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Research paper

Individualized *in vitro* and *in silico* methods for predicting *in vivo* performance of enteric-coated tablets containing a narrow therapeutic index drug

Frank Karkossa, Sandra Klein*

University of Greifswald, Department of Pharmacy, Institute of Biopharmaceutics and Pharmaceutical Technology, Center of Drug Absorption and Transport (C_DAT), Felix-Hausdorff-Straße 3, 17489 Greifswald, Germany

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ABSTRACT

The efficacy of narrow therapeutic index (NTI) drugs is closely related to their plasma concentration-time profile. Particularly for these compounds interindividual variability of gastrointestinal (GI) parameters relevant to *in vivo* drug release may result in fluctuations of the plasma concentration. The present study focused on assessing the influence of individual GI pH- and transit profiles on drug release of enteric valproate tablet formulations by means of individualized *in vitro* dissolution experiments. After initial experiments simulating GI passages in average healthy adults, a novel *in vitro* dissolution model was used to simulate individual GI pH- and transit profiles with physiologically relevant dissolution media. Based on the dissolution profiles obtained in these experiments, individual *in silico* plasma profiles were generated and compared to fasted *in vivo* data applying a mean Euclidean distance approach. Simulated individual gastric residence time was identified as crucial parameter determining the onset of absorption, whereas the shape of the plasma profile is mainly influenced by individual valproate pharmacokinetics. The novel *in vitro* and *in silico* methods used in this study are promising tools for estimating *in vivo* drug release and plasma concentration in individual subjects and thus may contribute to a prospective risk assessment for NTI formulations.

1. Introduction

In clinical practice, the administration of solid oral dosage forms is the route of choice for the therapy of numerous diseases [1]. After oral administration, solid dosage forms are exposed to various physiological conditions while passing through the gastrointestinal (GI) tract. As GI conditions can markedly differ among patients, drug release and consequently the onset and/or extent of the desired effect may differ between individuals. This might be of minor importance for immediate-release (IR) formulations that usually disintegrate in the stomach and release the active pharmaceutical ingredient (API) in proximal sections of the GI tract, but will be even more important for modified-release (MR), e.g. enteric-coated (EC) or extended-release (ER) formulations. Especially for EC formulations that are supposed to release the API above a certain pH, parameters such as gastric and intestinal pH and GI transit times can have a substantial impact on when and where the drug is released and thus can significantly affect the therapeutic outcome.

The bioavailability of APIs that have an absorption window in the upper small intestine (e.g. levodopa, acyclovir or gabapentin) and thus need to be released in the upper GI tract for proper action [2–4], may for instance be decreased, when the onset of drug release is located in distal parts of the GI tract. Other drugs might present with solubility or stability issues when they are released at GI sites that are not the target site of drug release and finally, orally administered dosage forms with local action in the GI tract might totally fail when the drug is not released at the target site.

A variety of inter- and intraindividual differences in GI physiology has been reported in the literature. These include variations in gastric emptying, small- and large intestinal transit times, composition and volume of fluid available in the different GI sections and in GI motility patterns in both fasted and fed state dosing conditions [5–9].

Besides differences in dosing conditions there is a variety of additional sources that can affect the intraluminal environment and GI motility and consequently can also impact *in vivo* drug release of oral dosage forms. It is obvious that diseases in the GI tract such as e.g. inflammatory bowel diseases or infectious diseases of the GI tract can have a huge impact on GI physiology [10]. However, a number of non GI-dependent diseases such as for instance cystic fibrosis or Parkinson's

* Corresponding author.
E-mail address: sandra.klein@uni-greifswald.de (S. Klein).

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disease as well as other drugs can have a marked impact on several GI parameters [11,12]. Finally, also a number of non-disease impacts, such as age, gender, ethnic and genetic factors, on GI physiology can be a source of intra- and interindividual differences in GI physiology [13,14].

As mentioned before, differences in the *in vivo* drug release behavior of oral dosage forms that are caused by interindividual differences in GI physiology can have a substantial impact on the plasma concentration-time profile API. This is of particular importance for narrow therapeutic index (NTI) drugs, i.e. compounds where small deviations of the targeted plasma concentration may lead to serious therapeutic failures resulting in a loss of effectiveness and/or adverse or even toxic drug effects [15]. Unfortunately, even though several APIs are known to have a NTI, a global list of NTI drugs that could be used for risk assessment does not exist. Health Canada, the Canadian health authority, lists a number of NTI drugs [16]. In the "Guideline for Bioequivalence Studies for Formulation Changes of Oral Solid Dosage forms", the Japanese National Institute of Health Science (NIHS) lists a total of 28 NTI compounds that are used for a variety of therapeutic indications ranging from asthma to immunosuppression [17]. In EMA and FDA guidelines, however, no information of which APIs belong to the group of NTI drugs is provided. However, from the lists available in other countries [16,17] and also based on the experiences made with these drugs over the past decades, antiepileptic/anticonvulsant compounds make up about one third of the currently marketed NTI drugs.

Within the group of anticonvulsants, the most commonly prescribed drugs are carbamazepine, phenytoin and valproate [18]. Carbamazepine and phenytoin are often administered in the form of solid oral IR and ER formulations or liquid suspensions. In contrast, due to the risk of gastrointestinal irritations when given as plain tablet [19], valproate is usually given as EC or ER formulation. The use of liquid valproate formulations is typically reserved for specific patient groups, e.g. paediatric and geriatric patients.

The anticonvulsant properties of valproate were discovered by Meunier et al. in the 1960s [20] and the compound was introduced into antiepileptic therapy in the 1970s. Since then, valproate administered as valproic acid, sodium valproate and divalproex sodium, became an important part of the therapy of bipolar disorders and generalized seizures. Because of its low solubility at low pH, but high permeability, valproate is categorized as a class II drug according to the Biopharmaceutics Classification System (BCS) [21]. When given orally, valproate is fully absorbed [22–25] and in adults has an elimination half-life of approximately 12 h [22,24]. Consequently, in chronic therapy valproate has to be administered at least twice a day to obtain sufficient plasma levels when using IR formulations. Rapid valproate release and absorption can cause fluctuations in plasma levels and thus bears the risk of high plasma peaks after dose administration and subtherapeutic plasma concentrations prior to next dosing. In order to overcome these issues, MR formulations comprising EC formulations that prevent the API from being released in the stomach and ER formulations that release valproate over an extended period of time, were developed and approved over the past decades.

As observed with other anticonvulsant drugs, the antiepileptic effect of valproate is more related to the plasma concentration over time rather than to the single dose administered. Consequently, when starting an anticonvulsant therapy with valproate, therapeutic drug monitoring is required to determine an appropriate dosing regimen for a successful therapeutical outcome in the individual patient to be treated. Once the patients are titrated to the targeted plasma level with a certain drug product, drug product, dosing schedule and dose should not be altered. Since the plasma concentration profile of poorly soluble but highly permeable drug compounds is strongly determined by the rate and extent of *in vivo* drug release/dissolution, generic substitution of oral valproate formulations presents with the chance of switching to a product with different *in vivo* release performance. This can result in toxic or subtherapeutic valproate plasma levels and thus bears a high risk for the individual patient.

Assessing the risks of generic substitution but also of how individual GI physiology will affect the *in vivo* performance of oral valproate formulations in an *in vitro* setup would be highly beneficial for a safe and effective oral valproate treatment. Ideally, such an *in vitro* setup should properly address the GI parameters relevant to *in vivo* valproate release and dissolution and, as possible, should also allow for simulating intra- and interindividual variabilities of these essential GI parameters.

In order to mimic *in vivo* conditions relevant to intraluminal drug release after oral administration in an *in vitro* setup, the simulation of residence times in the respective GI segments (stomach, duodenum, jejunum, ileum and colon) and the choice of appropriate dissolution media to be applied in the *in vitro* experiments are important facts to consider. As possible, *in vitro* dissolution media should reflect all essential properties of the fluids available at different sites in the GI tract. Thus, besides simply simulating pH changes along the GI tract, the use of physiological buffer systems is essential, as we recently could show for EC-coated aspirin formulations [26]. Whereas gastric fluid does not represent a buffered medium, the physiological buffer system in intestinal fluids is the bicarbonate buffer. Since the thermodynamic stability of this buffer system is limited, in the past the use of bicarbonate-based media in *in vitro* dissolution testing was nearly impossible. However, since the introduction of automated devices that allow for a proper pH-control of bicarbonate-based buffer media over the time of an *in vitro* experiment, it is possible to properly simulate intestinal conditions with regard to both pH and intraluminal contents [27–29].

