	-0.063	**1.30E-3**
x_2	-0.150	**<1.00E-3**	-0.066	**8.00E-4**
x_3	0.064	**<1.00E-3**	0.011	**5.56E-1**
x_1x_2	0.031	**5.55E-2**	-0.022	2.97E-1
x_1x_3	-4.16E-5	9.98E-1	0.380	6.63E-2
x_2x_3	0.019	2.32E-1	-0.017	4.15E-1
x_1^2	-0.026	**3.65E-1**	0.096	1.21E-2
x_2^2	-0.031	2.81E-1	-0.087	2.21E-2
x_3^2	-0.049	1.01E-1	0.039	**3.00E-1**

To evaluate the model significance, ANOVA was performed and a model was considered significant if the p-value is 0.05 or less, the adequacy of the models were estimated by $R^2$and the p-value of Lack of Fit (LOFT). The results obtained are described on Table 8.

TABLE 8.ANOVA of model significance

Response	DF	p-value (Model)	F-Ratio	R^2	p-value (LOFT)
y_1	5	<0.0001	20.51	0.72	0.1200
y_2	5	<0.0001	19.19	0.71	<0.0001
y_3	3	<0.0001	18.23	0.56	0.0828
y_4	5	<0.0001	48.18	0.86	0.1608
y_5	5	<0.0001	8.13	0.50	0.3756

The five models evaluated were considered significant with a p-value of less than 0.0001 for the model. For responses y_1, y_2 and y_4, the Pearson correlation coefficient (R^2), which shows how much of the data variability can be explained by the model, were considered satisfatory with values of 0.72, 0.71 and 0.86 respectively and model y_3 and y_5 were considered less adequate to explain data variability with an R^2 of 0.56 and 0.50. The LOFT of the models were not considered significant with the exception for the response y_2.

The beginning of the dissolution test of a immediate release dosage form is naturaly variable, therefore, it's expected that a part of this variability would not be explained by the factors evaluated at the model. At the end of a dissolution test, the assay of each tablet is variable either, been another variable that is not considered by the model which may affect the value of R^2.

The Normal probability plot of the residuals and the residual homocedasticity can be observed on Figures 28 to 37 for all models. All the residues have a normal distribution and are included at the interval of ±3 standard deviation, indicating the absence of outliers.

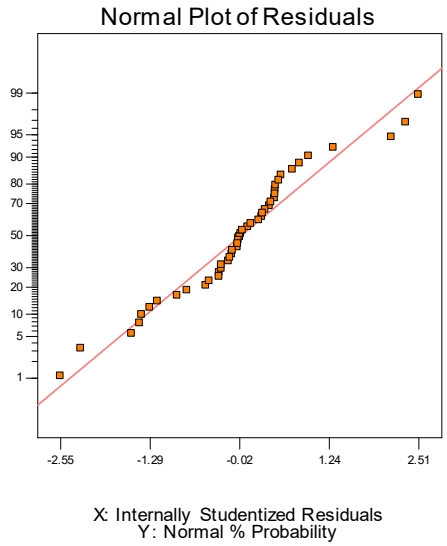

FIGURE 27. Normal probability plot of the residual for the response y1

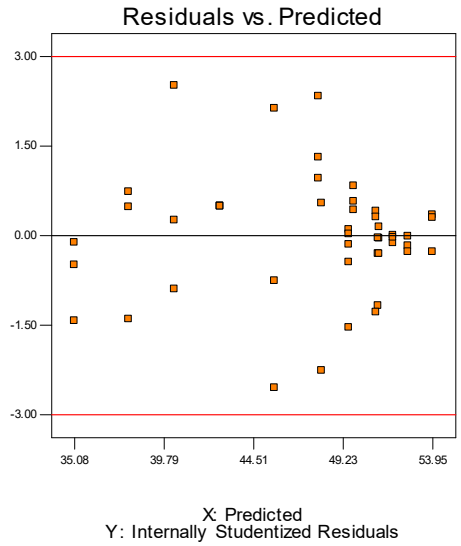

FIGURE 28. Predicted response in function of the proposed model residue for response y1.

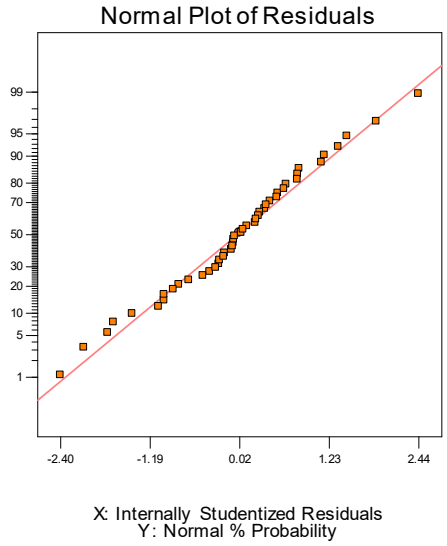

FIGURE 29. Normal probability plot of the residual for the response y2

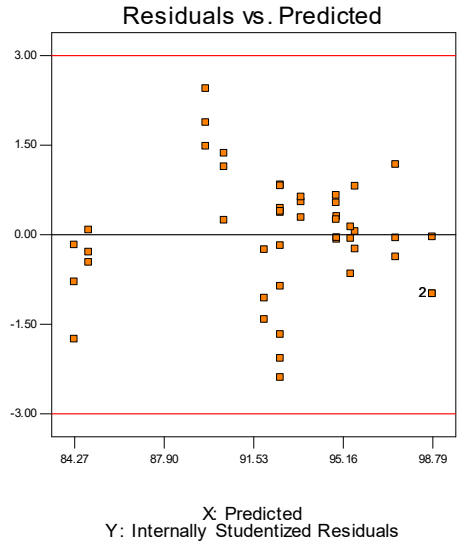

FIGURE 30. Predicted response in function of the proposed model residue for response y2.

