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Research paper

Predictive PBPK modeling as a tool in the formulation of the drug candidate TMP-001

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ABSTRACT

Since many drugs in the therapy scheme of multiple sclerosis (MS) are applied parenterally with significant side effects, oral treatment is the most accepted therapy option for chronic diseases like MS.

The drug candidate TMP-001, which has disease-modifying properties, can be applied orally. Beside other symptoms, swallowing disorders have a major impact not only on the health status and quality of life of MS patients, but also impede reliable drug therapy. Consequently, the development of an easy-to-swallow liquid oral dosage form supported by a combined PBPK-IVIVC model was approached. In this context, the impact of formulation parameters was studied. Biorelevant *in vitro* drug release studies resulted in an almost complete release of 96.91% ± 1.00% in the intestine which was translated to rapidly increasing *in silico* plasma profiles. The predictions were compared to the outcome of a phase I clinical trial. A partial parameter sensitivity analysis of the *in silico* model deepened our understanding of the physiological processes underlying human pharmacokinetics.

1. Introduction

TMP-001 is a drug candidate for the treatment of multiple sclerosis (MS). It has anti-inflammatory properties and a structure similar to other compounds in the group of nonsteroidal anti-inflammatory drugs. Recently, TMP-001 was tested for its disease modifying properties in patients with relapsing-remitting multiple sclerosis (RRMS) in a clinical phase IIa study (US National Library of Medicine 2017, NCT02686788). Approximately 85% of the MS patients are affected by RRMS which is characterized by subsequent periods of flare ups and alleviating symptoms [1]. In the clinical study, 600 mg of TMP-001 was orally administered twice a day to MS patients with an expanded disability status scale (EDSS) of 0.0 to 5.0 using hard gelatin capsules (3x 200 mg capsules). The EDSS measures the degree of disability originating from the disease on a scale from 0.0 to 10.0, where the grade 10.0 corresponds to death due to MS [2].

Swallowing disorder is a frequently underestimated symptom of MS. It is not only life-threatening during the later stages of the disease [3], but significantly reduces the patient's quality of life and can potentially affect *in vivo* pharmacokinetics due to the delayed intake of medication. Prosiegel and co-workers reported that the prevalence of swallowing disorders in MS patients varies considerably, depending on the age of the literature [4]. In older studies, a lower prevalence was documented, whereas in more recent publications, about one third of MS patients were diagnosed with dysphagia [5]. Even higher numbers were reported with increasing EDSS. At an EDSS value of 9.0, 95% of the

Abbreviations: AAFE, absolute average fold error; AUC, area under the curve; EDSS, expanded disability status scale; FaSSGF, fasted state simulated gastric fluid; FaSSIF-V2, fasted state simulated intestinal fluid version two; HPLC, high-performance liquid chromatography; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; LLOQ, lower limit of quantification; MMC III, phase III of the migrating myoelectric complex; MRT, mean residence time; MS, multiple sclerosis; PBPK, physiologically based pharmacokinetic model; PPSA, partial parameter sensitivity analysis; RH, relative humidity; RPT, reciprocal powered time model; RPTE, reciprocal powered time exponent model; RRMS, relapsing-remitting multiple sclerosis; SD, standard deviation

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patients were affected [5–7]. Further health risks associated with this symptom include aspiration pneumonia, dehydration and malnutrition [6].

In the light of high prevalence of dysphagia experienced by MS patients, authors are of the opinion that hard-gelatin capsules are not the most suitable dosage form for MS patients. Especially when using capsules, the oesophageal transit time markedly depends on body position and volume of liquids swallowed, along with the dosage form [8]. Also gelatin shell of the capsule can form an adhesive surface depending on the moisture content further delaying the drug release from dosage form [9]. Therefore, an easy-to-swallow liquid oral dosage form was developed which could serve as the investigational medicinal product in a second clinical trial. To support formulation development, the *in vivo* performance was predicted using biorelevant *in vitro* drug release testing in combination with *in silico* modeling. To meet all regulatory requirements for the clinical investigation, the oral suspension of TMP-001 complied with highest quality standards. The stability was tested over a period of three months under accelerated conditions following the specifications defined in the guideline specifications Q1A of the International Conference of Harmonisation (ICH) [10]. When testing the *in vitro* drug release properties, the biorelevant media, fasted state simulated gastric fluid (FaSSGF) and fasted state simulated intestinal fluid version two (FaSSIF-V2) were employed. A partial parameter sensitivity analysis (PPSA) and mechanistic modeling of gastric emptying were applied to carefully analyze the impact of physiology on pharmacokinetics *in silico*. Further, the effects of swallowing disorder on human pharmacokinetics were investigated *in silico* based on the non-public clinical experience with dysphagia in MS patients.

