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DOCTORAL SCHOOL OF PHARMACEUTICAL AND PHARMACOLOGICAL SCIENCES

IN VITRO MODELLING OF FOOD EFFECT INFLUENCING DISSOLUTION OF ACID-LABILE DRUGS

Ph.D. Thesis

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1. Introduction and Objectives

Studying food effect –or the lack of it– on drug release/absorption, and consequently drawing conclusions regarding the presence or absence of drug-food interaction, is mandatory when it comes to modified-release (MR) formulations [1, 2]. Some chemical entities in immediate release (IR) forms can also be subject to such investigation (e.g. buspirone hydrochloride). Chemical entities of special physico-chemical properties constitute a special class of drugs where investigating drug-food interaction might be inevitable, especially when an innovative drug substance is addressed. Among these, acid-labile drugs have been identified and continue to present as a special subclass of molecules that could necessitate particular pharmaceutical formulation for administration.

In vitro dissolution profiles provide invaluable information regarding rate of drug release in a given dissolution medium, such information could help predict the biological availability of the drug providing *in vitro* dissolution can be brought in association with events of the biological system. Generally applied *in vitro* dissolution media –though conform to the latest guidelines– lack consistency when it comes to similarity to naturally occurring fluids where genuine dissolution occurs. Collecting biological samples of gastric and intestinal fluids can only be accepted for experimental and/or investigational purposes [3]. Therefore, simulating biological environments offers a convenient alternative that could facilitate routine and experimental *in vitro* dissolution work.

The aim of our work was to:

- investigate the acid buffering capacity of the different dietary components, especially those components included in the so-called “Standard Test Meal”;
- examine the applicability of these dietary components to laboratory-prepared artificial gastric juice;
- analyse the suitability of end-result dissolution medium as one used to simulate fed-state for subsequent *in vitro* studying of drug-food interaction;
- apply newly developed model with the intention to investigate *in vitro* dissolution profiles of an acid-labile drug under both simulated fasted and fed conditions;
- explore the possibility of establishing an *in vitro-in vivo* correlation (IVIVC) between pharmacokinetic parameters calculated based on *in vitro* profiles data and those obtained from bioavailability studies in healthy volunteers.

2. Theory and Background

2.1. General considerations

In vitro dissolution testing has been applied in many fields within the pharmaceutical industry. These include optimisation of medicinal compounds during development [4 - 6]; quality control assurance [7 - 9]; employment as an adjutant in determination of bioequivalence [10 – 12] and, with increasing importance, application in regulatory approvals as well [13]. It is considered to be one of the most important quality control tests performed on pharmaceutical dosage forms [14]. In principle, it is aimed to assess the rate by which a chemical entity is released from a preparation of a certain pharmaceutical form; a step most investigators consider as rate-limiting during drug absorption in any biological system. In addition, *in vitro* dissolution has been used to compare two or more commercial products of the same active ingredient regarding release specifications. This is beneficial when comparing generic preparations either to each other or to the innovator [15, 16]. Some investigators have also considered *in vitro* testing of a drug to be a prognostic tool that could assist predict its *in vivo* performance [17 - 19]. Others have applied *in vitro* dissolution testing to investigate the effects of special pharmaceutical formulations on drug release and its role in assuring clinical requirements [20 – 22]. Strenuous efforts have been exerted to establish correlation –of reasonable extent– between *in vitro* release of a chemical compound and its *in vivo* behaviour. This was approached based on both *in vitro* dissolution profiles and pharmacokinetic data. The latter is collected either from animal studies [23 – 25], or healthy volunteers [16, 26 - 29]. The pursuit to substantiate an *in vitro-in vivo* correlation (IVIVC) has involved exhausting investigation of different *in vitro* models

applying various dissolution media [18, 19, 30 – 32]. Despite extensive studying, a well-developed method that predicts dissolution-absorption relationships has not been agreed upon yet [32 – 34]. Designing and performing *in vitro* dissolution testing with the intention to correlate to *in vivo* drug behaviour is further complicated due to the fact that it is practically unattainable to set one *in vitro* dissolution model that applies to all screened drug substances.

2.2. Factors influencing drug absorption

Before considering crucial aspects of *in vitro* modelling, it is advisable to have a closer look at the steps a drug undergoes before it appears in the systemic circulation. After oral administration of a solid form, first drug is released from its pharmaceutical form by disintegration. This step depends largely on the site of the gastro-intestinal tract (GIT) where it occurs, and on the physicochemical environment surrounding the drug. Next, and with the aid of naturally found juices in the GIT, a solution-containing drug is formed. It is either at absorptive site already, or reaching it through flowing in the digestive tract. Either way, a fraction of the drug undergoes decomposition. By coming into contact with the gut wall, another fraction of the drug is metabolised there while the rest is delivered to the systemic circulation. With the great majority of orally administered drugs being subject to liver metabolism, yet an additional drug fraction loss is accounted for (Figure 1.-adapted from Antal, 2001 [35]). This simplified description does not take into account the different pharmaceutical forms a drug substance is contained within, nor does it relate to the effects of any ingredients of the formula itself (e.g. coating) or the excipients neighbouring the drug. Nevertheless, and

based on the above mentioned description of events, it is accepted to relate inadequate bioavailability of a drug to one of the following factors [19]:

- the active ingredient is not released from the pharmaceutical form in time to constitute a solution that is easily absorbed;
- the amount of drug released through dissolution undergoes degradation, could become adsorbent or form an insoluble complex;
- the active ingredient fails to cross membranous lining of the GIT;
- intense metabolism or rapid elimination occurs right after the active ingredient is absorbed.

Even with the best designed *in vitro* dissolution testing models, some of the above mentioned factors can hardly be examined under experimental conditions and, consequently, are unlikely to be confirmed. Extensive studying has been done earlier to develop a dissolution model that focuses on analysing more than one physiological factor at a time [36, 37]. Despite the fact that it facilitated establishing correlation to some *in vivo* observations [38 – 40], it did not gain much popularity and is not applied world-wide nowadays.

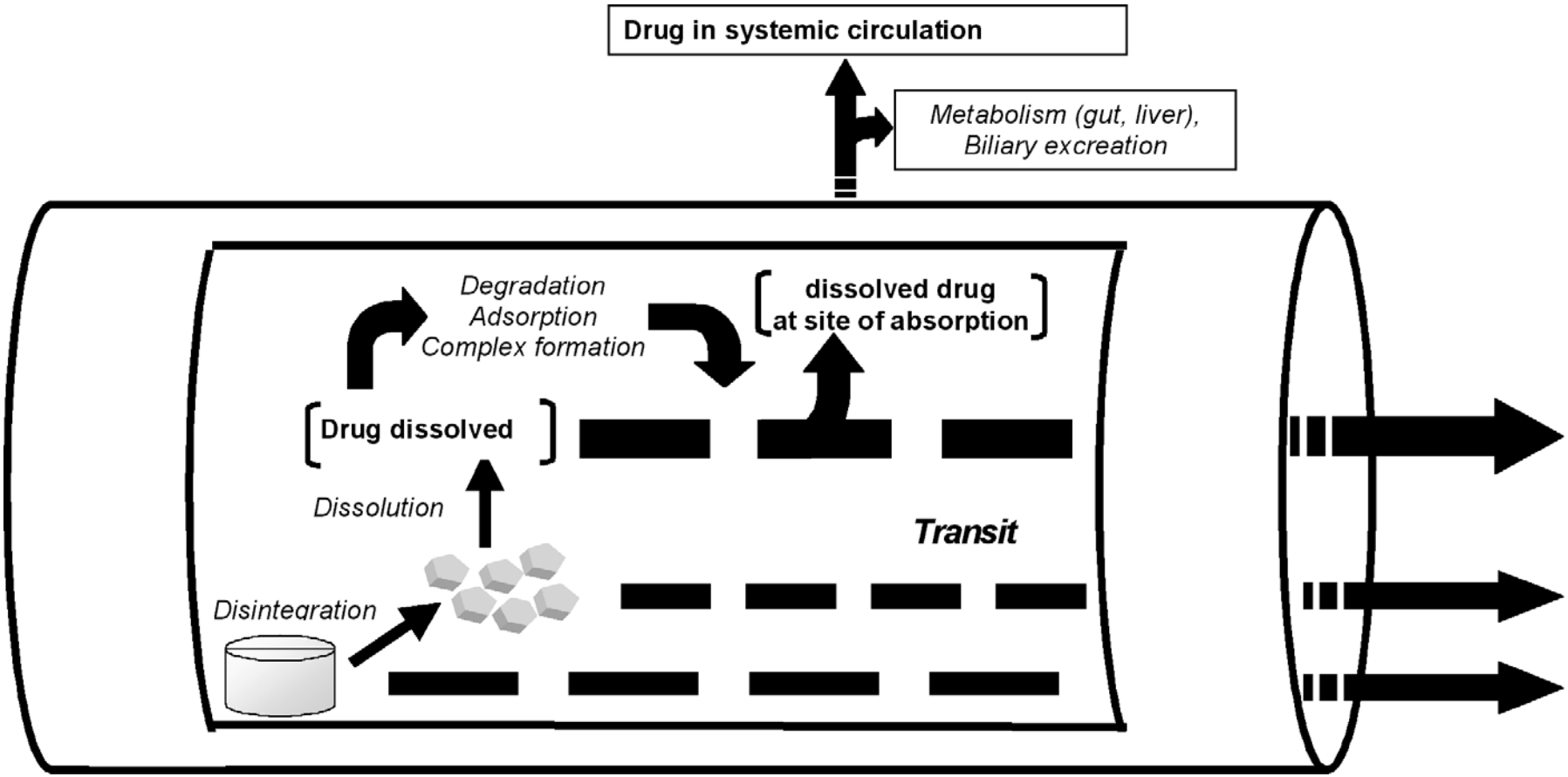

Figure 1. Factors influencing drug absorption all through the gastro-intestinal tract (GIT)

2.3. The basis of drug classification

Setting the theoretical basis for the Biopharmaceutics Classification System (BCS) of drugs [41] has elicited a series of experiments and arguments, each aiming at verifying the concepts and analysing the applicability of IVIVC. Amidon and co-workers (1995) have applied Fick`s First Law as a fundamental starting point for their analysis, that is;

$$J_w = P_w\, C_w \qquad \text{(Eq. 1)}$$

where J_w is the drug flux through the intestinal wall at any position and point of time; P_w is the permeability of this membrane and C_w is drug concentration at the membrane surface. In principle, this equation is perceived as a law pertaining to each point along the intestinal membrane. Drug absorption rate, or better to say, the rate of drug loss from the intestinal lumen at any time is;

$$\text{Absorption rate} = \mathrm{dm/\,dt} = \int\int_A P_w C_w dA \qquad \text{(Eq. 2)}$$

where the double integral is over the entire GIT surface, and assuming no luminal reactions take place. The total mass, M, of drug absorbed at time t is:

$$M(t) = {}^{t}\!\int_0 \int\int_A P_w C_w dA\, dt \qquad \text{(Eq. 3)}$$

Based on equations 1 and 2 above, it is reasonable to adopt the following principle for bioavailability:

If two products, containing the same drug, have the same concentration time profile at the intestinal membrane surface then they will have the same rate and extent of absorption.

Further interpretation of this statement could imply that; if two drug products have the same *in vivo* dissolution profile **under all luminal conditions**, they will have the same rate and extent of drug absorption.

The above discussion assumes all steps taking place in the GIT are not influenced by the fact that every drug formulation includes a number of excipients that might as well affect both dissolution and permeability. In addition, there is the variability of the GIT transit and lumen content at time of dosing as well as inter- and intra-individual GIT variations. The absence of a single model (mathematical, statistical or instrumental) that allows analysis of this series of complex events leads one to accept the above-mentioned approach. Consequently, variation in the rate and extent of absorption are to be expected.

The BCS helps group chemical substances into four major classes (Table I.), based on their solubility and permeability characteristics [19, 41].

Class I drugs: are highly soluble-highly permeable drugs. In this case, drug dissolution is considered to be the rate-limiting step for drug absorption (unless dissolution is very rapid then gastric emptying is the key factor). Drug substances of this class that are contained in immediate release (IR) forms which dissolve rapidly will be completely controlled by gastric emptying, and would show no correlation between *in vitro* dissolution and *in vivo* absorption rates. Nevertheless, Class I drugs in IR forms may insure bioequivalence if ~ 85 % of the active ingredient is dissolved in 15 minutes [42].

Class II drugs: are practically speaking water insoluble but of high permeability. These drugs serve as the best candidates for IVIVC studies. That of course does not exclude any other substance [43]. The hydrophobic character of Class II drugs makes their solubility too low to be consistent with complete absorption, and accordingly, their dissolution rate is the primary limiting step to absorption (except when high doses are administered). With this in mind, the circumstances under which the dissolution profile is performed should be broadened to include dissolution media of different pH ranges with special attention paid to physiological ones.

Class III drugs: represent the mirror image of class II drugs. Consequently, permeability –and not dissolution– is the rate-limiting step for drug absorption. The same facts applied for class I regarding gastric emptying and IR drug forms are also relevant here. The quest to establish an IVIVC for this class of drugs is rather limited and frequently unaccomplished [44]. Nevertheless, successful attempts with low permeability drugs (e.g. ranitidine) have been reported [45].

Class IV drugs*:* are practically insoluble and of low permeability. This class of drugs present with significant problems for oral delivery, let alone IVIVC studying.