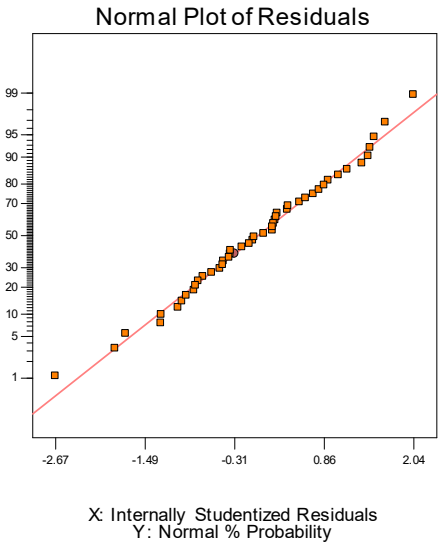

FIGURE 31. Normal probability plot of the residual for the response y3

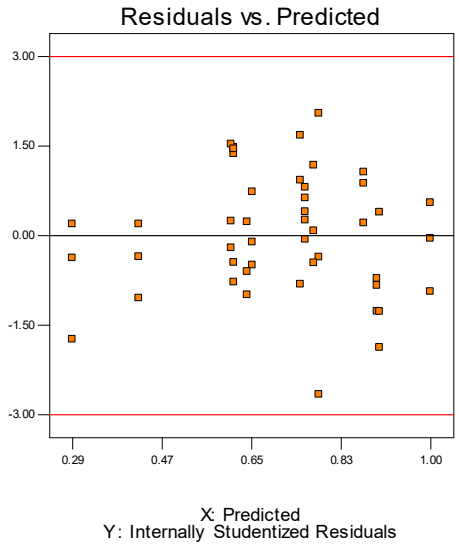

FIGURE 32. Predicted response in function of the proposed model residue for response y3.

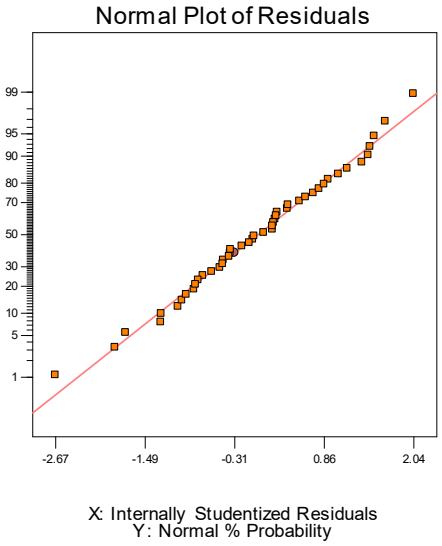

FIGURE 33. Normal probability plot of the residual for the response y4

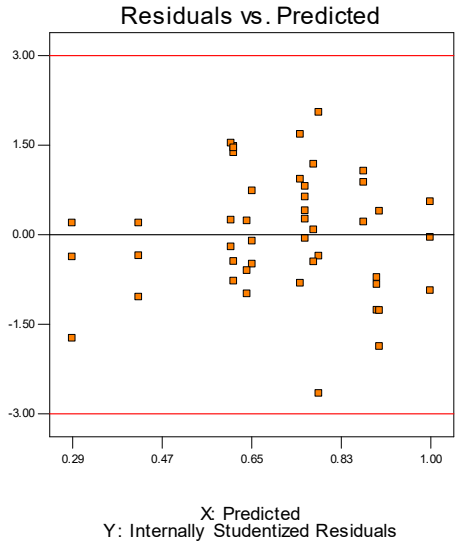

FIGURE 34. Predicted response in function of the proposed model residue for response y4.

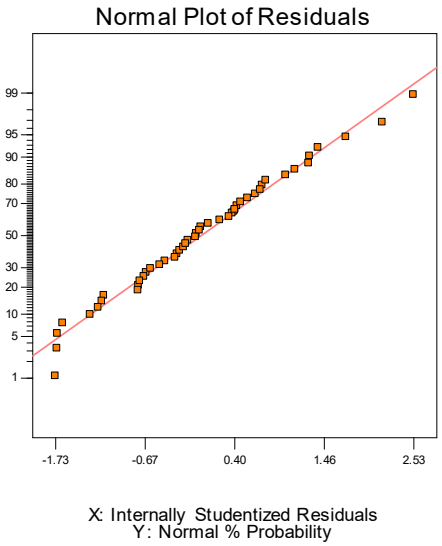

FIGURE 35. Normal probability plot of the residual for the response y5.

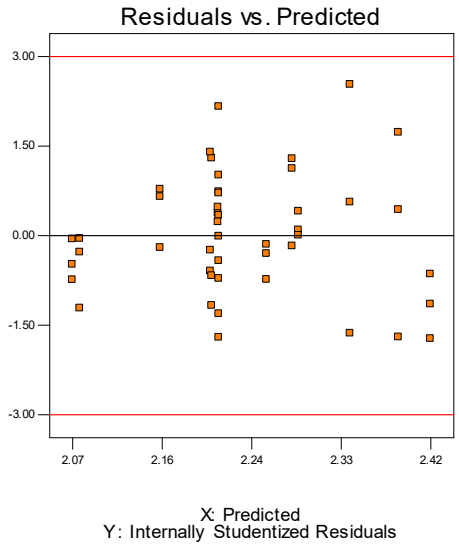

FIGURE 36. Predicted response in function of the proposed model residue for response y5.

For a better evaluation, three-dimensional (3D) and countor plots for the measured responses were prepared based on the proposed models. Since the model has more than two factors, one factor was held constant in zero for each graph.

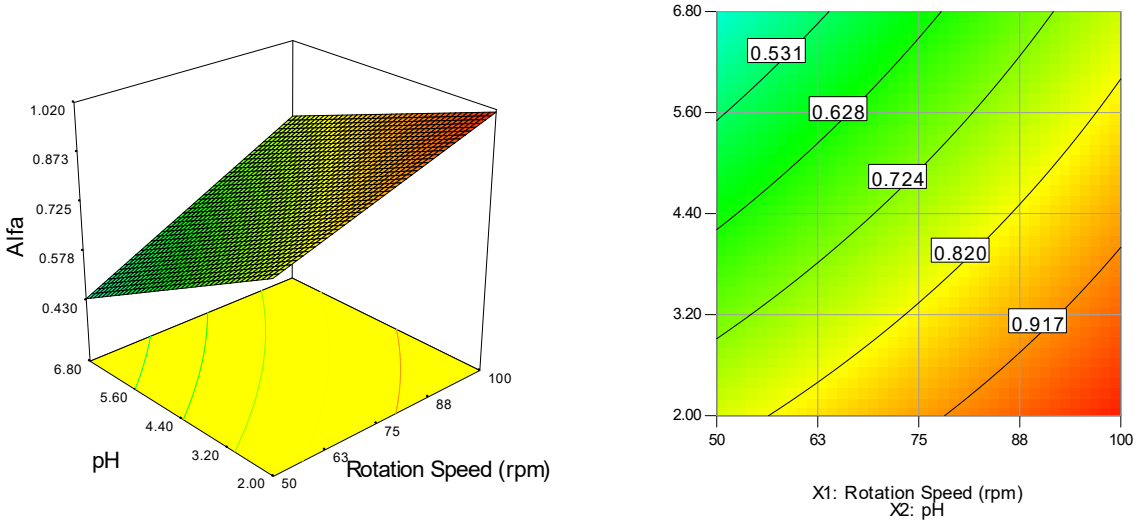

FIGURE 37. Response surface plot (3D) and countour plot showing the effect of pH of the dissolution medium (x2) and rotation speed of apparatus (x1) on the response y4 (α parameter of Logistic model).