To predict whether *in vivo* drug release from EC valproate formulations is sensitive towards interindividual variations in intraluminal pH-conditions and GI transit, a suitable *in vitro* model that closely resembles the *in vivo* conditions would be advantageous. Thus, the objective of the present study was to develop individualized *in vitro* dissolution test methods by implementing individual *in vivo* GI pH- and transit profiles into a physiologically relevant *in vitro* test model. *In silico* modelling based on the release profiles obtained in the dissolution experiments should allow for the prediction of individual plasma profiles of three enteric valproate formulations used in the study.

2. Materials and methods

2.1. Materials

The marketed enteric valproate formulations, Leptilan 300 (# 407999, Dolorgiet GmbH & Co. KG, Sankt Augustin/Bonn, Germany), Orfiril 300 (# 14005908, Desitin Arzneimittel GmbH,

Table 1
Composition of the tested EC formulations as described in the Summary of Product Characteristics (SmPCs).

Formulation	Excipients
Leptilan 300 tablets	carboxymethyl starch sodium, copovidone, silicon dioxide, hydroxypropylmethylcellulose, magnesium stearate, macrogol, methacrylic acid: ethyl acrylate copolymer (1:1), microcrystalline cellulose, polysorbate 80, povidone, shellac, talc, titanium dioxide, iron (III) oxide
Orfiril 300 tablets	calcium behenate, microcrystalline cellulose, gelatin, macrogol 6000, methacrylic acid: ethyl acrylate copolymer (1:1), sodium dodecylsulfate, polysorbate 80, silicon dioxide (methylated), talc, triacetin, titanium dioxide
Ergenyl 300 tablets	Povidone (K90), calcium trimetasilicate 5 x H_2O, talc, magnesium stearate (plant-based), methacrylic acid: methyl methacrylate copolymer (1:1), cellacefate, diethyl phthalate, hydroxypropylcellulose, titanium dioxide

Hamburg, Germany) and Ergenyl 300 (# J602, Sanofi-Aventis, Frankfurt/Main, Germany) were obtained from the local hospital pharmacy. The composition of these formulations is given in Table 1. Sodium valproate reference material was purchased from Sigma-Aldrich (# MKBS5723V, Steinheim, Germany). All other chemicals used in the study were of analytical grade and purchased commercially.

2.2. Methods

2.2.1. Drug release experiments simulating an "average" patient

The first set of experiments was performed in USP apparatus III, the reciprocating cylinder apparatus (RRT 10, Erweka, Heusenstamm, Germany), simulating fasted GI pH-conditions and transit times in an average adult as reported previously [30–32]. Based on the recommendations given in the Patient Information Leaflets (PILs) of the three EC formulations, they are to be administered in the fasted state. Consequently, a test design simulating fasted GI conditions was applied. Simulated Gastric Fluid without pepsin (SGF*sp*), a set of Blank Fasted State Simulated Intestinal Fluids (Blank FaSSIFs), i.e. FaSSIFs without bile components, and Simulated Colonic Fluid (SCoF) were used to simulate the changing pH-conditions during a passage through the fasted stomach, small intestine and proximal colon (Table 2). Since preliminary tests of the valproate formulations revealed that drug release of the EC tablets was not affected by the presence of bile components, we decided to use blank media for all dissolution experiments. In order to get an insight in how variations in GI transit times affect the drug release from the three tablet formulations, two test scenarios, one simulating a continuous passage of the tablets through the small intestine, the other simulating a discontinuous passage comprising a rapid passage of the dosage form through proximal parts of the small intestine and a prolonged residence in the distal ileum were applied (Table 2). In all experiments, the media volume per vessel was 200 mL and the temperature was set to 37 ± 0.5 °C. The reciprocating cylinders were equipped with 420 μm mesh screens on both top and bottom and the agitation speed was set at 10 dpm throughout each experiment. Samples were removed at predetermined time points via a sample cannula equipped with a 10 μm poroplast filter (Erweka). Following filtration through a 0.45 μm cellulose acetate syringe filter (Puradisc 30 FP 30/0.45 CA-S, Whatman/GE Healthcare Life Sciences, Buckinghamshire, UK) all samples were analyzed by HPLC-UV.

In the second part of the study a set of experiments with corresponding pH gradients and residence times was performed in USP apparatus II, the paddle apparatus (DT 626/DT 7R, Erweka). For this purpose, the EC formulations were initially exposed to SGF*sp* pH 1.8 to simulate gastric residence, before they were transferred into another vessel containing Blank FaSSIF pH 6.5. In order to simulate an intestinal passage, in accordance with the tests performed in USP apparatus III, at predetermined time points a small defined volume of 2 N sodium hydroxide solution (750–1500 μL) was added to the medium to stepwise raise the pH from 6.5 to 7.5 according to the test designs shown in Table 2. After a test duration of 240 min in both test scenarios 10 N acetic acid (1125 μL) was added to the medium simulating conditions in the terminal ileum. This decreased the pH to 5.8 and simulated entry into the proximal colon. In all experiments, the volume of sodium hydroxide- and acetic acid solution added to the test medium did not exceed 1% of the total media volume.

In the next part of the study, the release medium used for simulating intestinal conditions was switched from phosphate-based Blank FaSSIF to a recently introduced bicarbonate-based buffer system simulating the fasted state, i.e. Blank Carbonate-based Fasted State Simulated Intestinal Fluid (Blank CarbFaSSIF) [33], which is composed of 120 mM sodium chloride, 5 mM potassium chloride and 15 mM sodium bicarbonate. An automated and programmable pH-controller (pHysiograd®, Physiolution GmbH, Greifswald, Germany) was used to control and change the pH of the dissolution medium in pre-programmed intervals [27] to adjust the pH-conditions and residence times applied in the experiments with USP apparatus III.

2.2.2. Individualized dissolution tests

The focus of the last series of experiments was to simulate individual GI pH-profiles for estimating the variability of *in vivo* drug release of the EC valproate formulations in different subjects. Fasted individual GI pH-profiles were obtained from an *in vivo* study published by Koziolek et al. [34]. In the cited study, the Intellicap® system was used to record pH and temperature profiles in the GI tract of 20 healthy subjects after an overnight fast and the intake of 200 mL of water [34]. For the simulation to be applied in our *in vitro* dissolution experiments GI pH-profiles of four subjects were selected. The pH-profile of subject 1 (ID 1–2) was characterized by a long duration of high (distal) small intestinal (SI) pH-conditions, that of subject 2 (ID 1–3) by a long duration of low (proximal) SI pH-conditions. Subject 3 (ID 1–9) had a pH-profile that was similar to the mean pH-profile calculated from the individual pH-profiles obtained in the *in vivo* study and the GI transit profile of subject 4 (ID 1–10) was characterized by a rapid transit through stomach and small intestine and a short colon arrival time (CAT). The mean gastric pH, gastric emptying time (GET), small intestinal transit time (SITT) and CAT measured in the four simulated subjects are depicted in Table 3. For the *in vitro* simulation of the individual pH-profiles, a multiple-stage dissolution model in USP apparatus II was applied. In the first vessel, gastric residence was simulated in SGF*sp* at a static pH that was modified to match the mean fasted gastric pH of the respective subject. Simulated GETs were adapted to the individual *in vivo* GETs obtained in the Intellicap® study (Table 3)[35].

At the time point of gastric emptying, the dosage forms were transferred into a second dissolution vessel containing Blank CarbFaSSIF to simulate intestinal conditions. For the *in vitro* simulation of the small and large intestinal transit, the detailed pH-profiles obtained from the *in vivo* study, in which pH was recorded with the telemetric capsule at a frequency of 0.1 Hz, were subdivided in intervals of 5 min resulting in an *in vitro* pH-profile that properly matched with the *in vivo* profiles (Fig. 1). The pH of Blank CarbFaSSIF was altered according to this pre-programmed *in vitro* profile using the pHysiograd® device (Physiolution).

For all experiments in USP apparatus II the media volume for both

Table 2
Test conditions applied to simulate a fasted GI passage in USP apparatus III.

GI section	pH	Medium	Residence time	
			Continuous passage	Discontinuous passage
Stomach	1.8	SGF*sp* 1.8	60 min	60 min
Proximal Jejunum	6.5	Blank FaSSIF pH 6.5	45 min	15 min
Distal Jejunum	6.8	Blank FaSSIF pH 6.8	45 min	15 min
Proximal Ileum	7.2	Blank FaSSIF pH 7.2	45 min	30 min
Distal Ileum	7.5	Blank FaSSIF pH 7.5	45 min	120 min
Proximal Colon	5.8	SCoF pH 5.8	240 min	240 min

Table 3
Individual gastric pH, gastric emptying time (GET), small intestinal transit time (SITT) and colon arrival time (CAT) in the four selected subjects.