The above classification has helped regulatory authorities (namely the FDA) set the standards for drugs and drug products that could be considered as candidates for *biowaivers*. The **permeability** requirement states that drug permeability is commensurate with ≥ 90 % of an administered dose, based on a mass balance determination or in comparison to intravenous reference dose. The **solubility** requirement is that the dose-to-solubility ratio (D:S) of the drug must be ≤ 250 ml over a

pH range of 1-7.5, and the **dissolution** requirement for the drug product is that dissolution must be > 85 % complete within 30 min [46].

Chemical substances might also present with special physico-chemical characteristics that could constitute a challenge to the BCS. Among those lie drugs with pH-dependent solubility. *In vitro* dissolution testing of these drugs will necessitate tailoring of dissolution media so as to resemble *in vivo* dissolution before any IVIVC is attempted.

Table I. The Biopharmaceutics Classification System (after Amidon et al, 1995 / [41] and Dressman and Reppas, 2000 / [43]). Examples of orally administered drugs are adapted from Dressman et al, 2001 / [47]

	Class I	**Class II**	**Class III**	**Class IV**
Solubility	High	Low	High	Low
Permeability	High	High	Low	Low
Examples	paracetamol metoprolol theophylline	carbamazepine griseofulvin ketoconazole danazol	aciclovir atenolol cimetidine ranitidine	chlorothizide furosemide

2.4. Physiological events to be considered before setting *in vitro* models

When designing *in vitro* models to be involved in future IVIVC, it might be reasonable to consider those most likely simulating biological systems' environments - namely the GIT [48]. These, however, vary according to the site where drug dissolution

starts (i.e. stomach vs. upper small intestine); and show considerable physico-chemical diversity even within the same site (e.g. the stomach: fed vs. fasting conditions). Parameters to be considered when preparing dissolution media include volume, pH, osmolarity and biorelevant ingredients and/or surfactants to be added just to mention a few [43]. *In vitro* dissolution studies conducted as part of biowaiver applications include dissolution media showing certain similarity to GIT juices [46]. When it comes to associating *in vitro*-observed profiles and *in vivo*-calculated pharmacokinetic data, dissolution media better mimic biologic environments before ruling IVIVC in (or out). Investigations dealing with a chemical substance in a modified/extended release pharmaceutical form should include a food effect study [1, 49]. The same rule applies to innovative drug substances, regardless to its pharmaceutical form [2]. Moreover, when drug-food interaction is shown and is believed to be related to the chemical substance itself and not to the formulation, then detailed food-drug interaction for such drugs might not be necessary providing they are contained in IR formulae [49]. Nevertheless, comparative fasting and fed studies have been conducted with such drugs in spite of their IR pharmaceutical form (e.g. buspirone) [50].

The physiology of the GIT is greatly influenced by the administration of food [51]. Under fasting conditions, the pH of the stomach is estimated at 1-2 with only very small percentage of the population showing values as high as 3 [52, 53]. Food consumption acts first on elevating **stomach pH**, despite continuous acid secretion which is stimulated by food itself. This comes as a combined effect of both increasing the volume of stomach content as well as the acid buffering capacity of food particles. Stomach pH at 6.7 has been recorded minutes within ingestion of high-fat meal and needed more than 100 minutes to return to fasting pH [53]. Inter-individual variations

are, however, evident. In addition, the composition of ingested food and the size of the meal itself are also important. Another stomach-related factor is **gastric motility.** This is reinforced by food intake. With these steps happening in the stomach, other changes take place elsewhere in the GIT. **Bile secretion** is also stimulated by ingestion of food. This is of particular importance in case of lipophilic drugs ingested with high-fat meals. Such drugs are more likely to show modified bioavailability based on improved drug absorption [54]. Food-induced changes also include increased **blood flow** to the GIT and liver. In part, intensified circulation in the GIT could increase absorption and bioavailability of drugs. Yet, liver involvement might have opposite effects as it might enhance first-pass metabolism, and consequent reduction in drug bioavailability. Finally, **gastric emptying** is delayed after the consumption of food. Drugs absorbed in a later segment of the GIT (either due to physico-chemical features or to formulation type) might suffer decreased bioavailability when administered with food. The possibility of food-drug complex formation should not be ignored, either.

Noteworthy is the possibility of interaction between drugs and beverages as well. The use of grapefruit juice to mask the taste of ethanol in a drug-alcohol interaction study of felodipine has revealed a marked increase of felodipine`s bioavailability [55]. Similar findings were also reported with another calcium channel antagonist, nifedipine [56]. A direct inhibition of P450 –the cytochrome responsible for the metabolism of both drugs– is thought to be involved. The same findings have also been described for buspirone [57].

It is true though that most reliable information regarding possible food-drug interaction is usually obtained from studies performed in healthy volunteers [58 - 61]. Nevertheless, *in vitro* models simulating both the fed and the fasting states in the GIT

help explore factors related to food effect(s) on drug dissolution; and consequently, assist better insight into postprandial drug behaviour.

2.5. Modelling *in vivo* dissolution

2.5.1. The fasting state

While most investigators –from both control laboratories and industrial sections– agree that fluids used as dissolution media should be kept as simple in composition as possible, improvement of these fluids frequently require expenditure of basically applied ones [62]. Essentially applied dissolution media should be aqueous systems in nature, except when the drug substance itself is of extremely low solubility. Medium pH and ionic strength are also important. Early guidelines such as the F. I. P. joint report have encouraged the use of standard fluids, which cover the extreme ends found in the GIT. These include using an acidic fluid of pH between 1 and 2, and a neutral fluid of pH 6.8. A lot of studying has also been dedicated to apparatus methodology, but no standardisation has been finalised here. In general, USP dissolution apparatuses –or those based on one of the original apparatuses [63, 64]– are widely applied and accepted. Care, however, should be taken when adjusting the so-called "dynamic parameters" of the apparatus, namely the stirring speed, flow rate and volume for individual adaptation. The use of other devices have also been described [25, 67 - 71].

More physiological fluids that simulate the fasting state in the GIT have largely replaced the use of distilled water as a dissolution medium. The drive for complete simulation has lead to extensive analysis of the gastric juice of healthy human beings [3]. Simulated gastric fluid with added surfactant is now being applied to simulate the fasting stomach [19, 43]. For simulating the fasting state in the proximal intestine,

FaSSIF has been used. This medium contains phosphate buffer –instead of normally used bicarbonate– to achieve pH of 6.5 and a buffering capacity of 10 mEq per litre per pH unit [43]. It also contains bile salts and lecithin in amounts typical of those found in the intestinal. Application of these media during *in vitro* dissolution of poor water-soluble drugs have yielded acceptable IVIVC and provided a promising tool for forecasting *in vivo* performance of these drugs [30]. Other approaches have also been described. The studied tablet was embedded in a laminar flow device and aqueous solutions of an anionic surfactant, sodium dodecyl sulphate, were pumped over it to simulate flow in the human upper GIT [72].

2.5.2. The fed state

The complexities of events taking place within the GIT after food consumption have been described above in 2.4. For any positive outcome anticipated from an IVIVC attempt, most –if not all of– these events have to be taken into account when designing the *in vitro* dissolution model to be used. To start with, stomach pH rises after ingestion of a meal. This fact has to be taken into consideration when simulating the stomach in the postprandial state. *In vitro* dissolution medium applied to simulate this state is bound to show considerable pH elevation as compared to the medium used to simulate the fasting state. Another parameter that is readily affected by ingestion of food is volume. Physiologically speaking, the volume of stomach content –which is passed later to the small intestine– expands continuously; depending on the volume of co-administered fluids, stomach secretions and water flux across the gut wall [19]. While reproducibility of this is practically difficult during simulation, scientists agree that a volume of 300-500 ml could be used for the fasted stomach; to be increased to 900 ml

in the fed state. Fasted small intestine volume can be simulated with 500 ml, increased to 900-1000 ml in the fed state [43]. On the other hand, bio-relevant dissolution media (BDM) containing different amounts of lecithin and sodium taurocholate have been applied to simulate a fed state and have resulted in enhanced drug dissolution in certain cases [31]. Furthermore, adding bile salts to dissolution media designed to evaluate *in vitro* dissolution of certain Class II drugs have helped enhance solubility of an otherwise poorly soluble drug [73]. Long life milk of 3.5% fat content has also been applied to simulate fed state in the stomach [43]. They have also described FeSSIF medium that simulates fed state conditions in the small intestine. The main physico-chemical aspects of such simulation should include medium pH and osmolarity. Again, *in vitro* dissolution of some lipophylic drugs in these media has provided a considerable burden of evidence regarding possible enhancement of drug solubility.

2.6. *In vitro* modelling of food effect on drug dissolution/release

With all attempts devoted for setting the most accurate simulations, refinement of *in vitro* dissolution models continues to occupy much of investigators` attention. Currently applicable guidelines tend to describe in details all aspects of the test meal to be considered when designing and performing food-effect studies in humans [49]. Based on this, it is easy to conceive that the same *in vivo* parameters would necessitate considerable adjustment before applying to *in vitro* dissolution. With guidelines mainly focused on the role high fat / high calories diet plays in food-drug interaction studies, simulations had to follow the same trend as well. Early observations of food effect on the bioavailablity of theophylline after the administration of high fat breakfast to healthy volunteers have encouraged unique *in vitro* modelling [74]. To simulate high fat

breakfast, the preparations were pretreated with peanut oil for 2 hours before performing *in vitro* dissolution and release studies. An IVIVC was successfully established. The reproducibility of the method, after being subjected to further development, has also been reported [75]. Evaluation of food effect on a highly lipophilic drug has also included *in vitro* dissolution testing after the administration of mixed bile salt micellar system –4:1 sodium taurocholate:licithin– [76]. While IVIVC was not a goal here, *in vitro* studies did help provide an explanation for postprandial increase of drug bioavailability. On the other hand, some investigators ruled out the possibility of predicting food effect or bile influence on the absorption of sustained-release theophylline, based on *in vitro* dissolution of the drug in synthetic surfactants-containing test medium [77].

2.7. Acid labile drugs

2.7.1. General considerations

In vitro dissolution testing has also been applied to determine the stability of the active ingredient of a given preparation. Testing of the cardiac glycoside digoxin at different pH ranges provided reasonable explanations for the variation in its bioavailability, which had been witnessed upon oral administration of the drug to healthy volunteers [78 - 80]. Investigators observed a decrease in digoxin half-life from 131 min at pH 2 to 13.5 min at pH 1. It had been concluded that variations in the gastric pH could influence the extent to which hydrolysis occurred and effectively competed with absorption of the active drug. In order to avoid degradation in the stomach acidic pH, chemical moieties of acid-labile nature are manufactured in special pharmaceutical formulation, e.g. omeprazole [81], lansoprazole [82].

2.7.2. Deramciclane fumarate

Deramciclane fumarate (EGIS-3886; (1R, 2S, 4R)-(–)-N,N-dimethyl-2-{(1, 7, 7-trimethyl-2-phenylbicyclo-[2, 2, 1]-hept-2-yl)oxy}-ethamine-2-(E)-butendioate (1:1)); Fig. 2.), is a new putative non-benzodiazepine-like anxiolytic agent synthesized by EGIS Pharmaceuticals Ltd. (Budapest, Hungary). It shows high affinity for 5-HT_2 receptors *in vitro*, where it exerts antagonistic effects [83 - 85]. *In vitro* receptor binding studies indicate that deramciclane has no affinity for the $GABA_A$-benzodiazepine receptor complex, α_1-, α_2- receptors, β-receptors and histamine H_1 receptors [84]. Using positron emission tomography (PET) and [^{11}C]-*N*-methyl spiperone ([^{11}C]-NMSP) in an open exploratory study in healthy volunteers, interaction of deramciclane with cortical serotonin 5HT receptors was verified [86]. The drug caused a dose- and concentration-dependent displacement of [^{11}C]-NMSP binding in the frontal cortex. Unlike benzodiazepines, deramciclane does not induce muscle relaxation or sedation in animal models [87, 88]. Studies with side chain-radiolabelled deramciclane in rats proved cleavage to occur at the ether bond, with resulting dimethylglycine and dimethylamino-ethanol being incorporated into the synthesis of endogenous substances (e.g. proteins, choline/acetylcholine) or tightly bound to proteins [89 - 91]. These findings are of major investigational importance. It can be stated, however, that in humans, the pharmacological effect / anxiolytic activity of deramciclane requires the integrity of the molecule [91].

Extensive pharmacokinetic studies of deramciclane have been conducted in several animal species after oral [89, 90, 92, 93], subcutaneous [94], intravenous and intraperitoneal administration of the drug [95]. Deramciclane is readily and completely absorbed from the GIT in animal models studied [89, 96]. These observations were also

confirmed by clinical studies in healthy volunteers, which showed the compound was well tolerated and safe after the administration of both single [97] and multiple oral doses [98]. *N*-desmethyl deramciclane is the pharmacologically active metabolite that has been separated in all studied species so far [99 - 101]. As for the extent of deramciclane metabolism, substantial inter-species variation has been observed [92]. Several minor and major metabolites were separated from plasma and urine [102]. Based on studies conducted in animals, deramciclane has non-linear pharmacokinetics caused by a decrease in total body clearance at doses higher than 1 mg/kg [96]. In man, on the other hand, only slight -if any- non-linearity was reported over the single oral dose range of 3-150 mg [97].