It can be seen that for response y_4, the rotation speed of the basket have a positive effect (Figure 38). It also can be observed a positive interaction of rotation speed and pH of the dissolution media. Since the mechanism of drug release from the extend release layer is a swellable system, the hydrodinamic stress and intensity of fluid flow causes greate attrition at the swollen matrix periphery, changing the release mechanism from a difussion process to a erosion processof the matrix. The pH-dependent solubility of the drug affect release mechanism either.

The response y_1 is affected by the interaction of rotation speed and pH of the dissolution media. With a low hydrodinamic condition, as rotation speed of 50 rpm, the dissolution of the immediate release layer of the tablet is affected mainly by the drug molecule physicochemical properties, as solubility pH dependence, but when working with higher rotation speed, the dissolution is more dependent on the removal of the stagnant layer and the physicochemical property of Zolpidem becomes less important (Figure 39).

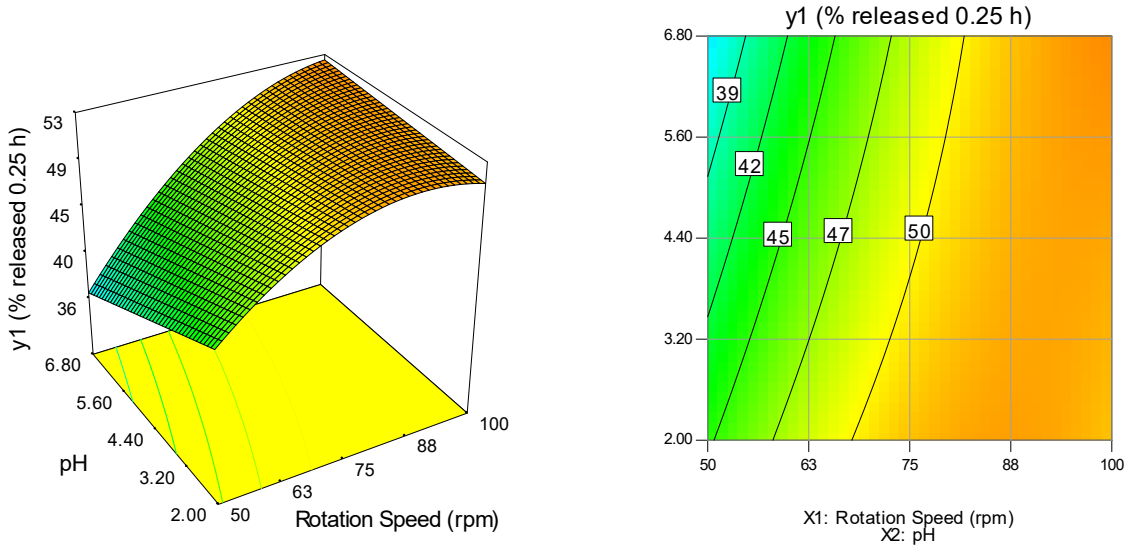

FIGURE 38. Response surface plot (3D) and countour plot showing the effect of pH of the dissolution medium (x2) and rotation speed of apparatus (x1) on the response y1 (% released at 0.25h).

The countour plot represented on Figure 40 allows to observe the interaction of dissolution media volume (x_3) and rotation speed (x_1) for the response y_2 with a fixed pH of 6.8. It's possible to optimize the percentage released at this pH rising the volume of the dissolution media and the aparattus speed. The higher the

volume of the dissolution media, the lower is the drug saturation solubility, therefore, faster is the dissolution of the drug.

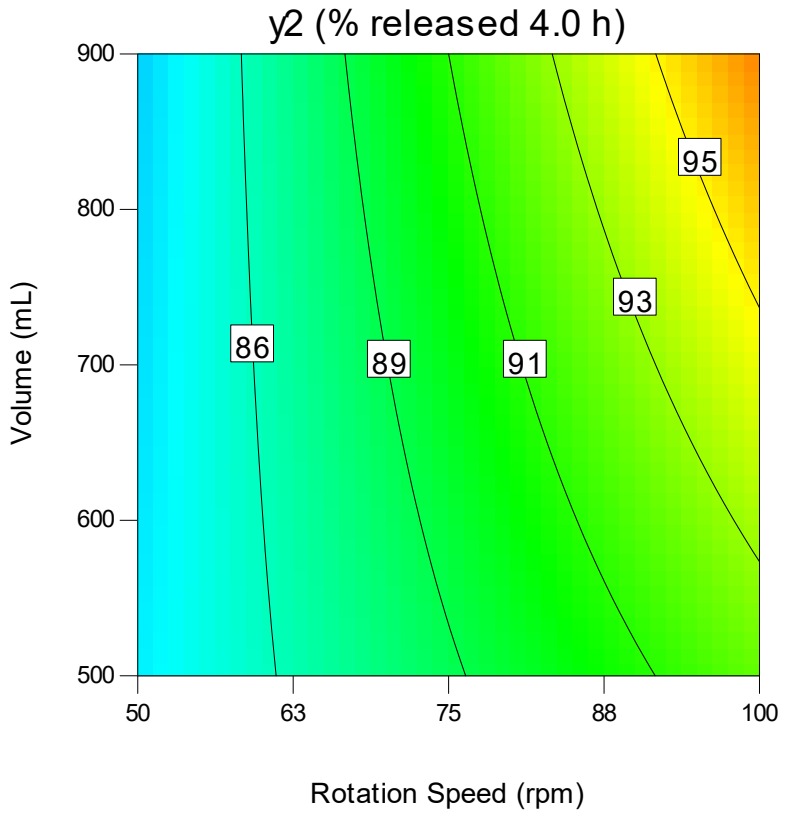

FIGURE 39.Countour plot showing the effect of rotation speed (x1) and the volume of the dissolution medium (x3) on the response y2(% released at 4.0h).