Subject No.	Subject ID [34]	Characteristics	Gastric pH	GET (min)	SITT (min)	CAT (min)
1	1–2	long duration of high (distal) small intestinal pH-conditions	1.8	129	314	443
2	1–3	long duration of low (proximal) small intestinal pH-conditions	2.0	39	347	386
3	1–9	mean pH-profile	2.0	8	240	248
4	1–10	short GET and SITT	1.9	7	67	74

gastric and intestinal conditions was 500 mL. The media temperature was set to 37 ± 0.5 °C and the paddle speed was 75 rpm. Samples were taken at predetermined time points via a sample cannula equipped with a 10 µm poroplast filter (Erweka) and after additional filtration through a 0.45 µm cellulose acetate syringe filter (Puradisc 30 FP 30/0.45 CA-S, Whatman/GE Healthcare Life Sciences) analyzed by HPLC-UV.

All experiments were run in triplicate and results were expressed as mean percentage of the dose released ± standard deviation (S.D.) at each sampling point.

2.2.3. Sample analysis

The samples obtained from all drug release studies were analysed on a Waters HPLC System consisting of an 1525 binary pump, a 2707 autosampler and a 2998 photodiode array (PDA) detector (Waters, Milford, MA, USA) using a previously described method [33]. Briefly, the stationary phase consisted of a LiChrospher® 100 RP−18 (4 × 125 mm, 5 µm) column equipped with a LiChrospher® 100 RP−18 (4 × 4 mm, 5 µm) guard column (both Merck KGaA, Darmstadt, Germany). The flow rate of the mobile phase (50 mM phosphate buffer: acetonitrile, 80:20) was 1 mL/min and the column temperature was set to 50 °C. Valproate was detected at a wavelength of 200 nm. The amount of valproate released in the different simulated GI sections was calculated using standard curves ($R^2 > 0.999$) prepared from sodium valproate reference material in the respective media.

2.2.4. Determination of tablet dimensions and coating thickness

To get a better understanding of the *in vitro* performance of the three EC valproate formulations, tablet dimensions and coating thickness were screened with a caliper or by scanning electron microscopy (SEM), respectively. For the determination of the coating thickness of the investigated formulations, cross sections of three individual tablets of each product were prepared vertically to the band. The tablet sections were then sputter coated with gold/palladium using a Mini Sputtercoater (SC7620, Quorum Technologies, West Sussex, UK) and images of the tablet coating were taken at 8 predetermined spots covering tablet bands, faces and edges using a table top SEM (Phenom, FEI Company, Hillsboro, USA). The coating thickness at each spot was determined via an integrated software. As a result of the coating process of the biconvex tablets, the tablet edges are likely to present the tablet segment with the highest variability in coating thickness or the smallest coating thickness, respectively. For EC formulations, beside the polymer type, coating thickness is an essential parameter for drug release [36] and can have a substantial impact on the time to onset of *in vivo* drug release and absorption. To get a better estimate of the coating thickness at these critical spots, the number of measurements at the tablet edges was higher (n = 4 per tablet) than that for tablet faces and bands (n = 2 per spot and tablet) (supplement – Fig. 1). Average coating thickness at the tablet edges, faces and bands as well as maximum and minimum coating thickness were documented. Results from the coating thickness determinations at the tablet edges were tested for significant differences between the tested EC valproate formulations with an one-way-ANOVA followed by Tukey's post-hoc test using the GraphPad Prism software (version 5.02, GraphPad Software Inc., La Jolla, CA, USA). Results were regarded as significantly different when $P < 0.05$.

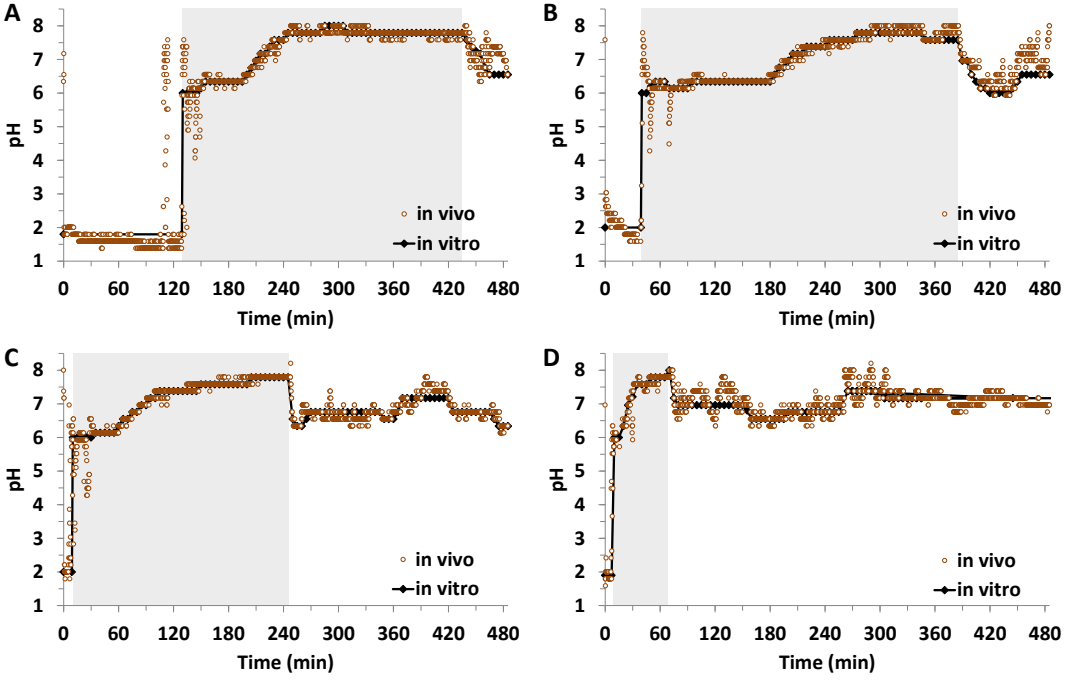

Fig. 1. Gastrointestinal pH-profiles recorded in the four selected subjects and their translation into the corresponding *in vitro* profiles (adapted from [34;35]): long duration of high (distal) small intestinal pH (A), long duration of low (proximal) small intestinal pH-conditions (B), mean pH-profile (C) and short gastric emptying time (GET) and colon arrival time (CAT) (D), shaded areas indicate the small intestinal transit.

Table 4
Individual valproate PK parameters of the 7 healthy subjects that were reported in [37] and used for the *in silico* simulations.

Subject #	Body weight (kg)	Volume of distribution ($L \cdot kg^{-1}$)	Clearance ($mL \cdot h^{-1} \cdot kg^{-1}$)
A	74	0.22	12.64
B	86	0.10	6.95
C	65	0.15	8.13
D	58	0.16	7.64
E	73	0.10	11.45
F	62	0.18	8.54
G	79.5	0.12	10.08

2.2.5. In vivo pharmacokinetics of the test formulations and in silico simulations

In order to link results of the *in vitro* release experiments with individual *in vivo* plasma profiles, the literature was screened for individual plasma profiles obtained after fasted administration of the three EC valproate formulations. Ideally, literature reports should also contain individual valproate pharmacokinetic (PK) parameters of the subjects enrolled in the fasted *in vivo* dosing experiments. Individual plasma profiles obtained from fasted administration were available for all formulations [37–39]. However, whereas for Leptilan the study report contained detailed information on gender, age, body weight and height, valproate clearance and volume of distribution of each of the 7 healthy subjects (subject A-G) enrolled in the study [37], in the study reports of Orfiril [38] and Ergenyl [39] individual PK parameters were not given. Hence, we decided to use the individual PK parameters from the Leptilan study as input data for the *in silico* simulation experiments of individual plasma profiles of all EC valproate tablets. The individual PK parameters reported for the 7 subjects (A-G) that were used for the *in silico*simulations are given in Table 4.

Since it might be a major determinant for the time to onset of *in vivo*drug release, individual GET is another fact to consider when simulating individual plasma profiles. To address this variation, six different GETs (15, 30, 60, 90, 120, 240 min) were used as input for each individual subject (A–G). Based on the physicochemical properties (Table 5) and the simulated intestinal *in vitro* valproate release profiles in physiological buffers, theoretical individual plasma profiles were calculated using the BDexpert software (JMC, Tokyo, Japan), a recently introduced software based on PK models developed by Sugano [40]. The software allows to predict individual plasma profiles by taking into account the drug properties, drug release characteristics and individual population data, e.g. bodyweight, drug clearance and drug volume of distribution. A more detailed description of the PK software and the parameters that were applied for the simulations are presented as supplementary material (supplement – Table 1). The resulting *in silico* plasma profiles were then compared with the corresponding *in vivo* plasma profiles reported in the literature [37–39]. In order to reflect the maximum *in vivo* variability in valproate plasma concentration, observed in each of the three *in vivo* PK studies, three individual valproate plasma profiles (*in vivo* 1–3) with marked differences in the maximum plasma concentration (c_{max}) and the time point of c_{max} (t_{max}) were selected for comparison with the *in silico* plasma profiles.