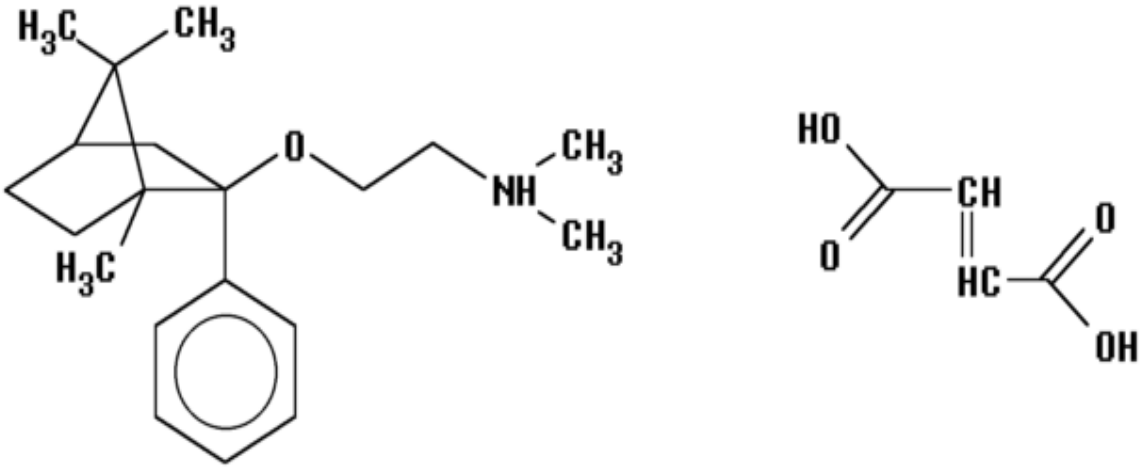

Figure 2. The structure of deramciclane fumarate

Regarding its physico-chemical properties, deramciclane is very slightly soluble in water (0.0088 g/100 ml, at 25°C). The aqueous pK_a value of deramciclane fumarate is 9.61; which means that practically all the compound is ionised in the stomach,

duodenum and in the plasma. Octanol / water partition coefficient (LogP) values for deramciclane and deramciclane salt are 5.9 and 1.41, respectively [103]. *In vitro* studies have revealed its acid liability nature to be most prominent over a pH range of 1.2-2.1 [104]. The use of different formulations of deramciclane-containing preparations in man (i.e. conventional capsules and enteric coated tablets) has yielded different drug pharmacokinetic profiles, allowing the speculation of a possible pH-effect involvement. The low pH of a fasting stomach in man might have caused degradation of deramciclane, with total amount of drug available for absorption from the intestinal tract being diminished [104]. Animal models, however, might not be suitable for evaluating the clinical relevance of this phenomenon, as they do not share the same gastrointestinal physiology with man [105, 106]. As far as acid-labile drugs are concerned, the use of animal models could after all constitute a limitation [104].

2.8. IVIVC or IVIVR?

The effort to connect *in vitro* dissolution and *in vivo* pharmacokinetics of a drug is often referred to as IVIVC. At this stage of analysis, it is obvious that extended release (ER) formulations have provided better grounds for successful IVIVC rather than IR ones. There are three often-used approaches to perform IVIVC [26, 107]:

- **Level A** where a one-to-one relationship can be found between absorption and dissolution.
- **Level B** correlation is based on statistical moments determined *in vitro* and after *in vivo* administration. Parameters used are:
  - *in vitro*: MDT (mean dissolution time)

- *in vivo*: MRT (mean residence time), MAT (mean absorption time), MDT (mean dissolution time)

- **Level C** point-to-point relationship is found between a dissolution parameter and a pharmacokinetic one. Parameters usually used are:
  - *in vitro*: % dissolution, T50 % (time of 50 % dissolution)
  - *in vivo*: C_{max}, T_{max}, AUC

Among the above mentioned three levels, Level A had been the one preferred method for IVIVC as it plots the fraction drug absorbed (Fa) against the fraction dissolved (Fd). The former is obtained by deconvolution (i.e. back calculation) of drug plasma profile. ER dosage forms are more likely to yield a linear (or almost linear) relationship between Fa and Fd. This type of analysis is where the term IVIVC evolved. For IR dosage forms, however, dissolution is more rapid than overall absorption. Therefore, a non-linear profile results [108, 109]. Nevertheless, it is still possible to establish a correlation. If this is conceived as the degree of relationship between two variables, then there should be a room for non-linear relations as well. Therefore, the term IVIVR might after all substitute the old one. The aim of any IVIVR attempt would be to learn about the relative contribution of dissolution to a product's overall absorption kinetics. Polli and co-workers [110] have set the basic mathematical model for that. It is as follows:

$$F_a = 1/f_a \, (1- \alpha/\alpha\text{-}1(1- F_d) + 1/\alpha\text{-}1(1\text{-}F_d)^{\alpha}) \qquad \text{Eq. 4}$$

where Fa is the fraction of the total amount of drug absorbed at time t; fa is the fraction of the dose absorbed at t = #; α is the ratio of the apparent first-order permeation rate constant to the first-order dissolution rate constant (kd) and Fd is the fraction of drug dose dissolved at time t.

Level A correlation can be analysed as a special (linear) case of equation 4. When complete absorption prevails, fa would equal 1.0; and in case of strongly dissolution rate-limited absorption, Fa would equal Fd.

The term "Relationship" between *in vivo* drug absorption and *in vitro* dissolution rates appeared in the literature as early as 1965 [111]. Levy et al tried verbalising the correlation of times as a method to reveal the relevance of *in vitro* dissolution properties for the *in vivo* concentration time course. They assumed that if the transformation of the time base is performed, then the correlation of amounts related to identical times observed in the *in vitro* and *in vivo* systems would give straight line with slope equal to unity and intercept equal to zero. That of course if and only if the *in vitro* release profile is equivalent to the *in vivo* one. The decades to follow have showed intensive efforts focusing on re-defining the term relationship, to be substituted with "Correlation". Evolutionary processing of mathematical tools applied for correlation had been the key technique in doing that. The "Linear system analysis" technique–which is based on the concept that the body reacts to any input as a linear system– was first introduced, only to be further developed using numerical algorithms to provide a handy tool for correlation [112]. It was then concluded that only linear and time-invariant behavior of the body system was pre-requisite and that employment of compartment modeling should be justified. It was then also suggested that this technique would be added to the

traditional “AUC” assessment and compartment modelling. Later on, The so-called “method of moments” was introduced. It put much emphasis on the correct transformation of the major part of the dissolution profile. Furthermore, it was confirmed that correlation of release times and the method of moments can be applicable even if the dissolution profile cannot be described by an algebraic equation [39].

IVIVC has been visualised as “the establishment of a relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical property of the dosage form” [113]. After countless discussions in this topic, it had been agreed upon that the overall pharmacokinetic profile of a drug product is probably too complex to be accurately described by *in vitro* data. Furthermore, dissolution specifications can not be globally described for all drugs. Instead, individual settings have to be considered for each compound addressed at-a-time. Nevertheless, dissolution specifications remain a core issue in the process of establishing, proving, or merely exploring the possibility of any *in vitro-in vivo* relationship (IVIVR). For the time being, this continues to be an extremely useful tool for comparing different formulations of a given compound and to be the surrogate of the *in vivo* release process of the product [114]. It is not surprising then that terms like “similarity” and “association” have already appeared in the literature and could as well soon be replacing the old or the even not so-old ones.

3. Experimental

3.1. Materials and Methods

3.1.1. Chemicals, standards, reagents and dietary components

Film-coated tablets each containing 30 mg deramciclane base as well as bencyclane fumarate Halidor® (internal standard for the analytical work) were synthesised by EGIS Pharmaceuticals Ltd. (Budapest, Hungary).

Methanol, ethylacetate and n-hexane were obtained from E. Merck (Darmstadt, Germany) and were of chromatographic purity. Ammonium hydroxide (approx. 25 (m/m) %) was of analytical purity (Fluka). Double distilled water was further purified using a Milli-Q equipment (Millipore, Milford, USA).

The extraction solvent was prepared by mixing 100 ml ethylacetate and 900 ml hexane (1:9 (v/v) ethylacetate-hexane). Dilute (0,25 M) ammonium hydroxide solution was prepared by diluting 19 ml of 25 (m/m)% ammonium hydroxide solution to 1000 ml using double distilled water.

Stock solutions of deramciclane and internal standard (Halidor®) were prepared by dissolving 10 mg deramciclane and the internal standard in 10 ml methanol-water (9:1, v/v), respectively. Further dilution was made in water at 2000 (I), 200 (II) and 20 (III) μg/ml. Internal standard was diluted in water at 100 μg/ml. Standard solutions were stored in a refrigerator (5°C) for up to 1 month.

Sucrose (USP 23), whole milk powder(s) (whole and skimmed) were purchased from commercial source (Hajdu Ltd., Debrecen, Hungary). For the preparation of oil emulsion 50 ml of sunflower oil (Ph. Hg. VII) was emulsified in 450 ml of distilled water containing 1% methylcellulose (Ph. Hg. VII) as stabilizer (giving a result of 10%

oil emulsion used to simulate dietary fat). Gelatine (USP 23), sucrose (USP 23), lactose monohydrate (USP 23), fructose (USP 23), glucose (USP 23), starch (Ph. Hg. VII). Unless stated otherwise, all chemicals and dietary components were purchased from commercial sources.

3.2. Instrumental

For both acid buffering capacity of dietary components and *in vitro* dissolution testing of deramciclane film-coated tablets, the USP dissolution apparatus II (Pharmatest PTWSII, Pharma Test GmbH, Hainburg, Germany) was used. During the analytical work, an HP 5890 gas chromatograph series II (Hewlett-Packard, Palo Alto, USA) equipped with an HP 7673 autosampler and nitrogen-phosphorous selective detector (NPD) was used. Data acquisition, instrument control and evaluation of results were performed by a Hewlett-Packard Chemstation software (version A 03.02.) running on HP Vectra VE computer.

3.3. *In vitro* acid buffering capacity test

Each solid food component was dispersed into 500 ml of distilled water while stirring for 5 minutes at 100 rpm, temperature was maintained at 37.0 ± 0.5°C. Afterwards, the system was titrated with 1 N HCl while the pH of the medium was continuously recorded using a computerised pH-meter (OP-550S, Radelkis, Budapest, Hungary). Hydrochloric acid was added in two ml portions allowing enough time to achieve equilibrium represented by a pH value remaining stable for 10 seconds. Six replications were made for each component studied. Readings were interpreted using

Table Curve 3D SPSS, version 2 for Win 3.2 (Chicago – USA). The use of the USP method had been similarly described elsewhere (Plachy et al, 1995).

3.4. Clinical study

The clinical study was conducted in accordance with the internationally accepted standards and the local regulatory requirements including the principles enunciated in the Declaration of Helsinki (as lately revised in Somerset West, 1996). Detailed study procedure is well documented and archived at EGIS Pharmaceuticals Ltd. [116]. The Clinical Study Protocol and the Written Informed Consent forms were reviewed and approved by relevant health authorities, and also by The Central Ethics Committee of the National Scientific Medical Council of Hungary. All procedures were carried out in accordance with the rules of the Good Clinical Practice (GCP) directives and the CPMP guideline "Investigation of Bioavailability and Bioequivalence". An open-label, randomised, two-way crossover, single-dose, food-interaction study (2-week washout period) was conducted in 18 healthy male volunteers. In one phase of the study, a single dose of one film-coated tablet containing 30 mg deramcicalne base was administered to volunteers after overnight fasting. In the other, the same treatment was administered after volunteers consumed a high fat-containing standard breakfast (based on specifications of Test Meal) [49]. Blood sampling took place before drug intake and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72 and 96 hours after it. Determination of plasma drug concentrations and detailed bioanalysis took place as described before [117].

Among all criteria of the clinical study, the dietary and fluid intake is by far the most important from IVIVC point of view. On the day of treatment, the investigational

product was given either in a fasting state or 10 minutes after the ingestion of a standard high-fat breakfast. This meal consisted of 2 eggs (80 g) and one slice of bacon (50 g), served with 2 slices of white bread (100 g) and with 2.5 dl milk (3.3 % fat content), contained 38 g protein, 41.85 g fat, 65.5 g carbohydrates with energy content of 3307 kJ (787 kcal).

3.5. *In vitro* dissolution testing

3.5.1. Preparatory procedures

Deramciclane-containing film-coated tablets used in the clinical study were collected from a batch manufactured earlier than those used for the *in vitro* dissolution testing. Hence, it was necessary to compare dissolution profiles of tablets from both batches after testing in phosphate buffer solution (38 g Na_3PO_4 x 12 H_2O, 12.6 ml 37 % HCl and distilled water to yield 2 litres, pH 6.8). Quality Assurance Division at EGIS Pharmaceuticals Ltd. kindly provided dissolution data and profile of tablets applied in the clinical study. *In vitro* dissolution of the tablets from the recent batch (that was also used for simulation studies) took place using the same instruments, for sake of accurate comparison.

3.5.2. *In vitro* simulation of *in vivo* conditions

The amounts of dietary components examined here were determined based on the so-called “Standard Test meal” given to subjects participating in the *in vivo* studies [49]. This meal should contain 38 g of protein, 41.85 g of fat, 65.5 g of carbohydrates with energy content of 3307 kJ = 787 kcal and 2.5 dl of milk (of 3.3 % fat content).

Accordingly, we used 30 g of whole-fat milk powder, 65.6 g sugar and 10% oil emulsion was used to simulate dietary fat.

The standard USP paddle method (USP 24) was used to perform dissolution tests of deramciclane-containing film-coated tablets at 100 rpm in artificial gastric juice (content detailed below) and in phosphate buffer solution separately.