All the results observed are in concordance with the expectations, the difference now is that the important factors were modeled and it is possible not only to understand how these factors affects the dissolution rate of the product, but to quantify how each factor and its interaction interfere on release rate. The next step consist in choosing the best dissolution condition using all the information generated.

5.6 – Optimization and Selection of dissolution Condition

After generating the polynomial model equations to relate the dependent and independent variables, the process was optimized for all five responses.The objective at this point is to find a dissolution condition that generate a

dissolution curve that is closer to the one observed *in vivo* throught the deconvolution of the meanzolpidem plasma concentration-time profiles.

In order to obtain a compromise among various responses and identify a committedcondition of factors levels that jointly optimize a set of response, satisfying the requirements for each one, two different methods were used.

The first one applied the Desirability Function. With this function the responses of the design of experiment are converted in values of 0 and 1 called individual desirability, which 0 and 1 represent undesirible and desirable responses, respectively. After the individual desiribility, the global desirability is obtained using geometric mean of individual desirabilities.

It was attributed different importance to the desired responses described on Table 2. Since it's desired that the *in vitro* dissolution curve fit to the Logistic model, responses y_3 and y_5 were given aimportance of 4. Figure 41 shows the countor plot obtained whith the highest global desirability space in function of Rotatin speed (x_1) and pH (x_2) with a fixed volume (x_3) of 500 mL.

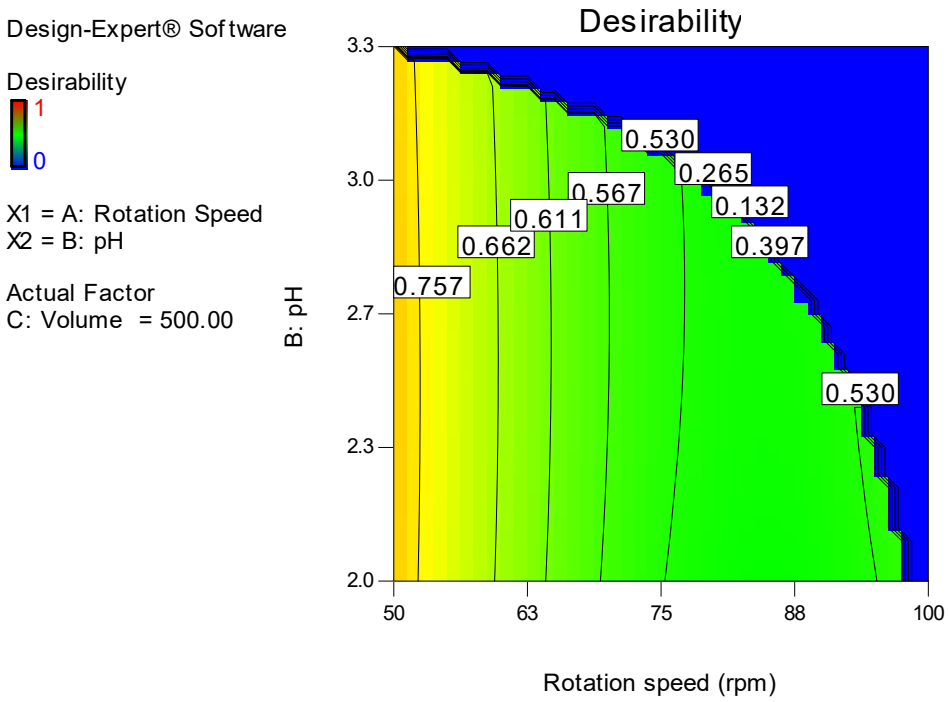

FIGURE 40. Contour plot with the highest global desiribility space in function of Rotation speed (x1), pH (x2) with a fixed volume of 500 mL.

It's possible to observe on Figure 41 that the optimun condition to obtain an *in vitro* dissolution result similar to the *in vivo*is working with a low rotation speed, i.e. 50 rpm, and a pH between 2.0 and 3.3 with a volume of 500 mL. Therefore, an acceptable dissolution condition would be working with 500 mL of dissolution media volume with pH of 2.0 and a rotation speed of 50 rpm with basket.

Another simple and easy to understand approach tested is based on the maximun difference accepted on each sampling point of deconvolutedzolpidem plasma concentration-time profiles. It was considered that a absolute difference of +/- 5 % at each sampling time would be considered acceptable, consequently, every dissolution condition that generate an*in vitro* dissolution curve inside this interval is considered a good method. The sampling times that measure the immediate release layer dissolution were not considered, it was stablished only a range of minimun dissolved. The representation of the deconvoluted zolpidem plasma concentration-time profiles with the intervals considered as acceptable is described on Figure 42.

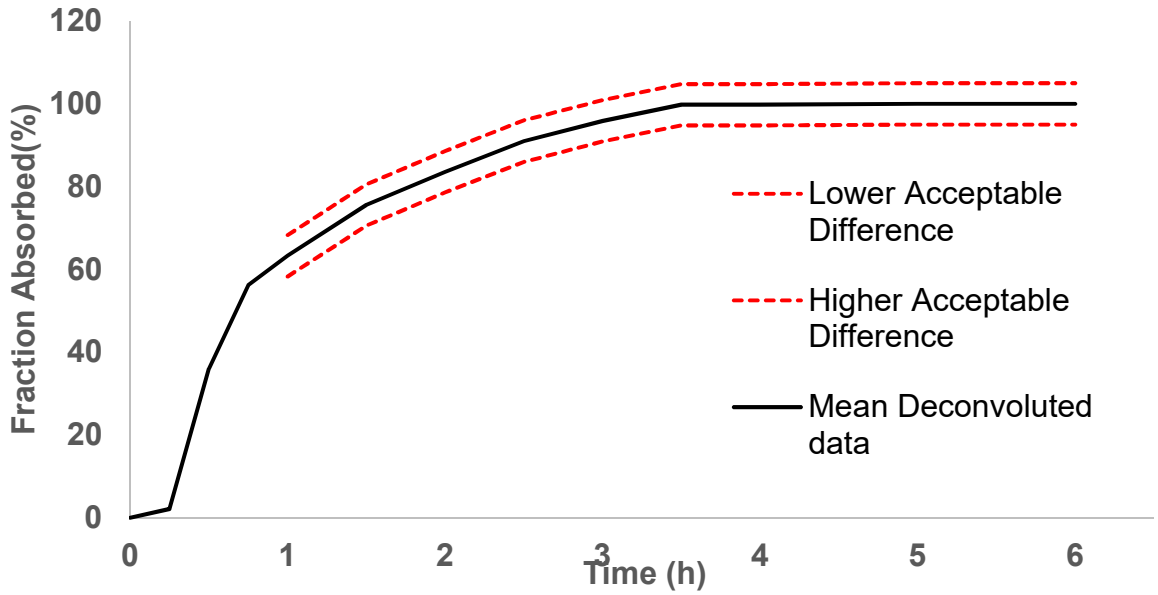

FIGURE 41.Deconvoluted data of the mean zolpidem plasma concentration profile with the acceptable difference range.