Table 5
Physicochemical properties of valproate that were used for the *in silico* simulations.

Physicochemical properties	Value	Reference
Molecular weight (g)	144.21	–
Water solubility ($mg \cdot mL^{-1}$)	1.3	[41]
pK_a	4.8	FDA label
logP	2.75	drugbank.ca
D_{mono} ($10^{-6} \cdot cm^2 \cdot s^{-1}$)	10.413	calculated from MW [40]

As a surrogate for the similarity of the individual *in silico* plasma profiles and the selected *in vivo* plasma profiles reported in the literature, the Euclidean distance of each *in vivo* plasma concentration point ($c_{in\ vivo}$) and its *in silico* counterpart ($c_{in\ silico}$) at a given time point was calculated. For the comparison of entire plasma profiles, the absolute values of the Euclidean distances of each $c_{in\ vivo}$: $c_{in\ silico}$-data pair were first summed up and then divided by the number of data pairs used for the comparison to obtain a number representing the mean Euclidean distance (MED) (Eq. (1)).

$$MED = \frac{1}{n} \sum_{t=1}^{n} |c_{t,invivo} - c_{t,insilico}| \quad (1)$$

From Eq. (1) it follows that if the MED is 0, *in silico* and *in vivo* plasma profiles are identical whereas with increasing MED they become less similar. Consequently, a low MED was regarded as an indicator for a high similarity of *in vivo* and *in silico* valproate plasma profiles. The *in silico* plasma profile obtained by combining individual PK parameters, GET and *in vitro* drug release characteristics resulting in the lowest MED was regarded as best match with the respective *in vivo* profile. In order to evaluate the predictivity of the *in silico* profiles, AUC and c_{max} of the *in silico* and *in vivo* valproate plasma profiles with the best match (lowest MED) were compared according to the recommendations given in the FDA guidance on *in vitro/in vivo* correlations (IVIVC) for ER oral dosage forms [42]. In this guidance, the method for evaluating an *in vitro/in silico* model with regard to predicting *in vivo* plasma profiles and obtaining a Level A IVIVC for NTI drugs permits an average absolute prediction error of 10%. In the present study, this criterion was also adapted for the EC valproate formulations. However, it should be noted that individual and not average plasma profiles were compared. An individual *in silico* plasma profile was considered as predictive, when the prediction error (PE) of AUC and c_{max} in comparison to the individual *in vivo* profile was $\leq \pm$ 10%.

3. Results

3.1. Drug release in simulated average adults (USP III experiments)

Fig. 2 displays the drug release profiles obtained in the experiments simulating a GI passage with a continuous (A) or a discontinuous (B) SI passage in an average healthy adult in USP apparatus III. All products showed enteric properties. The shape of the release profiles of the three EC tablet formulations was similar in both test scenarios. However, the profiles differed in the lag time before onset of drug release. In conditions of a simulated discontinuous SI passage, all lag times were shorter than those obtained when simulating a continuous SI passage. Independent of the test scenario, the lag time observed for Leptilan was shorter than those for Orfiril and Ergenyl.

3.2. Drug release in simulated average adults (USP II experiments)

Results from the paddle experiments in which media and simulated GI passage times corresponded to those in the USP III experiments were different from those obtained in USP apparatus III (Fig. 3A, B). The release profiles obtained in the USP III setup were almost superimposable, but those obtained in the paddle experiments could be clearly distinguished from each other. Whereas in the continuous test setup the onset of valproate release from the Ergenyl tablet was almost unchanged, the time to onset of drug release from the Orfiril and Leptilan was shorter in the paddle experiments. In contrast, in the discontinuous setup, the time to onset of drug release from Orfiril and Leptilan was similar to that in the USP III experiments, whereas the onset of valproate release from the Ergenyl tablet was slightly delayed. Overall and independent of the mode of simulated SI passage, all SI

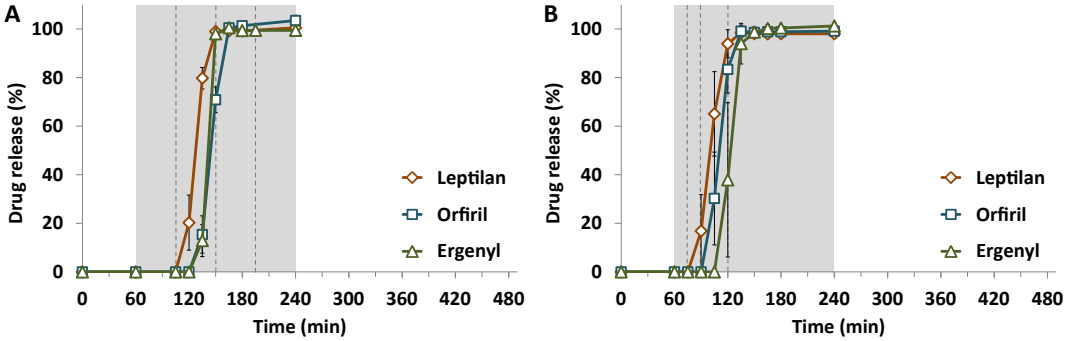

Fig. 2. Drug release from the EC valproate formulations in USP apparatus III simulating a continuous (A) and a discontinuous (B) SI passage, shaded areas indicate the simulated SI transit and dashed lines a pH change during SI transit, mean of n = 3 ± S.D.

release profiles in the paddle setup were not as steep as those obtained in the USP III experiments. The release profiles clearly indicate that the mode of agitation and the agitation speed had an impact on dissolution of the three EC valproate formulations. This effect was most pronounced for the Ergenyl tablet.

Results of the experiments where Blank CarbFaSSIF was used to simulate the SI milieu are shown in Fig. 3C and D. Again, there was only a minor impact of the mode (continuous or discontinuous) of the simulated SI passage on drug release from the Orfiril and Leptilan formulation. The lag times before the onsets of drug release from these formulations were similar to those observed in the experiments where Blank FaSSIF was used to simulate SI pH-conditions. Drug release from Ergenyl was, however, strongly affected by the buffer species. Thus, in the experiments with Blank CarbFaSSIF the lag time before onset of drug release was almost double as long as in the corresponding experiments with Blank FaSSIF. Interestingly, the overall release profile of Ergenyl in the Blank CarbFaSSIF-based setup was not affected by the mode of the simulated SI passage, since in both conditions, drug release initiated immediately after simulated colon arrival.

3.3. Drug release in simulated individual subjects

In a final set of experiments, drug release from the EC valproate formulations was screened in a test setup simulating individual GI pH-profiles. Results of these experiments are shown in Fig. 4A–D.

As already observed in the previous experiments, the release profiles of Orfiril and Leptilan were similar with a slightly faster onset of drug release of Leptilan in all individuals simulated. Overall, the simulation of individual pH-profiles had a minor impact on Orfiril drug release. Independent of individual GET and pH-profile simulated, drug release from these tablets initiated within 30–60 min after simulated gastric emptying and after dissolution of the enteric coating, was complete within 60 min.