To simulate *in vivo* conditions, the dissolution apparatus contained 500 ml of test media maintained at 37°C. Dietary components (summarised in Table II.) were added to test dissolution media and examined one-at-a-time as well as combined. Aliquots of dissolution media (5 ml) were collected 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40 and 45 minutes after initiation of test, diluted with equal volumes of 0.25 M ammonium hydroxide and analysed by gas chromatography – nitrogen phosphorous selective detection (GC-NPD).

Table II. Summary of the content of artificial gastric juice (500 ml) and the dietary components added during *in vitro* dissolution testing for simulated fasted and fed states*.

Dietary components	**Simulated fasting state Artificial gastric juice, pH 1.2**	**Simulated fed state Dietary components added to artificial gastric juice****
1 N HCl	94 ml	
NaCl	0.35 g	
Glycine	0.5 g	
H_2O	Add 1 liter, adjust pH	
Whole-milk powder		30 g
1% methylcellulose		450 ml
Sunflower oil		50 ml
Sucrose		65.5 g

* Based on Test Meal Specifications, Guidance for Industry. FDA-CDER, October, 1997.

** End-result pH 2.98

3.6. Analytical methods

3.6.1. Chromatographic conditions

A number of bioanalytical methods exist that are currently applied in pharmacokinetic practice [118]. Among these; a highly sensitive, validated and optimised gas chromatographic method with nitrogen selective detection (GC-NPD) using a solid-phase extraction technique has been used to determine levels of parent compound in rat plasma before [95]. Gas chromatography using mass selective detection (GC-MSD) has also been described for determination of deramciclane in rat and rabbit plasma [92]. For determination of the compound in human plasma, high-performance liquid chromatography-tandem mass spectrometry (HLPC-API-MS-MS) using selected reaction monitoring technique had been used [92]. Samples collected from *in vitro* dissolution tests, either those simulating the fasting or the fed states are of matrices that do not resemble plasma. Therefore, chromatographic analysis (GC-NPD) had to be adapted to suit the "non-biological samples".

3.6.2. Calibration samples

Calibration samples at the concentrations given in Table IV. below were prepared by adding the volume of deramciclane and internal standard using the actual dissolution model mixture as matrix.

The calibration samples were stored in a refrigerator in 1 ml aliquots. The internal standard is added at the time of sample preparation.

Table III. Summary of chromatographic conditions considered

Parameter	Specification
Column	Supelco SPB-1 (30 m x 0,25 mm x 0.25 μm); HP Retention Gap (5 m x 0.25 mm)
Carrier gas	He (4.6)
Carrier pressure	Isobar 115 kPa
Flow rate	1.95 ml/min (195 °C)
Temperature program	100 °C (0.05 min), 35 °C/min: 195 °C (8 min), 50 °C/min: 280 °C (2 min), 70 °C/min: 100 °C (1 min)
Injection method	split, split ratio approx. 2:1
Injector temperature	240 °C
Injection volume	2 μl
Detector temperature	300 °C
Detector make-up gas / flow rate	He, 30 ml/min
Detector auxiliary gas / flow rate	air, 110 ml/min
Detector auxiliary gas / flow rate	H_2, 4 ml/min
Septum purge gas / flow rate	He, 1.2 ml/min

Table IV. Calibration parameters

Calibration Level	Deramciclane concentration [µg/ml]	Standard solution	Standard solution volume [µl]	Volume of dissolution medium [ml]
1.	0.05	III.	25	9.98
2.	0.5	III.	250	9.75
3.	1.0	III.	500	9.50
4.	10.0	II.	500	9.50
5.	20.0	I.	100	9.90
6.	50.0	I.	250	9.75
7.	100	I.	500	9.50

3.6.3. Sample preparation

Deramciclane is extracted from the model dissolution media by liquid-liquid extraction at alkaline pH. All extractions are performed in duplicate.

One ml of calibration or dissolution sample is pipetted into a stoppered 10 ml test tube. 1 ml of 0.25 M ammonium hydroxide solution and 50 µl internal standard (corresponding to 500 ng bencyclane fumarate) are added. The sample is extracted for 30 seconds with 2 ml extraction solvent by vortexing. The two phases are separated by centrifugation (2000 min^{-1}, 15 min), the upper organic phase is pipetted into a clean tube and evaporated to dryness in a nitrogen stream at 50ºC. Evaporation residue is dissolved in 200 µl propanol and injected to the gas chromatographic system.

Deramciclane was extracted from the modelled dissolution media by liquid-liquid extraction and was assayed by capillary gas chromatography with nitrogen-phosphorus selective detection (GC-NPD). Dissolution media were neutralised at the time of sampling by mixing with equal volume of aqueous 0.25 M ammonium hydroxide solution. Internal standard, bencyclane fumarate [119], was added as 5 μg in 50 μl volume. The mixture was extracted with 2 ml 1:9 (v/v) ethylacetate-hexane solvent mixture for 30 seconds by vortexing. The organic phase was separated, evaporated to dryness and dissolved in 200 μl propanol and a 2 μl portion was submitted to gas chromatographic assay. Gas chromatography was performed on a Supelco SBP-1 (30 m x 0.25 mm x 0.25 μm) column, fitted with an HP retention gap (5 m x 0.25 mm) and using helium as the carrier gas at a flow rate of 1.95 ml/min. Analysis of samples was performed by temperature programming (100°C for 0.05 min, 100-195°C at 35°C/min, 195°C isotherm for 8 min, 195-280°C at 50°C/min, 280°C isotherm for 2 min). Linear calibration curves valid for the dissolution matrices were constructed in the range of 0.05-100 mg/l deramciclane. The method was found to be linear in the given range ($r \geq 0.998$). Limit of detection was 0.02 mg/l. Recoveries from neutralised artificial gastric juice simulating fasting and fed state were 93.5 and 78.7 %, respectively. Relative standard deviation of five repeated assays, from neutralised dissolution samples simulating fasting and fed status were 6.7 and 9.3 %, respectively.

3.7. Calculation of results

Linear calibration curves valid for the dissolution matrix were constructed using $1/y^2$ weighing, from analysis results of calibration samples in the range of 0.05 - 100 μg/ml. Results were calculated using these calibration curves, taking into account the

dilution factors and that 1 mg of deramciclane fumarate is equivalent to 0.72182 mg deramciclane base. Calculations and graphs were prepared using Microsoft Excel 97 (Microsoft Co., Seattle, USA) and Microcal Origin Version 6.0 (Microcal Software Inc., Northampton, MA, USA).

4. Results and Discussion

4.1. *In vitro* characterisation of dietary components regarding their acid buffering capacity

Dietary components were studied one at a time in order to elucidate each characteristic separately. For each component, the amount of hydrochloric acid (HCl) added was plotted against end result pH (witnessed as stable reading for 10 seconds). Titration results that reflect such plot are presented in Figure 3. Dietary components that are capable of neutralising the effects of added HCl acid are known to have acid buffering capacity. As expected, starch, oil and sucrose showed no such capacity. Protein-containing components (milk powder, gelatine), however, can first increase medium pH and then compensate pH-lowering effect of the added acid. Separate analysis of protein-containing dietary components reveals some differences between the studied elements. Adding milk powder has resulted in pH values almost close to neutral (initial pH of 6.4 ± 0.1 S.D.), with relatively large amounts of HCl acid needed to lower it. In the presence of gelatine, the initial pH was lower (5.5 ± 0.1 S.D.), but relatively large amounts of HCl were needed to lower the pH from 4 to 3 (Figure 4.), i. e. the main buffering capacity of gelatine is characteristic at this range. Further studying using two different types of milk powder (skimmed and whole-fat) showed some differences as well. The buffering capacity of skimmed milk powder was markedly higher between pH 5 and 3 (Figure 5.). The acid buffering capacity as a function of both pH and concentration for gelatine and skimmed milk is shown in Figures 6. and 7., respectively.

These observations concentrated on the effects exerted by dietary components themselves, without further complicating the test by adding a drug product.

Nevertheless, they provided substantial grounds to be taken into account when considering *in vitro* modelling of food-drug interaction studies.

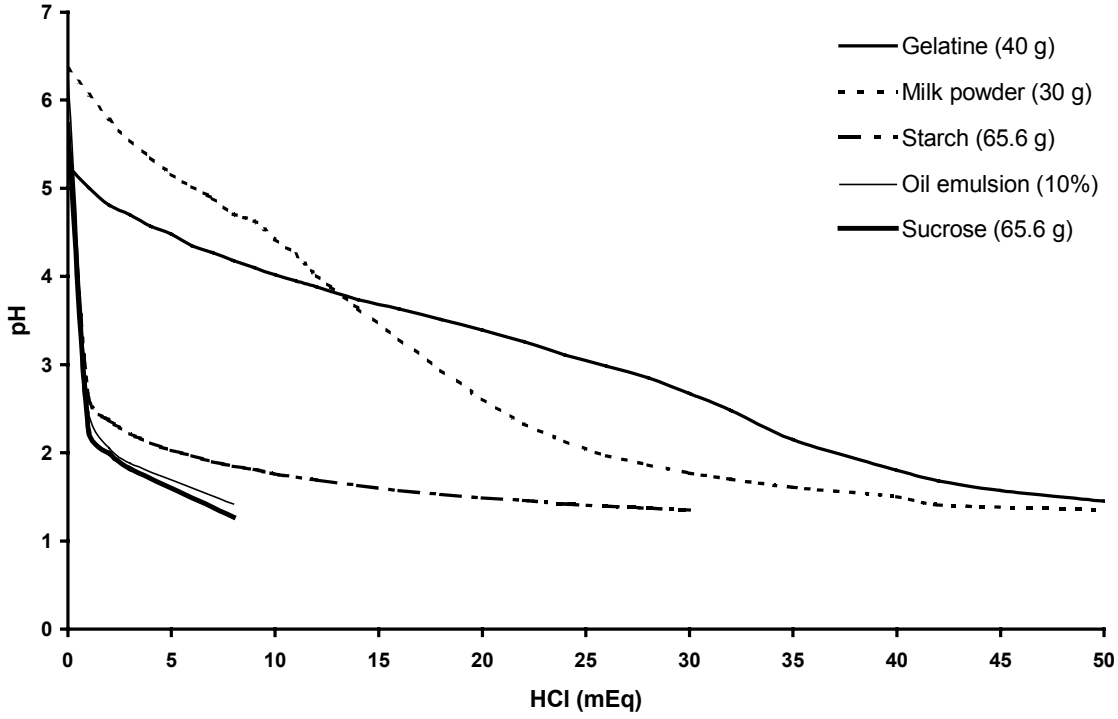

Figure 3. Titration results after adding HCl to the different dietary elements under simulated gastric conditions. Note short graph in case of oil emulsion, starch and sucrose

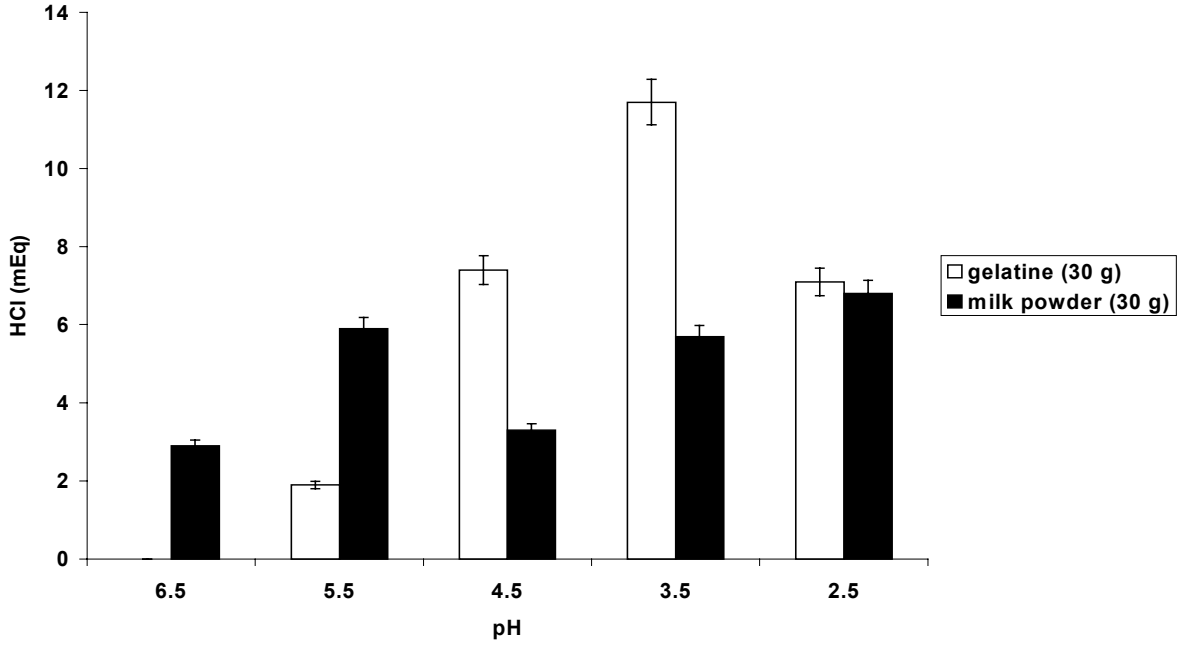

Figure 4. Acid Buffering Capacity of protein-containing dietary components: gelatine vs. milk powder