The working range that satisfy this condition of difference are represented in Figure 43. To construct this design space the sample times of 1.0, 1.5, 2.0, 3.0 and 4.0 h were modeled, the responses of % dissolved at 1.0, 1.5, 2.0 and 3.0 hours were indetified as y_6, y_7, y_8 and y_9, respectively.

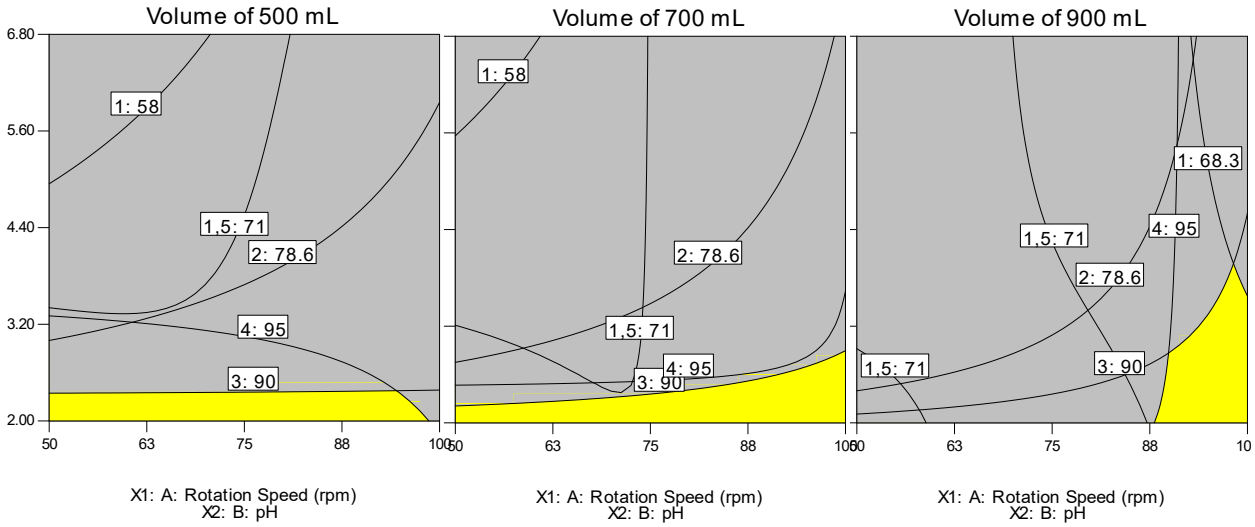

FIGURE 42.Design space (yellow) for response y_2 (% released at 4.0 h), y6 (% released at 1.0h), y_7 (% released at 1.5 h), y_8 (% released at 2.0h) and y_9 (% released at 3.0 h) with respect to rotation speed (x_1), pH (x_2) and volume (x_3).

There are many options to work that satisfy the similarity criteria stablished, including the one obtained by Desirability function (rotation speed of 50 rpm, pH 2.0 and volume of 500 mL). Working wih a dissolution volume of 900 mL, the working range of rotation speed become more restricted, while with volume of 500 and 700 mL, this working range is bigger.

The dissolution condition choosen to study the Zolpidem Hemitartrate Extended Release Rate 12.5 mg/tablet was with 500 mL of dissolution media volume with pH 2.0 and a rotation speed of 50 rpm with basket.

5.7 – Optimization and Model Validation

In order to validate the predicted optimal parameters condition and compare de obtained and theorethical responses, the drug release profile at choosen combination of physicochemical parameters was carried out. Since the condition choosen was performed during the execution of the factorial experiment (Experiment 05, see Table 2), this result was compared with the predicted by the model and can be observed on Table 9.

TABLE 9. Comparison of the predicted response of the model against the experimental results obtained.

Response	Predicted Response	Observed Response	Residual (absolute error)
y1	42.80(± 3.11)	44.38	3.6
y2	98.79(2.52)	97.16	1.7
y3	0.977(0.004)	0.979	-02
y4	0.650(0.067)	0.653	-0.6
y5	2.420 (± 0.076)	2.31	4.8

It can be concluded that optimized combination of investigated physicochemical parameters ensured a release profile which was very close to the predicted values.

Since the method development was performed with the reference product, it's expected that release rate of the product at the choosen method condition selected with the experimental design is similar to the deconvoluted zolpidem plasma concentration-time profiles. The result of this comparisson can be observed on Figure 44.

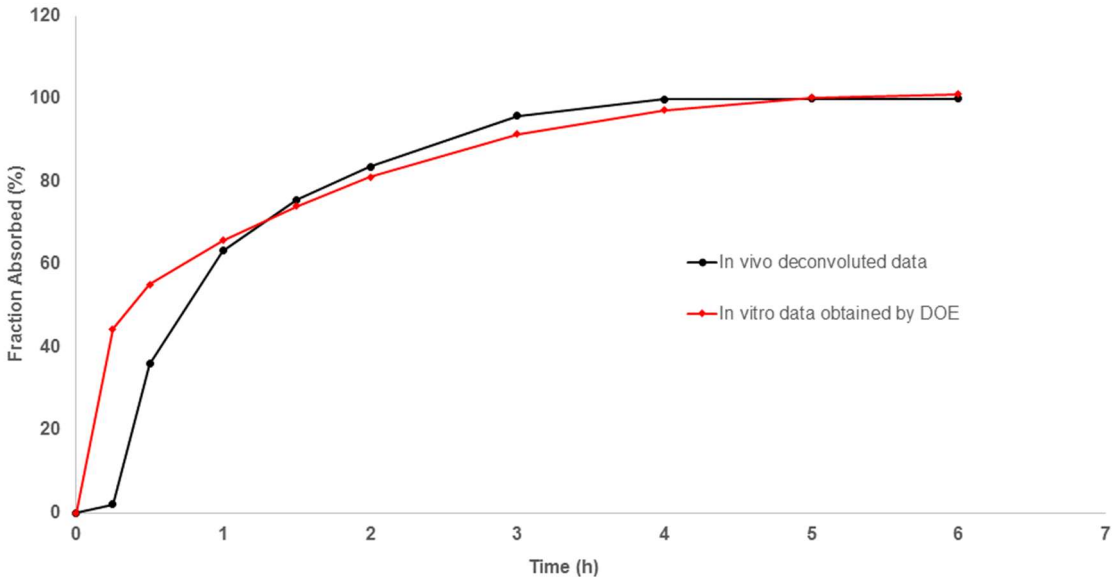

FIGURE 43. Comparison of the deconvoluted zolpidem plasma concentration-time profiles and the *in vitro* dissolution result obtained with the dissolution condition obtained by the design of experiment method.