The release performance of Ergenyl differed significantly from that of the other formulations and was strongly affected by the test conditions, i.e. the individual GI pH-profile applied. As expected and observed for all EC valproate formulations tested, no drug was released in simulated gastric conditions, independent of whether a short GET of 7–8 min or a long GET of 129 min was simulated. Overall *in vitro* valproate release from Ergenyl was affected both by simulated

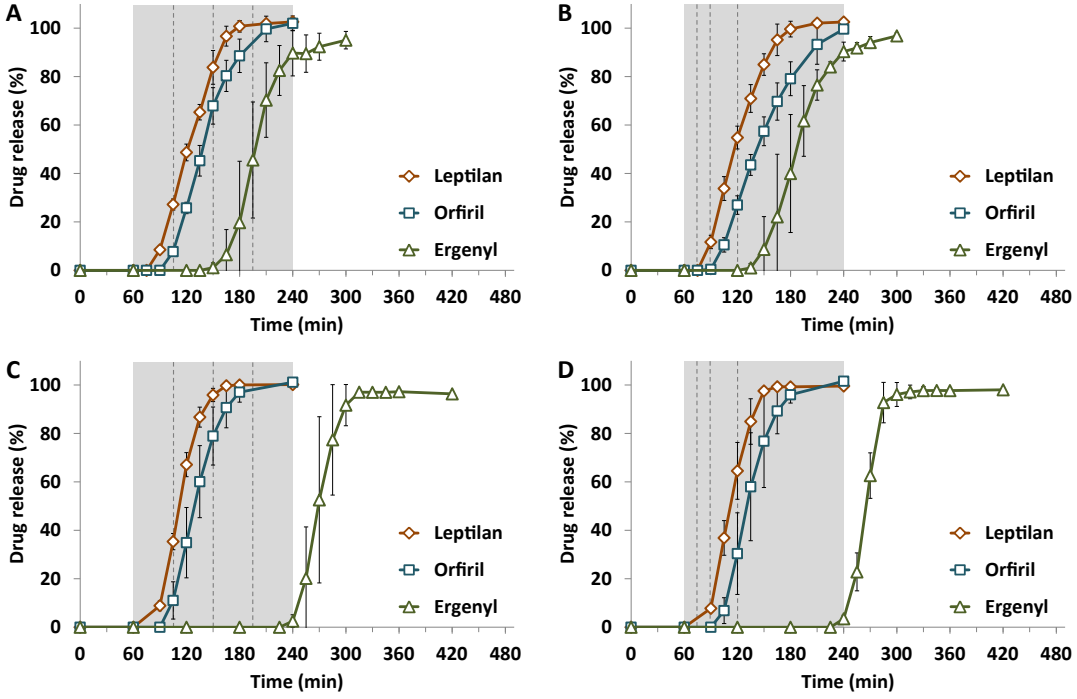

Fig. 3. Drug release from the EC valproate formulations in USP apparatus II simulating a continuous (A) and a discontinuous (B) SI passage in Blank FaSSIF and a continuous (C) and discontinuous (D) SI passage in Blank CarbFaSSIF, shaded areas indicate the simulated SI transit and dashed lines a pH change during SI transit, mean of n = 3 ± S.D.

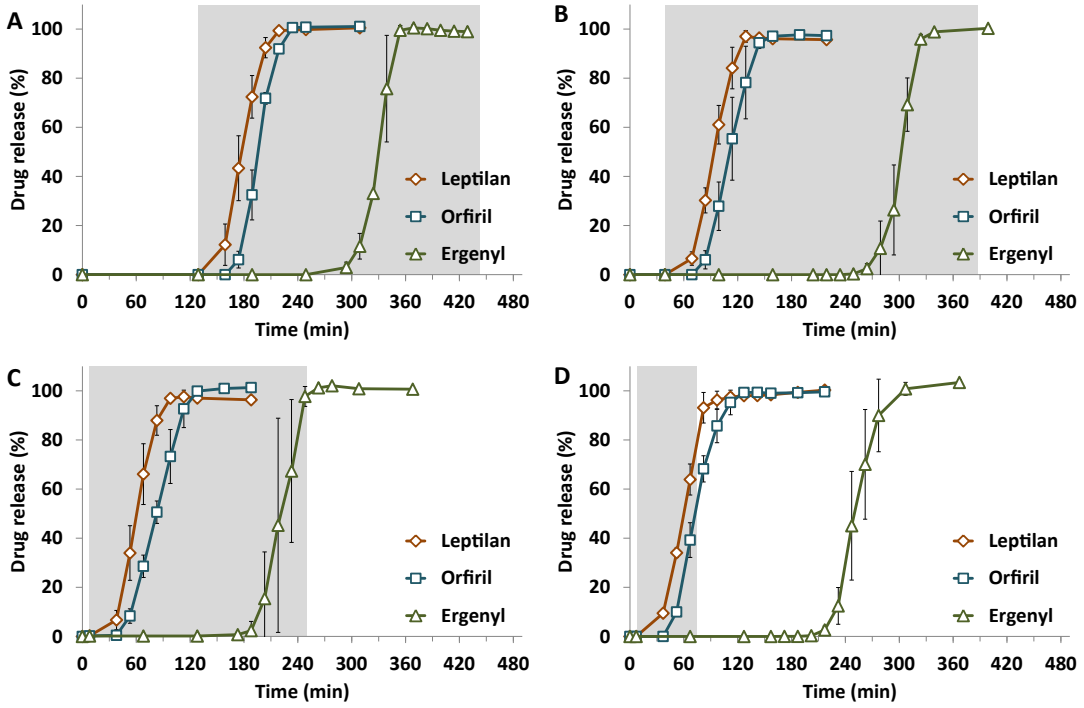

Fig. 4. Drug release from the EC valproate formulations in USP apparatus II simulating subject 1 (A), subject 2 (B), subject 3 (C) and subject 4 (D) with Blank CarbFaSSIF as release medium simulating intestinal conditions shaded areas indicate the simulated small intestinal transit, mean of n = 3 ± S.D.

individual SI transit time and SI pH-conditions. When simulating the subject with a long duration of high SI pH (subject 1) drug release initiated after a SI residence of 120 min. When simulating the subject with a long duration of low SI pH, the onset of drug release was, however, only observed after ~210 min residence in the SI. In the experiments where GI passages with similar GETs were simulated, i.e. subjects 3 and 4, both lag time before valproate release and site of release were affected by simulated SI conditions. When simulating subject 3, representing the individuum with the mean pH and transit profile of the *in vivo* study, drug release initiated after about 165 min simulated residence in the SI and was just completed before simulated ileocecal passage. When simulating subject 4, an individuum with a very short SITT, no drug was released during simulated SI residence and valproate release initiated after an additional 120 min simulated colonic residence time. However, independent of the duration of the lag time, similar to the observations made for Leptilan and Orfiril, once the coating had dissolved, drug release from Ergenyl was fast and complete within 60 min.

3.4. Tablet dimensions and coating thickness

Results obtained for tablet dimensions and coating thickness are given in Table 6. Diameter and height were similar for all EC valproate tablets. As expected, the coating layer was thinnest at the tablet edges in all formulations. Moreover, the average coating thickness at the tablet edges differed significantly for all EC valproate formulations ($P < 0.05$). Leptilan and Orfiril presented with a coating monolayer and the Leptilan coating was significantly thinner than that of Orfiril. Ergenyl, in contrast, had a bilayer coating, which at the same time was the thickest coating of all formulations.

3.5. Plasma profile simulations in individual subjects

Results of the drug release experiments with the EC formulations simulating average adults and individual subjects provided similar drug release profiles in simulated SI conditions independent of the individual pH-profile applied. It could also be noted that particularly for the Leptilan and the Orfiril formulation, the lag time before onset of drug release was mainly determined by the duration of the simulated gastric residence. Thus, instead of using the *in vitro* release profiles for the simulated individuals we decided to calculate individual *in silico* valproate plasma profiles for 7 healthy subjects with different age, body weight, valproate volume of distribution and clearance based on *in vitro* data from experiments simulating a continuous SI passage with Blank CarbFaSSIF in an average healthy adult but varying GET (15–240 min). This approach was supported by preliminary experiments indicating that the duration of simulated gastric conditions does not affect the drug release mechanism in the small intestine (supplement – Fig. 2). The resulting *in silico* plasma profiles were then compared with *in vivo* plasma profiles reported for the respective EC formulations using MEDs as surrogate for the similarity of the two data sets and the predictivity of the model. Fig. 5A displays the MEDs calculated for the Leptilan

Table 6
Dimensions (diameter and height, means of n = 3 ± S.D.) and coating thicknesses (means ± S.D. (*range*)) of the tested EC valproate formulations.