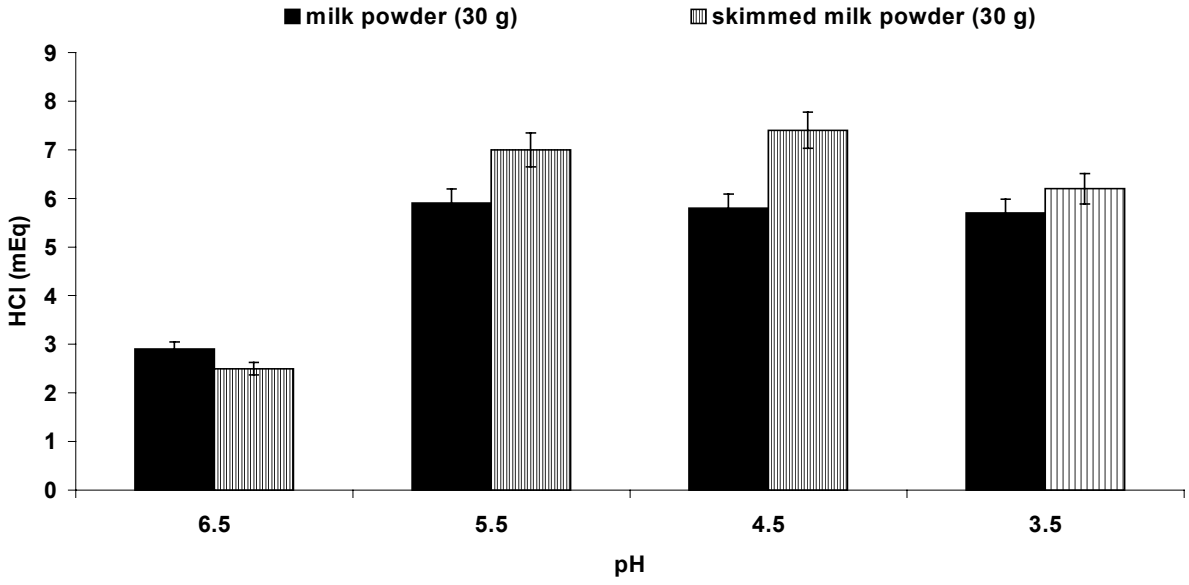

Figure 5. Milk powder *in vitro* acid buffering capacity: whole vs. skimmed

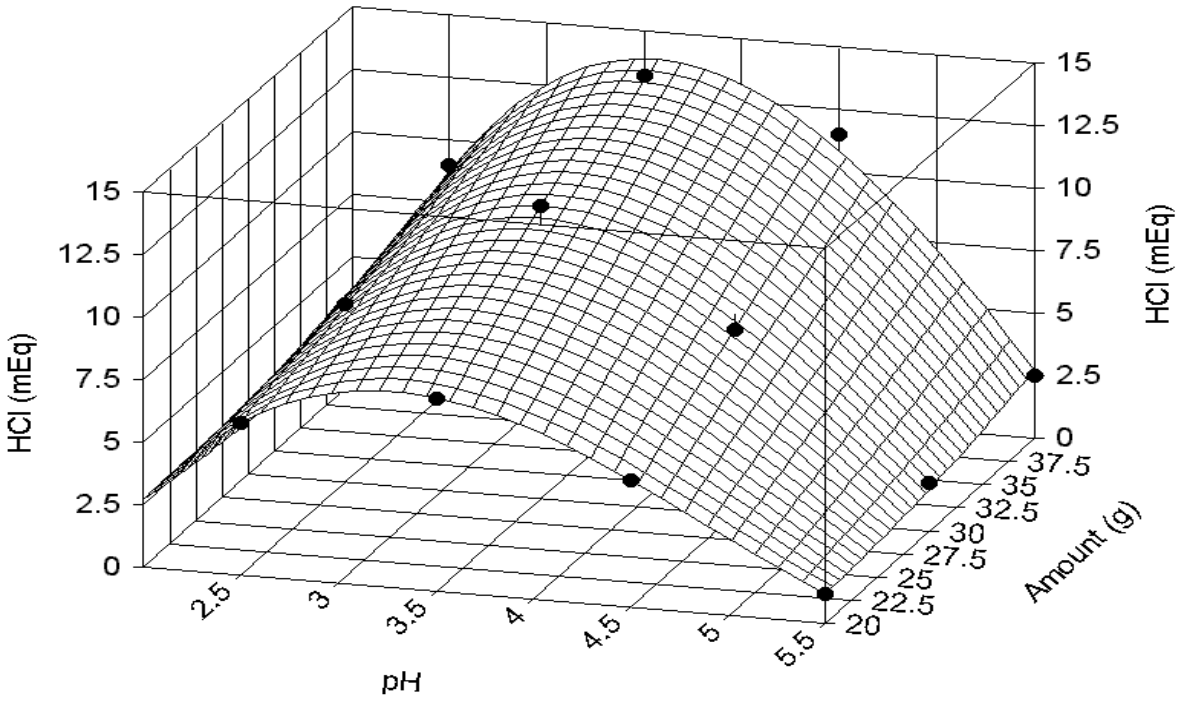

Figure 6. Acid buffering capacity of gelatine as a function of pH and concentration

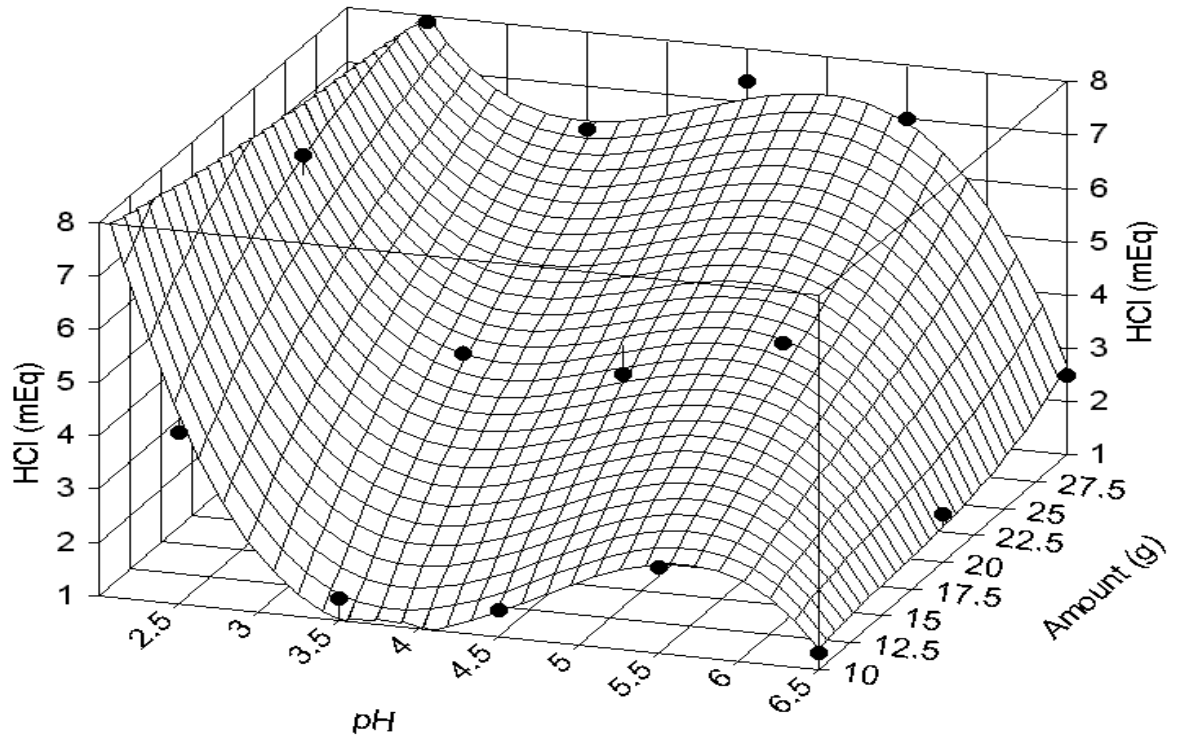

Figure 7. Acid buffering capacity of skimmed milk powder as a function of pH and concentration

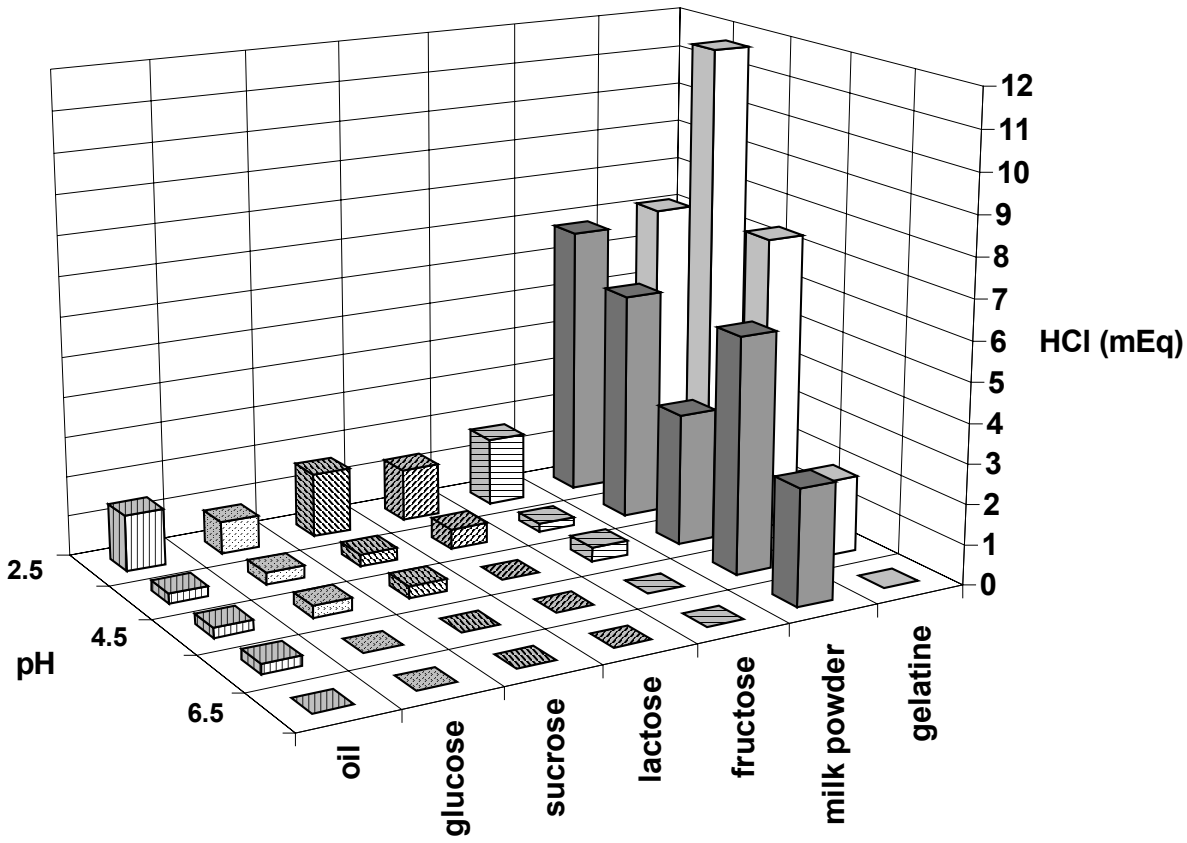

Figure 8. Summary of the acid buffering capacity of *in vitro* studied dietary components

4.2. Pharmacokinetic findings from the clinical study

4.2.1. Discussion of study design

To evaluate the influence of food on the bioavailability and pharmacokinetic parameters of deramciclane, the study was interpreted as a bioequivalence study including two treatments: drug administered to fasting volunteers was designated as reference treatment, while drug administered to fed volunteers was designated as test treatment. The two-way randomised cross-over design suits these kind of studies well.

4.2.2. Pharmacokinetic evaluation

Average values of deramciclane pharmacokinetic parameters are presented in Table V. The mean pharmacokinetic curves of deramciclane obtained under fasting and fed conditions are shown in Figure 9. Apparently, plasma concentration of deramciclane increased as a result of food intake prior to drug administration. Analysing obtained data show that $AUC_{0-\infty}$ of deramciclane increased by 32.8 % (this was calculated as $[AUC_{0-\infty\ fed} / AUC_{0-\infty\ fasting}] * 100$), whereas peak plasma concentration (C_{max}) increased by 27.3 % (calculated as $[C_{max\ fed} / C_{max\ fasting}] * 100$) under fed conditions. The presence of food interaction was verified. The 90 % confidence interval of the mean ratio (fed/fasting) for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values of deramciclane were 1.21-1.41, 1.21-1.41 and 1.20-1.38, respectively. This ranged outside the outer limit of 0.8-1.25 that is used for positive assessment of bioequivalence. At the same time, t_{max} of the parent compound did not change to any significant extent.