It can be observed that the *in vitro* data is very similar to the *in vivo* profile and main difference is observed at the sampling times of 0.25, 0.50 and 0.75h. Thesesampling times represent the percentage dissolved of the immediate release layer, therefore it was expected a poor correlation at thesepoints due to the lag time of absorption of the drug. The important observation is that during the *in vitro* test, the immediate release layer must be dissolve rapidly to simulate a process of bioavailability of the drug before the gastric empting.

A new dissolution testwith the best condition choosed was executed with six tablets and including the sampling times of 0.75, 2.5 and 3.5 hours to evaluate the correlation with the *in vivo* absorption curve. The correlation between the *in vitro* and *in vivo* data considering sampling times after 1h of test is represented on Figure 45.

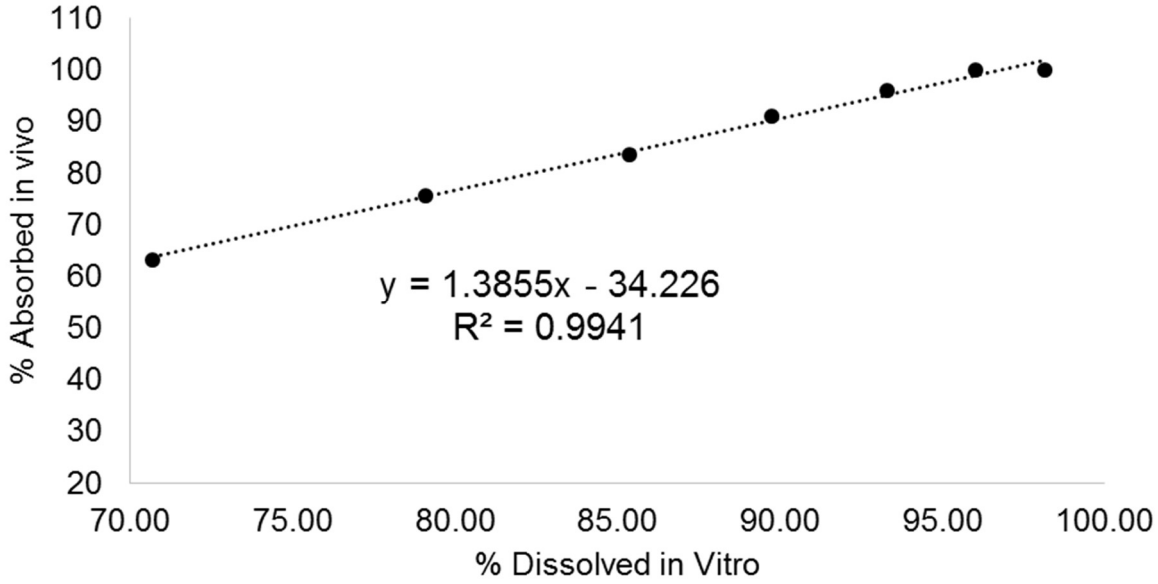

FIGURE 44. Correlation between the fraction dissolved *in vitro* and absorbed *in vivo* considering sampling times of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4 hours.

It was obtained a good correlation of the *in vitro* and *in vivo* data. It's important to point out that at this stage of product development, there is no objective to get a level A *in vivoin vitro* correlation, the objective here was to understand the

release mechanism of the reference product, understand how the dissolution conditions affect product release and propose an dissolution method that has good chances to predict *in vivo* behavior of the product.

5.8 – Comparison Between DoE and OFAT Method

A comparison was made between the Design of Experiment and the traditional one factor at time (OFAT) methods. This comparison was based on number of experiments, and samples, days to do the tests and quantity of the responses. All the evaluation is presented on Table 10.

TABLE 10. Comparison of OFAT and DOE method applied to dissolution method development

	OFAT	DOE	Reduction
Number of tests	38	24	37%
Sample quantity (tablets)	228	72	68%
Days to test execution	40	26	35%

The DOEmethod shows significant advantage over the OFAT, since the number of experiment is reduced significantly. The number of sample is reduced either and has important impact on costs of the method development, and would be a interesting approach when working with high cost drugs.

Another advantage of DOE is the quality of the experiment conclusion, with DOE method, it's possible to understand the impact of all factors evaluated, their significance and detect the interactions among them, possibilities that are not achieved with the OFAT method.

An important comparison to be made is which dissolution condition would be choosen using the OFAT method? Considering the usual procedure of the group to test and choose an adequate dissolution method, probably the dissolution condition choosen would be the basket aparratus with a rotation speed of 50 rpm and phosphate buffer pH 6.8 and 500 mL of dissolution media. The comparison of the

result obtained with this dissolution condition with the result condition obtained with the DOE method and *in vivo* can be observed on Figure 46.

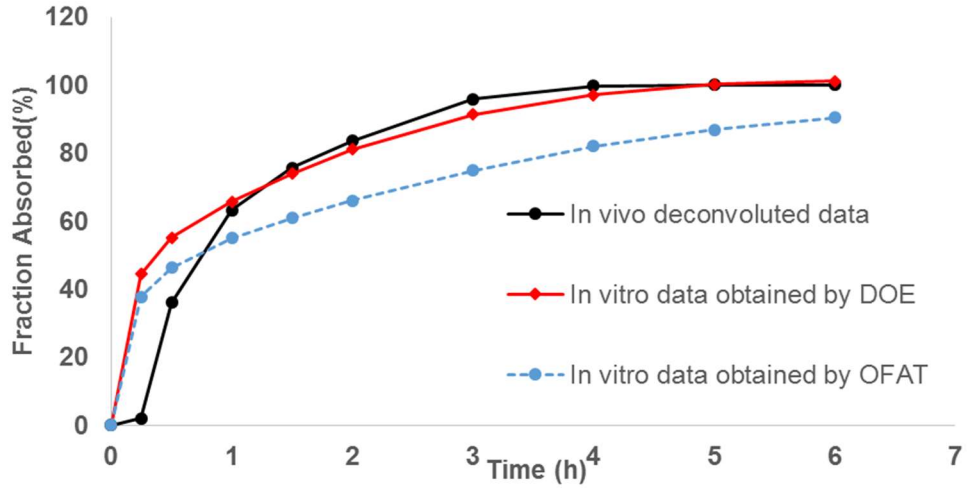

FIGURE 45.Comparison of the result obtained by the OFAT and DOE method with *in vivo* deconvoluted data.