Formulation	Diameter (mm)	Height (mm)	Coating thickness (μm)		
			Tablet faces (n = 6)	Tablet bands (n = 6)	Tablet edges (n = 12)
Leptilan 300	12.43 ± 0.03	5.93 ± 0.04	146 ± 7 (*132–153*)	114 ± 14 (*98–135*)	113 ± 16 (*91–138*)
Orfiril 300	10.46 ± 0.04	6.45 ± 0.08	151 ± 11 (*134–161*)	136 ± 18 (*112–166*)	136 ± 7 (*126–145*)
Ergenyl 300	11.04 ± 0.03	5.01 ± 0.04	211 ± 10 (*200–225*)	244 ± 16 (*215–259*)	203 ± 18 (*181–241*)

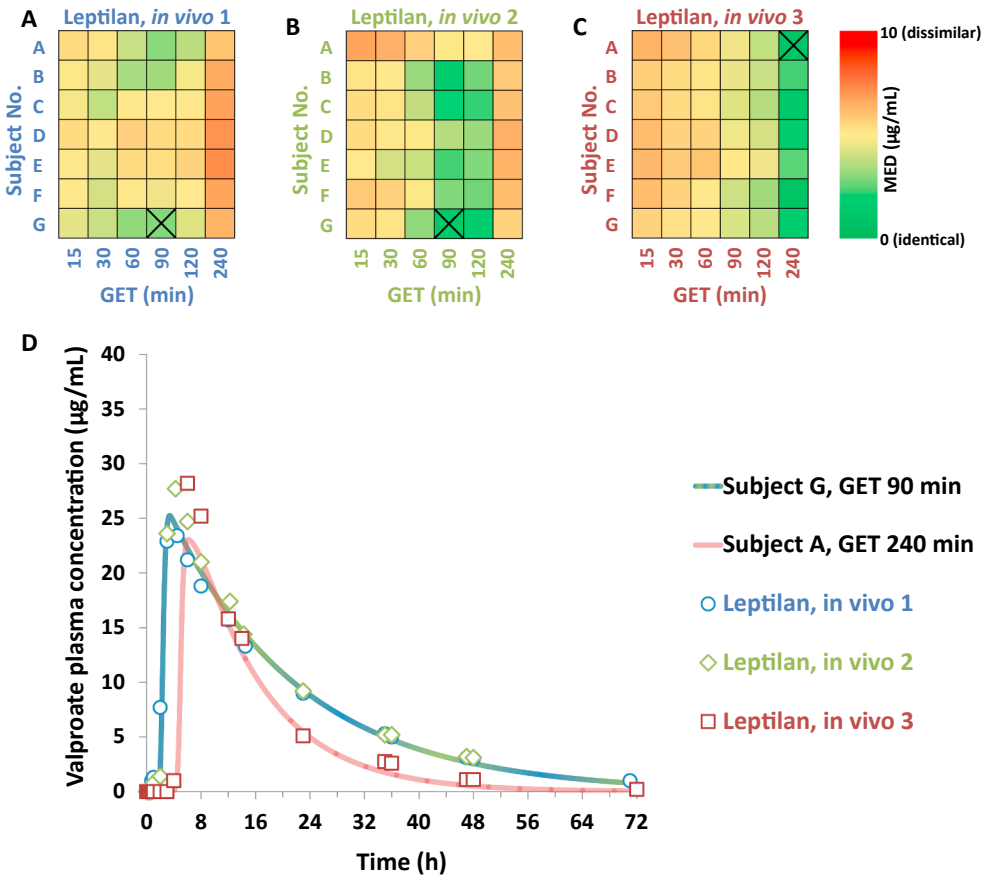

Fig. 5. Calculated Mean Euclidean distances (MED) for the comparison of the *in silico* valproate plasma profiles combining *in vitro* release profiles of Leptilan from experiments simulating a GI passage with continuous SI passage in an average healthy adult with individual PK parameters from subjects A-G and assuming different gastric emptying times (GET). The selected *in vivo* data sets were: Leptilan *in vivo* profile 1 (A), Leptilan *in vivo* profile 2 (B) and Leptilan *in vivo* profile 3 (C). Best match results of the *in silico* valproate plasma profiles are indicated with a cross (A-C) and plotted versus the corresponding *in vivo* plasma profiles (D).

formulation when comparing an individual *in vivo* plasma profile from the literature (*in vivo* 1) with each of the *in silico* plasma profiles calculated for the 7 healthy subjects (A-G) assuming different GETs. For Leptilan, the lowest MED, i.e. the best match, was obtained when using valproate PK parameters of subject G and assuming a GET of 90 min. Likewise, also a second plasma profile (*in vivo* 2), which was similar to the *in vivo* 1 profile could be matched with the *in silico* data obtained with the PK data set of subject G combined with a GET of 90 min (Fig. 5B). *In silico* data fit best with the plasma profile of a third individual (*in vivo* 3) characterized by a longer lag time before onset of drug release when using the PK data set of subject A and assuming a GET of 240 min (Fig. 5C). The three Leptilan *in vivo* plasma profiles (*in vivo* 1–3) taken from the literature and their respective best match *in silico* counterparts are shown in Fig. 5 D.

In silico data sets for Orfiril and Ergenyl were obtained as described for Leptilan and then processed in the same manner. The corresponding results are shown in Figs. 6A–D and 7A–D. In contrast to the Leptilan and the Orfiril formulation, *in vitro* drug release profiles of the Ergenyl formulation were characterized by a pronounced lag time and the onset of valproate release could only be observed when switching from SI to colonic conditions. Since the lag times observed in the *in vitro* experiments were not reflective for the selected *in vivo* valproate plasma profiles of Ergenyl, the *in silico* valproate plasma profiles resulted in poor predictions, reflected by higher MEDs when comparing the *in silico* and *in vivo* Ergenyl plasma profiles (Fig. 7A–C).

Table 7 displays the PEs for AUC and c_{max} obtained when comparing the *in silico* profiles shown in Figs. 5–7 with *in vivo* plasma profiles (*in vivo* 1–3). For Orfiril both AUC and c_{max} PE was ≤ ± 10% for all selected *in vivo* plasma profiles. For Leptilan two of three *in vivo* plasma profiles could be predicted with a PE ≤ ± 10%. Expectedly and for the aforementioned reasons, for Ergenyl the PE for AUC and c_{max} was > 20% for all of the three profiles.

4. Discussion

The objective of the present study was to estimate the *in vivo* performance of three EC valproate formulations by applying physiologically relevant (individualized) *in vitro* dissolution test methods. For this purpose a number of different dissolution setups simulating GI conditions in an average adult or individual subjects was applied and *in vitro* release profiles were used as input for *in silico* simulations and subsequent comparison of *in silico* and *in vivo* data.

4.1. Drug release under conditions simulating an average adult

With the drug release experiments in GI conditions simulating an average healthy adult, two key factors influencing the drug release characteristics of the tested EC formulations could be identified: These were the means of media agitation, i.e. whether drug release experiments were performed in USP apparatus II or III, and the buffer system that was used to simulate intestinal conditions, i.e. phosphate- or bicarbonate buffer, respectively. The tested formulations were sensitive to these parameters to a different extent. Onset and rate of valproate release from Leptilan and Orfiril were similar under all experimental setups applied, indicating a robust release behavior of these formulations. In contrast, the time to onset of valproate release from Ergenyl was strongly affected by the experimental conditions. Since all three EC tablet formulations have similar dimensions (Table 6), the surface area available for the dissolution of the enteric polymer will also be similar. Consequently, drug release behavior should primarily be attributed to the composition of the coating and/or the coating thickness. The three tested EC valproate tablets differ in the type of polymers that are used to ensure enteric properties. Leptilan and Orfiril bear a coating of methacrylic acid: ethyl acrylate copolymer (1:1) (PMAA:PEA), which, based on the excipients provided in the SmPCs has most probably been