Table V. A summary of deramciclane mean ± S.D pharmacokinetic parameters as evaluated in the clinical study (*n* = 18)

Pharmacokinetic Parameter	Fasting State	Fed State
C_{max} (ng/ml)	**43.68** ± 15.12	**54.15** ± 17.85
t_{max} (h)	**2.69** ± 0.52	**3.17** ± 0.89
AUC_{0-t} (h*ng/ml)	**657.95** ± 454.63	**837.22** ± 452.28
$AUC_{0-\infty}$ (h*ng/ml)	**745.67** ± 629.21	**942.25** ± 654.32

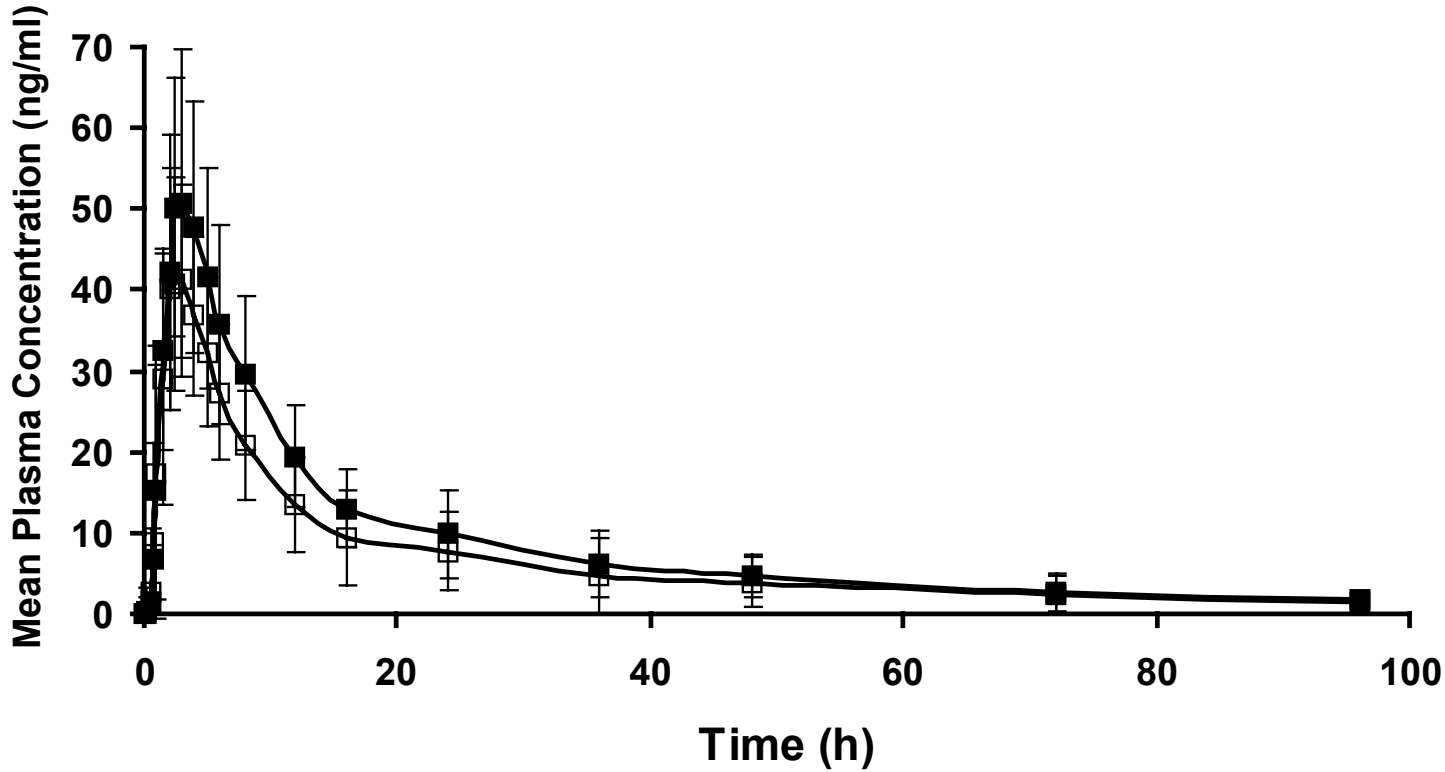

Figure 9. Mean plasma concentrations of deramciclane in human plasma after the administration of a single 30 mg dose (as base in film-coated tablets), either after fasting overnight (□) or after fat-rich standard breakfast intake (—■—)

4.3. *In vitro* modelling of *in vivo* conditions

4.3.1. Simulated fasting vs. simulated fed

Because clinical studies were already accomplished by the time *in vitro* modelling was planned the same conditions had to be literally considered. Bioavailability studies were conducted under fasting conditions and after the ingestion of a high-fat breakfast [116]. The differences between *in vitro* dissolution profiles of deramciclane-containing film-coated tablets in simulated fasting conditions and simulated fed conditions are significantly obvious (Figure 10.). In artificial gastric juice (pH 1.2), the amount of deramciclane traced in dissolution medium hardly reached 66% of tablet content during the first 10 min of dissolution to be followed with a marked decrease in collected drug. The term "collected" is used instead of "released", as speculations have it that in such a strong acidic environment the acid-labile drug would be released only to undergo rapid degradation. This observation is consistent with previously published *in vitro* analysis of the drug [104]. In simulated fed state, and despite the fact that collected amount of deramciclane did not reach the total tablet content of the drug (i.e. percent of drug retrieved from dissolution medium with added dietary components did not reach 100 % of the 30 mg drug base), more interesting observations are recorded. Drug dissolution is delayed and a plateau is reached. This outcome is relatively similar to drug dissolution profile in buffer (i.e. pH 6.8), where drug degradation is unlikely to happen. With acid buffering capacity findings in mind (from previous section), differential analysis was undertaken.

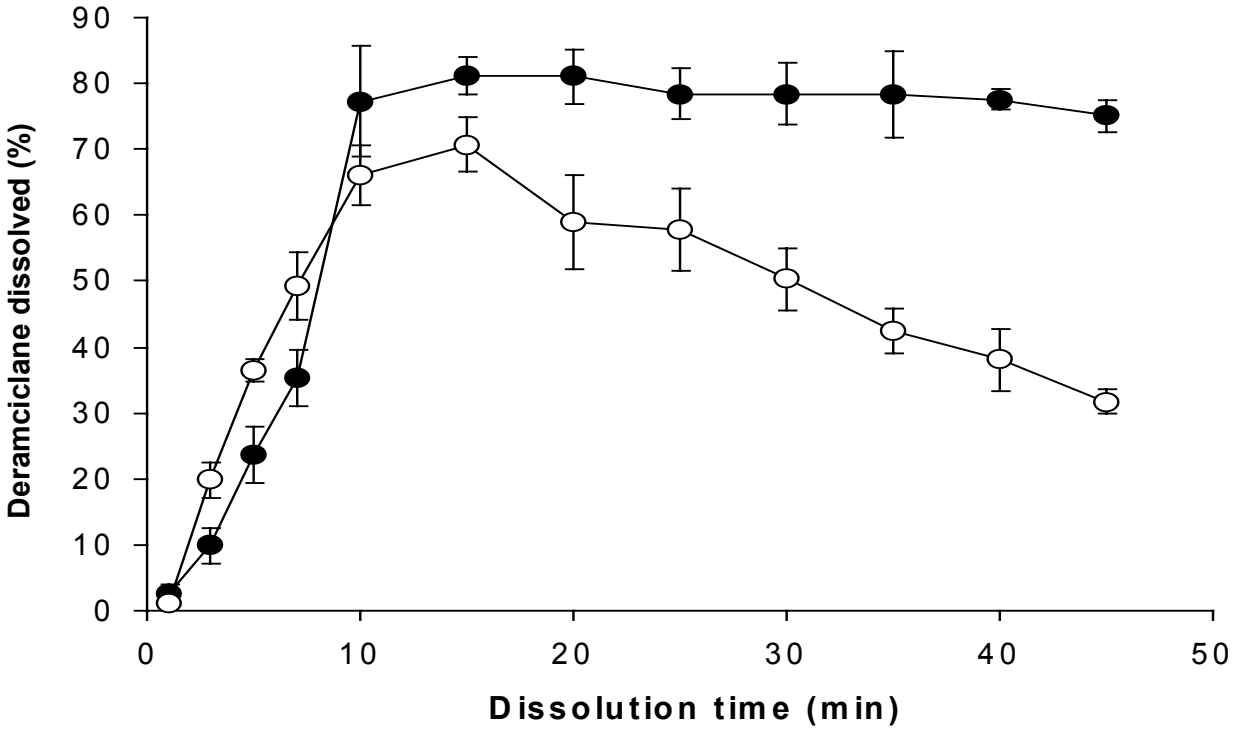

Figure 10. Mean dissolution profile of deramciclane from 30 mg film-coated tablets as studied with the USP dissolution apparatus II. (-●- fed state simulated with dietary components, -o- fasting state simulated with pH=1.2 buffer)

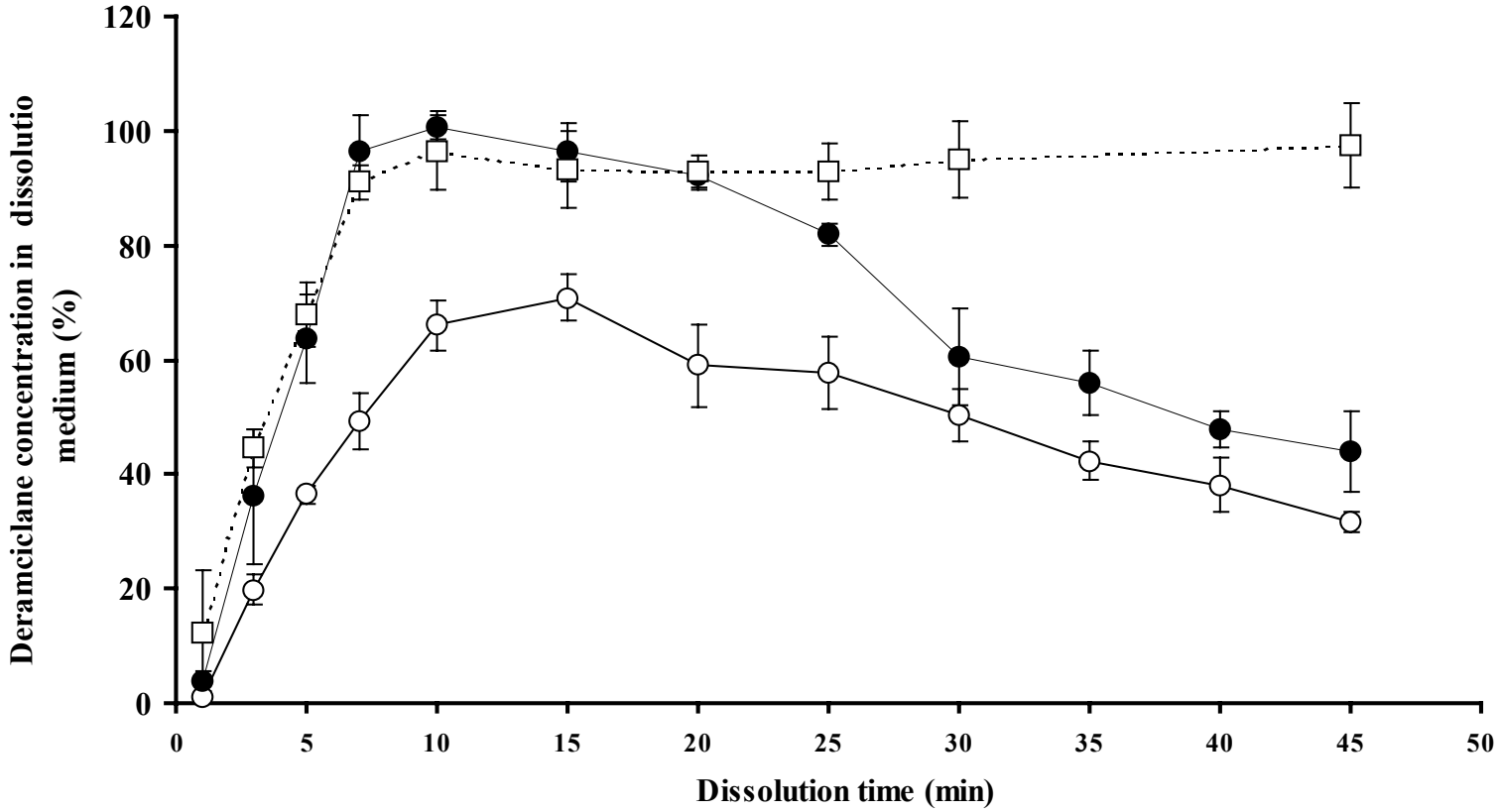

Figure 11. Deramciclane concentration as a function of time after *in vitro* dissolution of film-coated tablets containing 30 mg drug base, studied with the USP dissolution apparatus II. (⊖ fasting state simulated with pH 1.2 buffer, — fed state simulated with pH 6.8 buffer, ● fed state simulated with pH 1.2 buffer + oil)

4.3.2. *In vitro* dissolution of deramciclane-containing film-coated tablets in oil emulsion

Despite lacking acid buffering capacity, oil added to the simulated gastric fluid has altered the dissolution medium to the extent that influenced deramciclane *in vitro* dissolution profile. A comparative plot of the dissolution profile is shown in Figure 11. The acid-labile drug is presumably stable and should not undergo any degradation in dissolution media with pH higher than 3 (i.e. simulated fed state). This is confirmed when studying the curve representing deramciclane dissolution in buffer (pH 6.8). In strong acidic medium, however, a steep increase of the amount of drug is short and reflects only small amounts of the drug are traced in the dissolution medium. Adding oil has first provided some protection, though short-lived, as seen in the high readings during the first 10 minutes of dissolution. This was then followed by the expected degradation in the simulated gastric juice. It might be reasonable then to assume that deramciclane released from the film-coated tablets had been temporarily incorporated into oil particles, and got dissociated later.

4.3.3. Dissolution profiles in milk powder

Bioavailability studies have been utilising high-fat breakfast before the administration of the drug to healthy volunteers. This meal has included –among others- milk with high fat content. Whole milk is not only rich in fat content, but also in protein. And so it was used during *in vitro* simulation of the fed state. To analyse drug dissolution with respect to this dietary component alone, it was added to simulated gastric juice (i.e. pH 1.2 + 30 grams whole milk powder dissolved in a volume of water identical to that of the gastric juice). As expected, the acid buffering capacity of milk powder was evident, as no degradation of deramciclane was observed. Ten minutes after

starting *in vitro* dissolution, the percent of drug dissolved was recorded at as high values as 70-80 % and lasted all through the 45th minute of the study (Figure 12.).