The dissolution method proposed by the OFAT procedure is very different from one obtained by DOE. The release rate is slower than what is observed *in vivo* and seems like a zero-order release rate, while *in vivo* release is more like a first-order release rate.

CONCLUSION

6 – CONCLUSION

The dissolution method developed describes the dissolution release rate characteristics of the reference product and can be used during early stage development in formulation screening. This method have a higher probability of getting an *in vitroin vivo* correlation.

The design of experiment methodology proved to be faster, less expensive and more rational for dissolution method development when compared to the tradition OFAT method. Besides all the advantages, the DOE represented a new culture of work in method development on Research and development group, since its applicability is not restricted to dissolution method development, but to formulation and analytical method either.

Some improvements can be made in data evaluation, since always will depend on the goal of the dissolution method developed. An interesting characteristic of a good dissolution method is the discriminative power. Therefore it’s suggested to evaluate a differente response, studing two or more batches with proposital changes to have different release rate of the drug. A good dissolution method would be the one that detect these differences.

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APPENDIX I

Apparatus	Rotation speed (rpm)	pH	Volume (mL)	0,25	0,5	1	1,5	2	3	4	5	6	R2	α	β
Pá	100	2	900	50,02	55,62	63,39	69,06	73,84	81,15	86,83	90,75	93,89	0,9663	0,6943	1,9496
Pá	100	2	900	50,81	56,13	64,46	70,00	74,82	81,83	87,21	90,79	93,64	0,9699	0,7612	1,9608
Pá	100	2	900	52,24	57,76	65,71	71,73	76,46	84,08	88,88	92,43	94,95	0,9696	0,8741	2,0533
Cesto	100	2	500	51,68	57,99	67,45	75,28	81,60	91,42	96,45	98,39	99,19	0,9715	0,8856	2,2466
Cesto	100	2	500	51,21	58,06	68,60	76,68	82,93	91,47	95,48	97,55	98,26	0,9795	0,9325	2,2332
Cesto	100	2	500	52,55	58,87	68,37	76,02	82,11	91,32	96,15	98,27	98,96	0,9726	0,9457	2,2558
Pá	100	6,8	500	46,84	54,36	60,68	65,26	69,29	75,10	80,59	84,79	88,64	0,9653	0,6197	1,8261
Pá	100	6,8	500	46,76	52,75	59,20	64,12	68,34	75,29	80,99	85,63	89,77	0,9564	0,5293	1,8461
Pá	100	6,8	500	47,80	52,87	58,98	63,80	68,12	74,82	80,39	85,01	95,81	0,9320	0,4303	1,8588
Cesto	100	6,8	500	50,94	56,19	62,68	68,13	73,15	81,74	88,56	93,85	97,57	0,9498	0,5971	1,9689
Cesto	100	6,8	500	47,15	53,85	62,34	69,57	74,46	82,26	89,41	94,32	97,65	0,9634	0,5696	2,0753
Cesto	100	6,8	500	50,06	55,67	64,07	70,20	76,29	85,26	91,36	95,43	97,67	0,9636	0,6558	2,0544
Pá	50	2	900	49,05	55,05	61,82	68,07	72,53	80,98	86,81	91,86	95,50	0,9579	0,5830	1,9545
Pá	50	2	900	48,07	54,21	61,78	67,82	72,72	80,87	87,13	91,40	94,95	0,9617	0,5885	1,9851
Pá	50	2	900	39,16	46,63	54,25	60,44	65,49	73,91	80,80	86,24	90,70	0,9560	0,3495	2,1000
Pá	50	2	500	52,03	57,90	66,18	72,40	77,51	85,34	91,55	96,52	100,26	0,9530	0,6082	1,9423
Pá	50	2	500	52,43	58,45	66,89	73,13	78,27	86,26	92,06	96,77	99,96	0,9574	0,6393	1,9381
Pá	50	2	500	51,68	57,85	66,36	72,59	77,67	85,49	91,26	95,55	98,73	0,9598	0,6509	1,9436
Cesto	50	2	900	37,11	47,93	60,81	70,60	78,04	88,25	94,14	96,76	98,11	0,9774	0,5937	2,5695
Cesto	50	2	900	52,70	58,36	68,42	76,15	81,87	90,47	94,96	97,09	98,45	0,9695	0,9267	2,1892
Cesto	50	2	900	43,08	53,79	65,95	73,92	80,47	89,85	94,76	97,13	98,42	0,9799	0,7563	2,3897
Cesto	50	6,8	900	33,26	44,91	55,29	61,33	66,53	75,53	82,36	86,63	89,61	0,9762	0,3998	2,3245
Cesto	50	6,8	900	40,37	48,10	56,90	62,76	67,77	77,13	83,84	88,47	92,09	0,9626	0,4387	2,1448
Cesto	50	6,8	900	39,52	46,00	53,01	58,75	63,60	72,22	80,06	85,43	89,48	0,9484	0,3510	2,0992
Cesto	50	2	500	44,40	54,73	65,68	73,83	80,93	90,95	96,40	99,24	102,14	0,9785	0,6421	2,3108
Cesto	50	2	500	44,38	54,68	65,66	73,83	80,93	90,95	96,40	99,24	100,64	0,9788	0,7014	2,3568
Cesto	50	2	500	44,37	56,19	65,98	74,29	81,49	92,35	98,68	102,12	100,64	0,9787	0,6149	2,2581