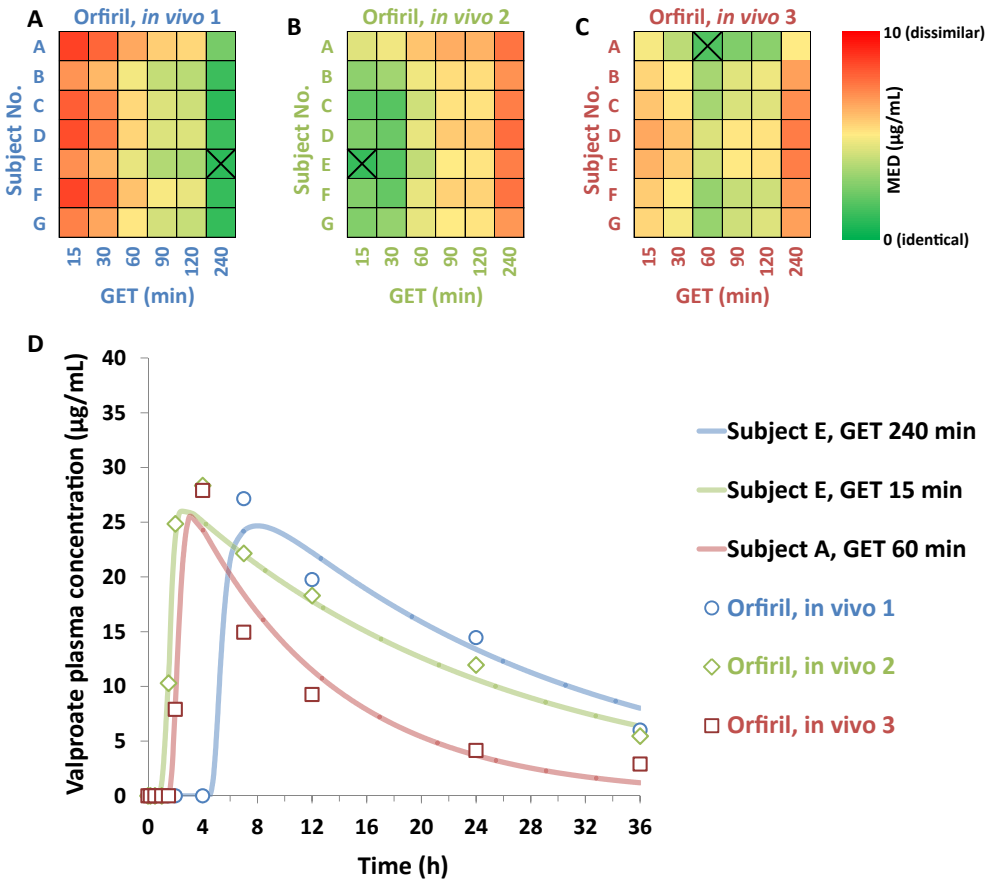

Fig. 6. Calculated Mean Euclidean distances (MED) for the comparison of the *in silico* valproate plasma profiles combining *in vitro* release profiles of Orfiril from experiments simulating a GI passage with continuous SI passage in an average healthy adult with individual PK parameters from subjects A-G and assuming different gastric emptying times (GET). The selected *in vivo* data sets were: Orfiril *in vivo* profile 1 (A), Orfiril *in vivo* profile 2 (B) and Orfiril *in vivo* profile 3 (C). Best match results of the *in silico* valproate plasma profiles are indicated with a cross (A-C) and plotted versus the corresponding *in vivo* plasma profiles (D).

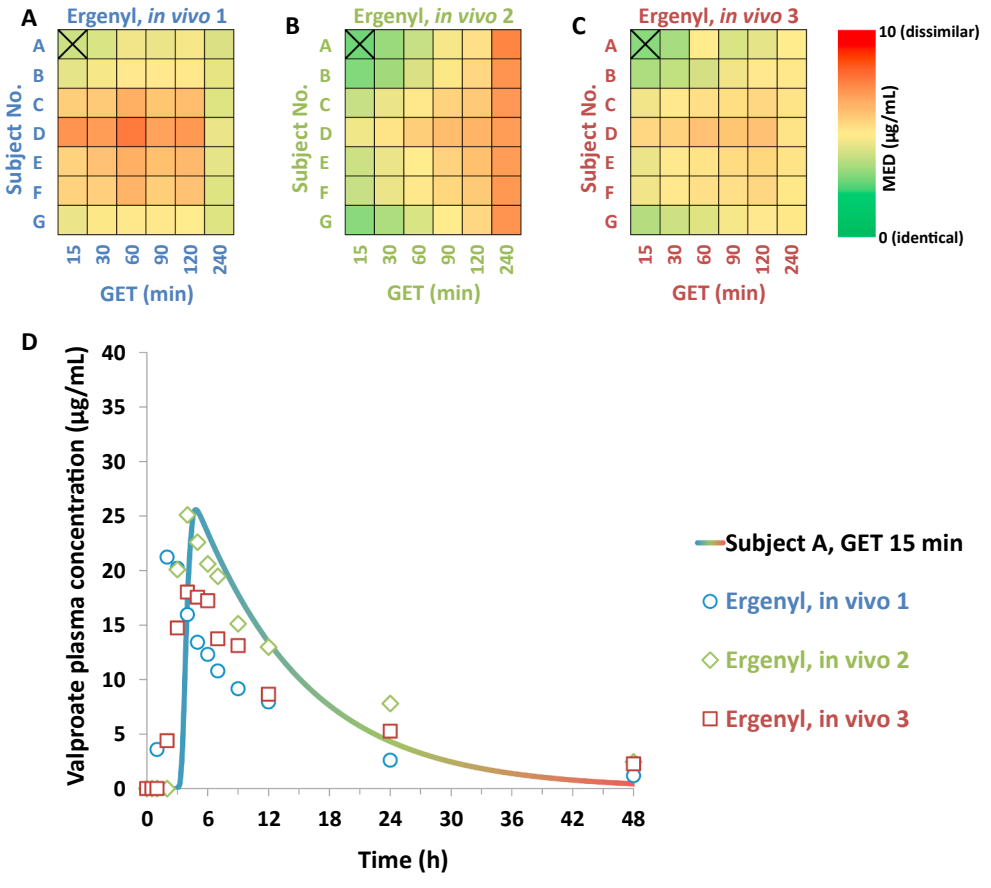

Fig. 7. Calculated Mean Euclidean distances (MED) for the comparison of the *in silico* valproate plasma profiles combining *in vitro* release profiles of Ergenyl from experiments simulating a GI passage with continuous SI passage in an average healthy adult with individual PK parameters from subjects A-G and assuming different gastric emptying times (GET). The selected *in vivo* data sets were: Ergenyl *in vivo* profile 1 (A), Ergenyl *in vivo* profile 2 (B) and Ergenyl *in vivo* profile 3 (C). Best match results of the *in silico* valproate plasma profiles are indicated with a cross (A-C) and plotted versus the corresponding *in vivo* plasma profiles (D).

Table 7
Prediction errors (PE) for AUC and c_{max} obtained when comparing *in vivo* profiles 1–3 (*in vivo* 1–3) with the selected *in silico* plasma profiles shown in Figs. 5–7.

Plasma profile		AUC			c_{max}		
		in vivo ($\mu g \cdot h \cdot mL^{-1}$)	*in silico* ($\mu g \cdot h \cdot mL^{-1}$)	PE (%)	*in vivo* ($\mu g \cdot mL^{-1}$)	*in silico* ($\mu g \cdot mL^{-1}$)	PE (%)
Leptilan	*in vivo* 1	512.96	505.00	+1.55	23.40	25.24	−7.86
	in vivo 2	494.91	469.60	+5.11	27.70	25.24	+8.88
	in vivo 3	367.68	303.50	+17.45	28.20	23.04	+18.30
Orfiril	*in vivo* 1	485.88	493.80	−1.63	27.15	24.67	+9.14
	in vivo 2	527.34	498.50	+5.47	28.35	26.00	+8.29
	in vivo 3	285.25	292.10	−2.40	27.90	25.56	+8.37
Ergenyl	*in vivo* 1	245.64	299.50	−21.93	21.22	25.59	−20.57
	in vivo 2	422.58	299.50	+29.13	25.09	25.59	−1.97
	in vivo 3	312.27	299.50	−4.09	18.02	25.59	−41.99

applied as aqueous dispersion and will dissolve at a pH ≥5.5. The Ergenyl formulation is coated with methacrylic acid: methyl methacrylate copolymer (1:1) (PMAA:PMMA) and cellulose acetate phthalate (CAP), both are reported to dissolve at a pH above 6.0 when applied as an organic solution, which was likely the case for the Ergenyl formulation. During SEM imaging two coating layers were identified suggesting one layer for each enteric polymer.

In previous studies the sensitivity of drug release from polymethacrylate-coated formulations towards the composition of the dissolution medium has been shown for both EC [26,43–45] and ER formulations [46–50]. In the experimental setup of the present study, two different buffer systems, i.e. Blank FaSSIF, a phosphate-based buffer, and Blank CarbFaSSIF, a bicarbonate-based buffer, were applied to simulate intestinal conditions. For the Ergenyl formulation, the type of buffer system used for maintaining a predetermined pH in the dissolution medium had a remarkable impact on the drug release properties. Release experiments with Ergenyl in Blank CarbFaSSIF resulted in significantly longer lag times before the onset of drug release than those in Blank FaSSIF. These observations were in good agreement with the results of a study reported by Liu et al. [51], who compared drug release from prednisolone tablets bearing different enteric coatings, e.g. PMAA:PEA applied as an aqueous dispersion (Eudragit L 30D-55) or PMAA:PMMA (Eudragit L 100) and CAP applied as organic solutions, at the same coating level in phosphate- and bicarbonate-based buffers. While the release profiles of all formulations in compendial 0.05 M phosphate buffer pH 6.8 were similar for all coating polymers tested, experiments in *m*Hanks buffer pH 6.8, a bicarbonate-based dissolution medium, resulted in more discriminative results. As observed in the present study, when compared to the PMAA:PEA-coated formulations the lag times before onset of drug release under SI conditions were significantly prolonged for the PMAA:PMMA- and the CAP-coated formulations [51]. Liu et al. attributed these differences in polymer dissolution to the polymer pK_a. Consequently, the pH required for polymer dissolution was regarded as the key factor for the accelerated dissolution of PMAA:PEA compared to PMAA:PMMA [51,52].