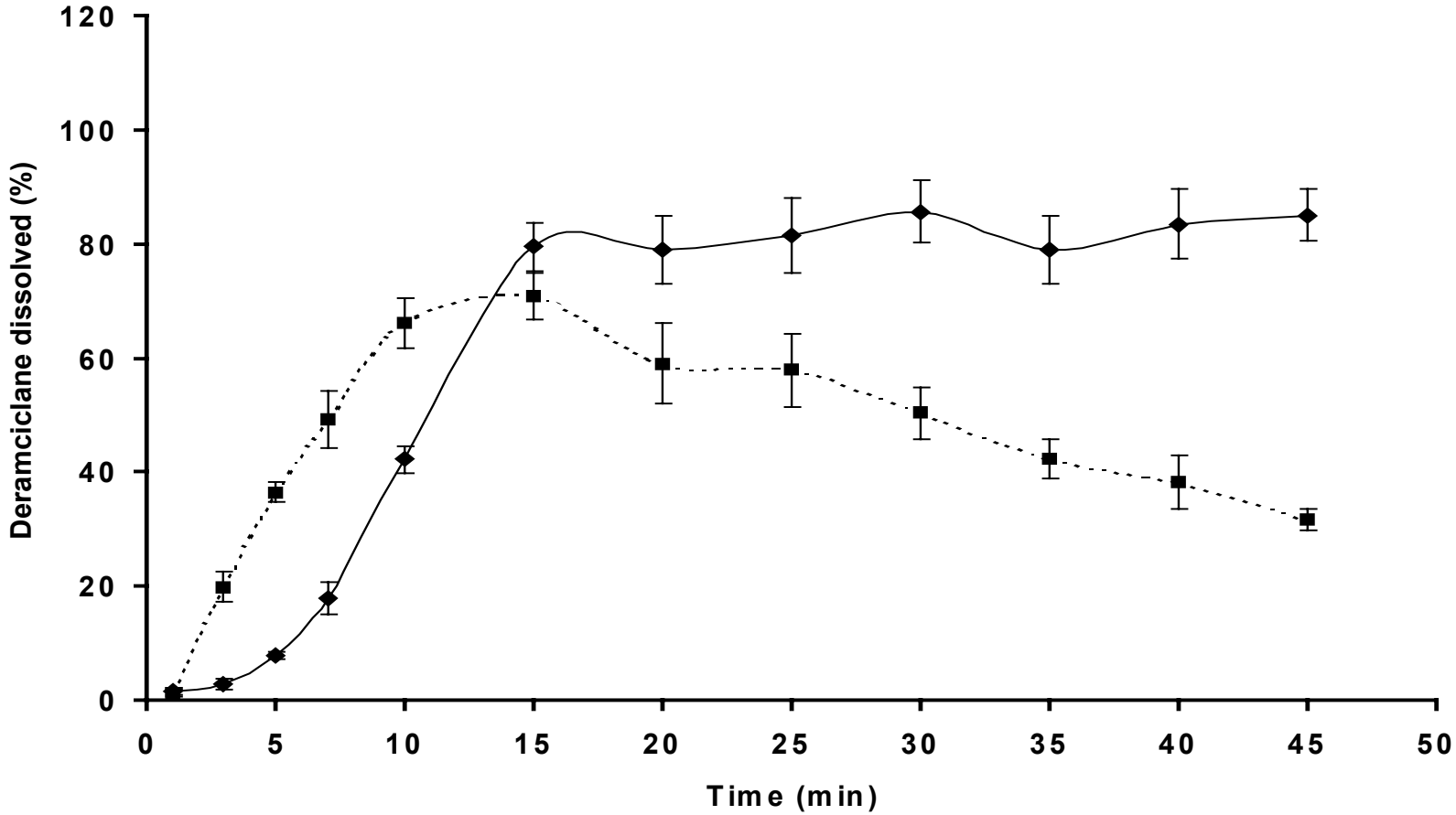

Figure 12. Deramciclane concentration as a function of time after *in vitro* dissolution of film-coated tablets containing 30 mg drug base in artificial gastric juice: pH 1.2 (—■—) and with whole milk powder added (◆) as studied with the USP dissolution apparatus II

Collecting all the pieces discussed so far could lead one to the following concept. Under fasting conditions, a strong acidic environment dominates in the stomach giving little chance to the acid-labile drug administered in a conventional pharmaceutical form to thrive. A high fat-breakfast alters this acidic environment at different steps. With its hydrophobic characteristic, deramciclane is incorporated into oil particles. This is only a temporary compartment during the process of dissolution. By the time it is "freed" from this compartment, the rest of the dietary components will have worked through their acid buffering capacity to encounter the environment into a less "acidic" one. While this seems to be a well-organised process, a small amount of the drug remains unaccounted for -probably through degradation- as percent of drug dissolved/detected does not reach 100 % of that provided. In reality, bioavailability studies do not encounter for all amount of drug administered. Furthermore, they are not even designed to do so. From this perspective, an absolute 100 % drug detection *in vitro* might not be the goal; and the lack of it should not necessarily be interpreted as failure of the model.

4.3. *In vitro-in vivo* correlation

To facilitate IVIVC establishment, cumulative area under the time-concentration curve (AUC_{cum}) for both *in vitro* (Table VI.) and *in vivo* (Table VII.) studies with deramciclane were analysed and showed similar tendencies. During the first 15 minutes of the dissolution process, higher AUC_{cum} values demonstrated faster release under simulated fasting conditions as compared to simulated fed conditions. On the other hand, AUC_{cum} values were markedly higher under fed conditions beyond that point of time. Similarly, fasting conditions have yielded higher AUC_{cum} values as compared to fed state values during the first 2 hours of the *in vivo* study. After that, AUC_{cum} values

obtained under fed conditions were higher. To establish a correlation, equal AUC_{cum} ratios (AUC_{cum} fed/AUC_{cum} fasting) seen at certain *in vitro* dissolution time(s) and *in vivo* blood sampling time(s) were plotted (Figure 13.). Logarithmic plotting of *in vivo* blood sampling time results in a linear relationship between *in vitro* dissolution profile and *in vivo* pharmacokinetic study as a function of AUC_{cum} (Figure 14.).

Table VI. Summarised AUC_{cum} values obtained from *in vitro* dissolution of deramcicalne 30 mg film-coated tablets

In vitro dissolution time (min)	AUC_{cum} (simulated fed)	AUC_{cum} (simulated fasting)
1	1.295	0.588
3	13.801	21.611
5	47.340	77.929
7	106.229	163.656
10	274.999	336.684
15	671.376	678.821
20	1077.486	1015.717
25	1476.495	1320.261
30	1868.731	1590.554
35	2260.859	1822.279
40	2650.776	2023.618
45	3032.460	2198.187

Table VII. Summarised *in vivo* AUC_{cum} values calculated after the administration of single oral dose of deramciclane 30 mg film-coated tablets under fasting and fed conditions*

In vivo blood sampling time (h)	AUC_{cum} (fed)	AUC_{cum} (fasting)
0.25	0.013	0.023
0.5	0.225	0.358
0.75	1.269	1.756
1.0	4.000	5.006
1.5	15.923	16.581
2.0	34.615	33.854
2.5	57.718	54.074
3.0	82.940	74.559
4.0	132.145	113.539
5.0	176.755	148.109
6.0	215.350	177.959
8.0	280.720	226.199
12	378.860	294.899
16	443.740	340.799
24	535.260	409.679
36	630.540	484.499
48	694.740	536.579
72	783.300	611.579
96	837.180	657.899

* [116]

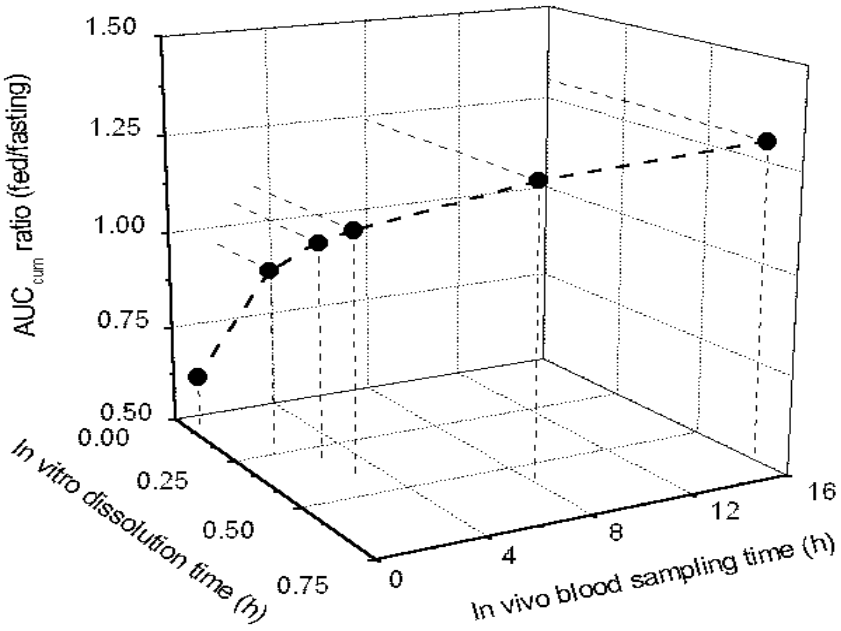

Figure 13. AUC_{cum} ratio in relation to both *in vitro* dissolution time and *in vivo* blood sampling time

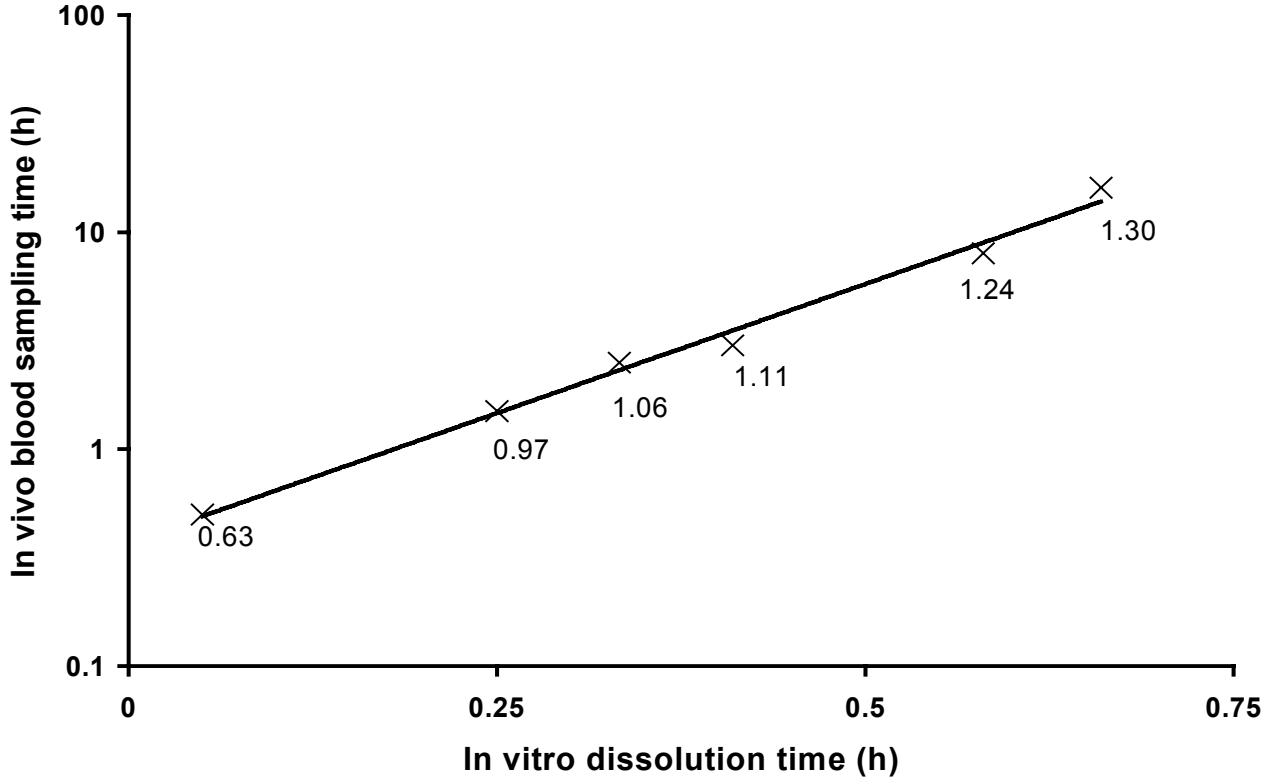

Figure 14. The correlation between logarithmic *in vivo* blood sampling time and *in vitro* dissolution time assigned to equal AUC_{cum} ratio ($AUC_{cum, fed}$ / $AUC_{cum, fasting}$)

y (*in vivo* blood sampling time) = $0.3723e^{5.4784 \times (in\ vitro\ dissolution\ time)}$,with r = 0.9956

Studies published so far do not seem to agree upon one method for establishing correlation, even when focussing on immediate release dosage forms only [16, 43]; let alone other dosage forms. In principle, *in vitro* dissolution kinetics is compared to *in vivo* absorption parameters. Nevertheless, interpretation of the term "correlation" lies usually with the investigators' abilities to spot one and, not surprisingly, could prove "non-established" or "non-existing" unless drawn by simple point-to-point correlation (see 2.8. IVIVC or IVIVR in **Theory and Background** above). The case of deramciclane-containing film-coated tablets discussed here suffered a lack of intravenous data. In addition, a compartment independent pharmacokinetic evaluation was chosen for sake of simplicity. Both facts meant that calculating the percent of drug absorbed *in vivo* would not be possible [120, 121]. Therefore, AUC_{cum} ratios between fed and fasting conditions were used to evaluate a correlation. By doing that, new approaches have hopefully been allowed to emerge adding to already existing -less complicated- ones. This could (and would) enable the meaning of correlation to eventually be broadened, perhaps to the extent where "relationship" rather than "correlation" would be put into use.