Pá	50	6,8	500	34,00	42,99	49,53	53,92	57,62	63,60	68,11	72,45	75,97	0,9670	0,4082	1,9501
Pá	50	6,8	500	51,59	57,43	63,65	68,63	71,68	80,03	85,16	90,58	93,50	0,9578	0,6549	1,8290
Pá	50	6,8	500	42,80	50,21	56,59	61,28	64,65	71,82	76,63	81,51	84,74	0,9626	0,5419	1,8797
Pá	100	2	500	53,09	59,18	66,95	73,17	78,06	85,65	91,00	89,91	92,56	0,9801	0,9332	1,9337
Pá	100	2	500	53,00	59,06	66,93	73,11	77,99	85,57	90,86	94,38	96,99	0,9705	0,8249	2,0042
Pá	100	2	500	51,65	56,41	63,57	69,47	73,89	81,59	87,44	91,85	95,19	0,9603	0,6803	1,9229
Pá	100	6,8	900	53,90	60,20	65,78	71,51	68,84	89,26	91,90	97,39	98,35	0,9600	0,8087	2,0497
Pá	100	6,8	900	56,35	62,14	67,97	73,41	77,67	87,64	94,04	98,25	105,06	0,9462	0,6237	1,8972
Pá	100	6,8	900	59,67	64,69	71,51	77,66	83,05	85,22	96,34	102,00	109,69	0,9486	0,7261	1,8970
Cesto	50	6,8	500	30,30	41,28	51,10	57,81	63,51	77,20	84,13	88,74	91,84	0,9673	0,1704	2,4005
Cesto	50	6,8	500	34,68	47,24	54,86	60,55	65,74	75,41	83,72	90,56	94,99	0,9559	0,2665	2,2671
Cesto	50	6,8	500	33,42	43,04	53,52	60,28	66,65	78,53	85,02	89,31	92,48	0,9699	0,3067	2,3856
Cesto	100	2	900	52,07	58,76	68,46	75,93	82,45	91,45	95,77	97,33	98,01	0,9746	0,9955	2,2933
Cesto	100	2	900	52,60	59,32	69,50	77,09	83,14	91,32	95,31	96,80	97,49	0,9796	1,0231	2,2378
Cesto	100	2	900	51,73	57,32	65,74	72,35	78,83	88,71	93,89	95,88	96,74	0,9646	0,9203	2,2609
Pá	50	6,8	900	45,12	52,16	56,93	60,07	63,08	67,94	72,59	76,49	79,47	0,9664	0,7376	1,7287
Pá	50	6,8	900	36,14	50,33	56,57	62,06	69,25	80,92	87,91	91,97	94,69	0,9622	0,4615	2,3797
Pá	50	6,8	900	49,67	53,79	59,57	64,63	68,23	74,78	79,90	84,17	87,41	0,9585	0,6861	1,8028
Cesto	100	6,8	900	55,09	62,31	72,01	77,79	82,82	85,22	96,39	107,72	106,58	0,9582	0,7773	2,1384
Cesto	100	6,8	900	53,02	62,16	70,87	76,64	80,43	93,90	100,10	104,18	108,15	0,9568	0,7393	2,2276
Cesto	100	6,8	900	54,93	59,86	70,58	77,25	83,60	91,80	97,15	102,45	104,15	0,9653	0,8550	2,2162

APPENDIX II

Rotation speed (rpm)	pH	Volume (mL)	0,25	0,5	1	1,5	2	3	4	5	6	R^2	alfa	BETA
100	2	500	51,68	57,99	67,45	75,28	81,60	91,42	96,45	98,39	99,19	0,9715	0,89	2,2466
100	2	500	51,21	58,06	68,60	76,68	82,93	91,47	95,48	97,55	98,26	0,9795	0,93	2,2332
100	2	500	52,55	58,87	68,37	76,02	82,11	91,32	96,15	98,27	98,96	0,9726	0,95	2,2558
100	6,8	500	50,94	56,19	62,68	68,13	73,15	81,74	88,56	93,85	97,57	0,9498	0,60	1,9689
100	6,8	500	47,15	53,85	62,34	69,57	74,46	82,26	89,41	94,32	97,65	0,9634	0,57	2,0753
100	6,8	500	50,06	55,67	64,07	70,20	76,29	85,26	91,36	95,43	97,67	0,9636	0,66	2,0544
50	2	900	37,11	47,93	60,81	70,60	78,04	88,25	94,14	96,76	98,11	0,9774	0,59	2,5695
50	2	900	52,70	58,36	68,42	76,15	81,87	90,47	94,96	97,09	98,45	0,9695	0,93	2,1892
50	2	900	43,08	53,79	65,95	73,92	80,47	89,85	94,76	97,13	98,42	0,9799	0,76	2,3897
50	6,8	900	33,26	44,91	55,29	61,33	66,53	75,53	82,36	86,63	89,61	0,9762	0,40	2,3245
50	6,8	900	40,37	48,10	56,90	62,76	67,77	77,13	83,84	88,47	92,09	0,9626	0,44	2,1448
50	6,8	900	39,52	46,00	53,01	58,75	63,60	72,22	80,06	85,43	89,48	0,9484	0,35	2,0992
50	2	500	44,40	54,73	65,68	73,83	80,93	90,95	96,40	99,24	102,14	0,9785	0,64	2,3108
50	2	500	44,38	54,68	65,66	73,83	80,93	90,95	96,40	99,24	100,64	0,9788	0,70	2,3568
50	2	500	44,37	56,19	65,98	74,29	81,49	92,35	98,68	102,12	100,64	0,9787	0,61	2,2581
50	6,8	500	30,30	41,28	51,10	57,81	63,51	77,20	84,13	88,74	91,84	0,9673	0,17	2,4005
50	6,8	500	34,68	47,24	54,86	60,55	65,74	75,41	83,72	90,56	94,99	0,9559	0,27	2,2671
50	6,8	500	33,42	43,04	53,52	60,28	66,65	78,53	85,02	89,31	92,48	0,9699	0,31	2,3856
100	2	900	52,07	58,76	68,46	75,93	82,45	91,45	95,77	97,33	98,01	0,9713	1,00	2,3265
100	2	900	52,60	59,32	69,50	77,09	83,14	91,32	95,31	96,80	97,49	0,9762	1,04	2,2978
100	2	900	51,73	57,32	65,74	72,35	78,83	88,71	93,89	95,88	96,74	0,9606	0,94	2,2896
100	6,8	900	55,09	62,31	72,01	77,79	82,82	85,22	96,39	107,72	106,58	0,9582	0,78	2,1384
100	6,8	900	53,02	62,16	70,87	76,64	80,43	93,90	100,10	104,18	108,15	0,9568	0,74	2,2276
100	6,8	900	54,93	59,86	70,58	77,25	83,60	91,80	97,15	102,45	104,15	0,9653	0,86	2,2162
75	4.4	700	49.6	55.0	61.5	67.4	72.3	80.0	86.2	90.4	93.3	0.957	0.803	2.051
75	4.4	700	47.9	53.1	60.8	67.3	72.4	81.2	88.1	92.0	93.6	0.954	0.785	2.212