When comparing the release characteristics of the PMAA:PEA-coated valproate formulations, in all experimental setups drug release of the Leptilan formulation initiated somewhat faster than that of the Orfiril formulation. Since the two formulations are both coated with the same enteric polymer, the observed differences seem to primarily relate to the coating thickness. Observations during the *in vitro* drug release studies indicated, that onset of drug release is related to the formation of cracks at the tablet edges, i.e. the spot where the lowest coating thickness could be measured. Thus, coating thickness at these spots was likely to determine the time to onset of drug release [36]. This hypothesis was confirmed when determining the coating thickness of the Leptilan and the Orfiril formulation. The average coating thickness at the tablet edges of the Leptilan tablet was significantly ($P < 0.05$) lower than that of the Orfiril tablet (113 ± 16 µm *vs.* 136 ± 7 µm, Table 6). Consequently, dissolution of the coating of the Leptilan tablet initiated earlier than that of the Orfiril tablet.

4.2. Drug release in simulated individual subjects

With the experimental setup targeting to simulate individual GI passages interindividual variabilities of *in vivo* GI pH-profiles and transit times were simulated using a compendial dissolution test system as described earlier [35]. As observed in the experiments simulating a GI passage in an average healthy adult, drug release of the Leptilan and Orfiril formulation was hardly affected by pH-differences during simulated SI transit. These observations are in good agreement with results from other studies investigating the impact of intestinal pH-profiles on drug release of formulations coated with PMAA:PEA [27,28,53]. In the course of developing the pHysio-grad® device, which was used to create intestinal pH-profiles in physiologically relevant media in the present study, drug release from PMAA:PEA-coated esomeprazole beads was screened in Hanks Buffer of static pH (pH 6.8) and in two dynamic (pH 6.5–7.4) intestinal pH gradients. Application of different pH gradients had only a minor impact on the drug release behavior of the EC esomeprazole formulations [27]. Similar observations were made by Wulff et al. who characterized drug release from formulations bearing a coating blend of Eudragit RL and Eudragit L 55 and in the course of their experiments also compared dissolution in static and dynamic intestinal pH-conditions [53]. Using the Auto pH System® to modulate the pH of *m*Hanks buffer in a range of pH 5.6–6.8 to simulate proximal SI pH-conditions, Merchant et al. [28] investigated the dissolution behavior of EC prednisolone tablet formulations with different enteric polymers and which prior to that had been tested in static (pH 5.6 and 6.8) conditions [51,54]. Interestingly, in this study the lag time before onset of drug release of the PMAA:PEA–coated formulation observed in experiments with a pH gradient markedly differed from those observed at a static pH [51] and better reflected *in vivo* disintegration times reported for EC prednisolone products [55]. Another outcome of the Merchant study was that drug release from CAP-based formulations presented with a higher sensitivity towards the experimental conditions, i.e. whether static or dynamic SI pH-conditions were applied, than that from the PMAA:PMMA-coated candidates [28,51]. This indicates that in the present study the high variability of valproate release from the Ergenyl formulation might primarily be attributed to the CAP part of the coating.

4.3. Simulation of individual in silico plasma profiles

Valproate release of Orfiril and Leptilan turned out to be robust towards individual SI pH-conditions and transit times. The lag times before onset of valproate release were mainly determined by the duration of gastric conditions and the rates of drug release were similar for all subjects simulated *in vitro*. Consequently, differences observed in the *in vivo* plasma profiles might predominantly relate to (i) different GET of the tablets *in vivo* and (ii) individual differences in the valproate

PK parameters of the subjects enrolled in the reported *in vivo* studies [37,38]. For the *in silico* valproate plasma profile simulations we decided to use the drug release profiles from the experiments simulating a fasted GI passage comprising a continuous SI passage of the EC tablets in an average healthy adult. In order to reflect the *in vivo* variability in GET and valproate pharmacokinetics, for establishing the *in silico* models different theoretical GETs were combined with intestinal *in vitro* release profiles and individual data sets of valproate PK parameters from 7 healthy subjects [37]. In order to identify the best match results, *in silico* valproate plasma profiles were compared with a selection of *in vivo* plasma profiles obtained after fasted administration of the different EC tablet formulations (*in vivo* 1–3) [37,38]. An MED approach was applied to assess the similarity of *in silico* and *in vivo* data sets. To our knowledge, to date the MED approach has not been used to link individual *in silico* and *in vivo* plasma profiles. Since as a result of combining a range of different GETs and individual valproate pharmacokinetic parameters numerous *in silico* plasma profiles were generated, a fast, simple and reliable method for distinguishing between good and poor predictions and to finally identify the best match was required. Calculation of the MED was a simple and straightforward way for plasma profile comparison.

Expectedly, the GET was the major determinant for the lag time before valproate appeared in the systemic circulation, while the shape of the plasma profile was primarily defined by the individual valproate pharmacokinetics of the simulated subjects. Nevertheless, from the *in vivo* valproate plasma profiles data of all EC valproate formulation alone, it is not possible to distinguish whether the lag time observed *in vivo* can be solely attributed to gastric emptying or a combination of GET and the time before dissolution of the enteric coating under SI conditions.

The comparison of AUC and c_{max} of the best matching *in silico* and *in vivo* plasma profiles provided promising results for the Leptilan and the Orfiril formulations with PEs $\leq$10% (with one exception) indicating that the individual *in silico* plasma profiles are likely to be regarded as predictive. As already indicated by higher MED values, the *in silico* plasma profiles for Ergenyl were not predictive, since PEs for both AUC and c_{max} markedly exceeded 10%. Since for Ergenyl, *in silico* lag times before onset of *in vitro* drug release were markedly longer than the corresponding *in vivo* lag times, the *in vitro* test setup used to generate the input data for the *in silico* simulations, in this case, might slightly over-discriminate drug release from CAP-coated formulations and thus might require some additional fine-tuning in future experiments.

An alternative approach for estimating the impact of varying individual pharmacokinetics of enteric diclofenac tablets was reported by Kambayashi et al. who predicted individual plasma profiles by combining dissolution data under fasted and fed conditions in a PBPK model [56]. However, in contrast to the present study, the Kambayashi dissolution setup utilized “standard” biorelevant media to simulate upper GI conditions in average adults. Furthermore, Kambayashi used a virtually generated study population and focused on explaining the variability of *in vivo* plasma profiles by applying GETs that were randomly generated based on an algorithm derived from literature data for gastric emptying of monolithic solid oral dosage forms. In the present study, besides variations in GET further aspects that contribute to individual plasma profiles variability, such as individual API clearance and volume of distribution, were taken into account.

Since drug release from monolithic EC tablets may not only be affected by the physicochemical properties of the GI fluids and the passage times through the GI tract, GI motility and especially mechanical forces acting on the dosage form during their GI passage are further important factors to consider when developing predictive *in vitro* dissolution methods. In order to assess the sensitivity of the EC valproate formulations towards mechanical stress, a preliminary study was performed in physiological buffers in the stress test apparatus developed by Garbacz et al. [57] (data not shown), that allows for the *in vitro* simulation of mechanical stress acting on the dosage form during a GI passage. Neither the PMAA-PEA-coated formulations, nor the PMAA:PMMA/CAP-coated tablet presented with an altered drug release after pressure events simulating pyloric and intestinal passage indicating that *in vivo* drug release is unlikely to be influenced by mechanical forces acting on the valproate EC formulations.

Whereas for the valproate tablets screened in the present study GET turned out to be the major determinant for the onset of *in vivo* drug release and consequently the variability in individual plasma profiles, drug release from other types of oral dosage forms might be affected by other GI parameters that are subject to intra- and interindividual variability. The ability to address such variabilities in *in vitro* and *in silico* models, as shown in the present study is essential for a proper risk assessment in estimating safety and efficacy of oral dosage forms. Therefore, the present study presents a first step towards a better design of individualized / patient-specific *in vitro*- and *in silico* models and the models will be fine-tuned to address individuals of other patient groups in future studies.

5. Conclusion

Individualized dissolution test methods are an appropriate means for estimating *in vivo* drug release of orally administered dosage forms. In the present study results from individualized *in vitro* release experiments indicated that gastric residence time rather than the SI pH-profile is the essential parameter for the time to onset of *in vivo* valproate release. A novel PK software was applied to predict individual oral PK profiles based on average intestinal release profiles obtained in the *in vitro* experiments combined with different GETs, valproate characteristics and subject-specific PK parameters published in the literature. Results were promising, i.e differences observed in a number of fasted valproate plasma profiles could be correlated with individual GETs and individual differences in valproate PK parameters. Results from the study clearly indicated that both individualized *in vitro* and *in silico* methods present valuable tools in assessing the safety and efficacy of NTI drug formulations with regard to interindividual variability in GI parameters affecting *in vivo* drug release and individual drug pharmacokinetics.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2018.12.004.

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