Another aspect to be considered here is the *in vitro* model used for dissolution studies. A detailed description of simulated gastric and intestinal fluids during both fasting and fed conditions exists [19]. Both qualitative and quantitative application of the dietary components stated in a guideline governing bioavailability studies has not been attempted before. Special care was given to some elements of the so-called "standard meal", namely fat. The influence of high-fat meals on dissolution has appeared in the literature [74, 75]. Yet, a high-fat-containing model for *in vitro* dissolution has not been described to date. As a matter of the fact, some investigators

have ruled out the possibility of predicting food effects from *in vitro* characterisation of dosage forms, although accepted the idea as far as pH-dependent release properties are concerned [122]. Since these investigators specified their observations to modified-release dosage forms, it might be reasonable to accept different standards to be applied to IR dosage forms. In our work, the use of 10 % oil emulsion has served several goals: (a) implacability of dissolution media that is based on natural occurring components; (b) applicability (and hence validity) as a standard method for "oily/fatty" media; and (c) further refinement of models intended for simulating biological systems.

While not all dissolution trials successfully yield IVIVC, attention could be focused on model development since it plays a major role in the fate of any attempt described. Instead of applying an approach similar to the one used in the case of deramciclane, a new system that takes into account drug dissolution and pH changes in the GIT was developed and published [123]. In that system, a drug product was added into a drug-dissolving vessel with a pH of 1.0. The dissolved drug was then transferred into a pH adjustment vessel (pH 6.0). The resulting drug solution was then transferred to the apical surface of Caco-2 cells, with the permeation rate of the drug across Caco-2 mono-layer determined. The aim of this experiment was to predict oral absorption ratios of a number of water-soluble drugs in humans. Although successfully applied, the limitations of this system are numerous. First of all, a special drug group fits into this screening model, with more challenging ones (e.g. poorly water-soluble drugs) passing such test only after subjection to enhanced absorption. This was accomplished using solid dispersion of polymers that improve water solubility. In addition, the system takes only the fasting state as a basic condition for testing. Screening for new drug substances

might as well include the food-effect studies. And finally, this system can hardly be applied for IVIVC.

For people either involved or interested in IVIVC, several problems are addressed every time an attempt that relates *in vitro* observations to *in vivo* events is undertaken. First, there is the *in vitro* dissolution model chosen together with its mathematical interpretation. Most attempts –especially those with unsuccessful outcomes– tend to apply statistical interpretations for a more or less dynamic model; or falsely imply a stationary environment as a base for transformation between the systems studied. Some, however, have described mathematical and statistical "Model Dependant" approaches as better tool for IVIVC/R [124]. Although flexible and reliable, it has been concluded that it is primarily targeted for scale-up and post approval variations of IR products. Others have tried mathematical models that predict drug release from dissolution of gel micelles [125]. Experimental results were in agreement with model prediction for certain stirring speeds, nevertheless, discrepancies between actual and predicted values were also found and related to diffusion components. Once again, model limitation is concluded.

Second, IVIVC trials have to deal with the time-scaling problem and try hard to solve it [126, 127]; or assume analysed parameters to be time invariant and end up with imperfect approaches. *In vitro* dissolution –even for the slow–release formulae– takes far less time than that needed for the same dosage form to undergo *in vivo* dissolution. Events affecting this process should always be considered as a function of both time and location within the GIT. With this in mind, alteration in pH environment and/or enzyme content should also be taken into account. Unfortunately, this is not usually the case during *in vitro* modelling. And last but not least, there is the issue of inter-individual

variability that is usually incorporated *in vivo* but all too easily overlooked *in vitro*. It is therefore fair to conclude that even well designed models fail to provide accurate simulations, as it is not feasible for any given *in vitro* model to imitate the complex events occurring in reality-at least not simultaneously.

The scope of *in vitro* dissolution modelling lies far beyond experimenting and, hence, continuous analysis serves as a need rather than an accessory. Besides providing rich material for scientific research, it might as well give grounds for industrial achievements and regulatory breakthroughs [128 - 131]. Nowadays, and for many years yet to come, clinical applications for a given pharmaceutical dosage form will continue to include detailed bioavailability studies. It is also reasonable to say that the cost of such studies will only continue to rise. Another alternative would be newly addressed individual bioequivalence studies on case-by-case evaluation [132]. Whatever choices are at hand, the dilemma grows only larger. It might be naive to assume that serendipitous success –such as the one related to deramciclane IVIVC– can grant standard methodology in the field. But it is still possible to state that it could help waive unnecessary human studies in the future. Whether this can be applied to other drug products, probably of another pharmaceutical form or of a differently challenging physico-chemical property, is yet to be explored.

5. Conclusions

A simple and reproducible *in vitro* dissolution method was applied to define the acid-buffering capacity of different dietary components. In addition, the dissolution profile of a highly permeable, poorly water-soluble acid labile drug molecule in a conventional immediate release pharmaceutical form was investigated under simulated fasting and fed conditions.

Dietary components usually consumed as part of the so-called "Standard meal" given prior to drug administration were investigated regarding their acid buffering capacity. As expected, protein-containing dietary components showed considerable, concentration dependent acid buffering capacity when examined at simulated-biological environment. Although standard diets often emphasise the use of whole milk, our findings revealed that skimmed milk (i.e. milk of low fat content) shows more intense acid buffering capacity as compared to that of the fat-rich milk. Oil as well as sugars and starch, on the other hand, lacked such capacity.

With the aid of USP dissolution apparatus II (paddle method), biological reservoir-like environment was simulated all through experimenting with special care given to volumes, temperature and composition of gastric fluids expected at fasting and fed states. To perform a close-to-real simulation, dissolution media set for postprandial drug dissolution studies contained qualitatively and quantitatively identical dietary components to those included in the meal administered to the healthy volunteers. The pattern of drug dissolution in the simulated fasting state was in line with the acid-labile nature of the drug. In the simulated fed state, however, food components did delay drug dissolution and helped increase the percent of drug retrieved in dissolution medium.

Calculated AUC_{cum} ratios (i.e. AUC_{cum} fed/AUC_{cum} fasting) from *in vitro* data showed similar tendencies to those already observed in *in vivo* studies. A basis for *in vitro-in vivo* comparison was found and a correlation was established.

Our findings succeeded in defining an *in vitro* model that could be applied for future food-effect and/or food-drug interaction studies. They have also helped explore the postprandial environment together with its anticipated effects on an acid-labile drug.

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List of Original Publications

I. Balogh Nemes, K., Abermann, M., Bojti E., Grézal, Gy., ***Al-Behaisi, S.***, Klebovich, I. (2000). Oral, Intraperitoneal and Intravenous Pharmacokinetics of Deramciclane and Its *N*-desmethyl Metabolite in the Rat. J. Pharm. Pharmacol. 52: 47-51

II. ***Al-Behaisi, S.***, Antal, I., Morovján, Gy., Szúnyog, J., Drabant, S., Marton, S., Klebovich, I. (2002). In vitro Simulation of Food Effect on Dissolution of Deramciclane Film-coated Tablets and Correlation with In vivo Data in Healthy Volunteers. Eur. J. Pharm. Sci. 15: 157-162

III. ***Al-Behaisi, S.***, Antal, I., Morovján, Gy., Marton, S., Klebovich, I. (2002). The Effect of Oil as a Dietary Component on In vitro Dissolution of an Acid-Labile Drug. ^{Die} Pharmazie *(in press)*.

IV. ***Al-Behaisi, S.***, Antal, I., Morovján, Gy., Drabant, S., Plachy, J., Marton, S., Klebovich, I. (2002). Studying the Acid Buffering Capacity of Dietary Components Regarding Food-Drug Interaction (in Hungarian). Submitted in January 2002 to Acta Pharm. Hung.

V. Drabant, S., Balogh Nemes, K., Horváth, V., Tolokán, A., Grézal, Gy., Anttila, M., Gacsályi, B., Kanerva, H., ***Al-Behaisi, S.***, Horvai, G., Klebovich, I. Influence of Food on the Oral Bioavailability of Deramciclane from Film-Coated Tablets in Healthy Volunteers. Prepared to be submitted to Eur. J. Pharm. Sci.

Summary

In vitro dissolution testing is applied to evaluate drug dissolution/release from a given pharmaceutical form into a given medium. Investigators have utilised a number of methods to standardise test parameters. Despite their efforts, a standard scheme for *in vitro* dissolution is still lacking. A number of studies have been published that successfully establish a correlation between *in vitro* dissolution data of a drug product and its *in vivo* pharmacokinetics. Such correlation continues to prove of importance from both developmental and regulatory aspects. This kind of work could also be extended to alteration of drug bioavailability after food intake. To define a correlation between data collected from *in vitro* dissolution of a drug product and *in vivo* absorption of the same drug after the ingestion of a high-fat meal, an *in vitro* food-drug interaction model has first to be described. A simple and reproducible method that incorporates the USP dissolution apparatus II (paddle method) was applied. Dietary components of the so-called "test meal" were added –both qualitatively and quantitatively– to artificial gastric juice and a close-to-real simulation was attempted. The drug chosen for this series of experiments, deramciclane fumarate, is a model acid-labile drug in a conventional film-coated tablet. *In vitro* dissolution profiles in both testing media (i.e. simulated fasting and simulated fed states) showed similar tendencies to pharmacokinetic observations in healthy human volunteers. A correlation –though non-linear– exists. Analysing food components one at a time reveals new findings. Guidelines governing food-drug interaction studies highlight the role fat plays in food-effect. The case of deramciclane, however, proved that oil/fat could play a different role. The lipophilic character of the molecule enables temporary deramciclane-oil complexes to form, while the rest of dietary components are increasing stomach pH through their acid buffering capacity. Consequently, following dissociation from oil, less amount of the drug undergoes degradation. This could give reasonable explanation for the increase in bioavailability of the drug after food intake. In conclusion, refinement of any *in vitro* model to fit to the drug product at question is essential before correlation studies start. Utilising "genuine" dietary components could prove to be the bases for food-drug interaction modelling, which could facilitate better understanding of post-prandial drug behaviour.

Összefoglaló

Egy adott gyógyszerkészítmény *in vitro* kioldódási profilja és *in vivo* farmakokinetikája közötti korrelációt (IVIVC) számos kioldódási közeg alkalmazásával sikerülhet elérni. Olyan általánosan alkalmazható szabványos eljárást, amely a kioldódásnak és az abszorpciónak az előrejelzésére alkalmas, az eddigi irodalomban még nem ismert. Nyilvánvaló az igény olyan kioldódás vizsgálati módszerekre, amelyek megfelelő mértékben képesek előrejelezni a hatóanyagok biológiai hatékonyságát. Alapvetően, egy *in vivo* kioldódási modellnek szimulálnia kell a biológiai rendszerben lévő nedveket, figyelembe véve a kioldódás kezdetét (pl. a gyomor, ill. a vékonybél), és a fiziko-kémiai eltéréseket egy adott rendszerben (pl. éhgyomri ill. étkezés utáni állapot). A kioldódási vizsgálatok megtervezéséhez jelentős segítséget nyújt a Biofarmáciai Osztályozási Rendszer. Alapelvei lehetőséget adnak a kioldódás vizsgálati körülményeinek új szempontok alapján történő megközelítéséhez, figyelembevéve az *in vitro* modell felállításához nélkülözhetetlen biofarmáciai vonatkozásokat. Ezeken belül, egy speciális gyógyszercsoport létezik, azaz a sav-érzékeny hatóanyagok (pl. digoxin, omeprazol és az EGIS által fejlesztett új molekula, a deramciclan). Jelentős ételinterakciót észleltek a korábban végzett humán vizsgálatok során, amelyet markáns plazmaszint növekedést jelentett az úgy nevezett zsír-dús reggeli bevétele után. Az *in vitro* kioldódás tervezése az *in vivo* vizsgálatban alkalmazott étel komponensek figyelembevételével történt, különös tekintettel az arányokra. Az önmagában újdonságnak számít, mivel hasonló modellezést még nem írtak le. Továbbá, az analitikai módszer kifejlesztésére is szükség volt a magas zsírtartalmú minták esetében. Az *in vitro* kioldódási profil alapján kiszámolt görbe-alatti terület (AUC) értékek között és a korábbi humán vizsgálatok során szerzett eredmények között sikerült összefüggést találni. Eredményeink arra utalnak, hogy az IVIVC alkalmazása számos hasznos adatot szolgáltathat bizonyos gyógyszerek fizikai-kémiai tulajdonságai tisztázásában és annak emberi szervezetben való viselkedésének vonatkozásaiban. Ez utóbbi rendkívűl hasznos, ha étkezés utáni állapotra vonatkozik. Egyszerű, validált módszert sikerült alkalmazni, amely hasznosnak mutatkozhat egyéb készítmények esetében is. Következésképpen a bioreleváns modellezés nem csak az IVIVC vizsgálatokban bizonyult hasznosnak, hanem segít az *in vitro* ételinterakciós modellek megalapozásában, amely egy új vizsgálati irányt biztosíthat.