2. Materials and methods

2.1. Materials

The drug candidate TMP-001 was purchased from Aesica Pharmaceuticals (Hertfordshire, UK), methylparaben (lot 345231067), propylparaben (lot 36216958), citric acid (lot 464215351), sodium chloride (lot 464219160), sodium saccharin (lot 5532053), trisodium citrate dihydrate (lot 504219376), glycerol (lot 55223902) and polysorbate 80 (lot 65220269) from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Maltitol (lot 10200922) was obtained from ThermoFisher GmbH (Karlsruhe Germany), hydroxyethyl cellulose (lot 16100613) from Caesar Loretz GmbH (Hilden, Germany) and calcium chloride dihydrate (lot 16G084109) from VWR Chemicals (Leuven, Belgium). Citric flavor (lot 213841/10906185/4) was a generous gift from Symrise AG (Holzminden, Germany).

The biorelevant media FaSSGF and FaSSIF-V2 employed were kindly provided by biorelevant.com Ltd. (London, UK) and were prepared according to the instructions of the manufacturer.

An Ultra Clear® water purification system (Evoqua water technologies, Günzburg, Germany) was used to obtain high purity water. All other organic solvents were of high-performance liquid chromatography (HPLC) grade.

2.2. Quantification of TMP-001 using high performance liquid chromatography

To quantify TMP-001, a HPLC system (Chromaster, VWR Hitachi, Tokyo, Japan) was used. It consisted of an HPLC pump (no. 5160), a column oven (no. 5310), an auto sampler (no. 5260) and an UV–Vis detector (no. 5420). For the stability studies, an enantioselective method was developed to quantify the enantiomeric purity of the drug. A chiral HPLC column (Lux Amylose-2, 150 × 4.60 mm, particle size 5 µm) and a precolumn of the same material (Phenomenex Ltd., Aschaffenburg, Germany) maintained at 25 °C were employed for the separation.

A mobile phase comprising 60% [v/v] acetonitrile and 40% [v/v] of an aqueous formic acid solution (0.1% [v/v] formic acid in purified water) was pumped at a flow rate of 1 mL/min.

The drug was detected at a wavelength of 247 nm after injection of a 20 µL sample. The total run time was 6 min. Linearity was demonstrated in a concentration range between 0.025 µg/mL and 50 µg/mL. Any interference between the excipients of the formulation and the quantification method was excluded. For quantification of TMP-001 from biorelevant media, a reversed phase column (Gemini NX-C 18, 250 × 4.60 mm, particle size 5 µm) and a precolumn of the same material (Phenomenex Ltd., Aschaffenburg, Germany) were used. The mobile phase was pumped at a rate of 1 mL/min through the system and contained 58% [v/v] acetonitrile and 42% [v/v] of an aqueous trifluoracetic acid solution (0.1% [v/v] trifluoracetic acid in purified water). The injection volume was set to 20 µL and detection of the compound was carried out at a wavelength of 247 nm. The total run time was 10 min at 25 °C. Both quantification methods were validated according to ICH standards [11]. All samples containing biorelevant media were diluted with mobile phase prior injection.

2.3. Determination of thermodynamic drug solubility in biorelevant media

A modified “shake-flask” method was employed to determine the solubility of TMP-001 in the biorelevant media. An excess of drug was added to a volume of 5 mL of each release medium (n = 5) and incubated in an orbital shaker at 37 °C. After 24 h and 48 h, 2 mL dispersion was withdrawn and filtrated using a Whatman Anotop™ 25 Plus 0.02 µm syringe filter (lot A10105280, VWR International GmbH, Darmstadt, Germany), which was saturated with 3 mL concentrated drug solution prior to filtration. Solubility were quantified using HPLC (see Section 2.2).

2.4. Formulation of an oral suspension of TMP-001

An oral suspension of TMP-001 was successfully developed. All excipients complied with the current European regulations for investigational medicinal product. Sufficient microbial quality was guaranteed by using a combination of 75 mg of methylparaben and 25 mg propylparaben in aqueous solution. For taste masking purposes, 30 g maltitol, 100 mg sodium saccharin and 800 mg of lemon flavor were added. A 10 mM citrate buffer system was employed to stabilize the pH of the suspension in a pH range of 3 to 5 (50 mg of citric acid, 200 mg of trisodium citrate dihydrate). The gelation agent hydroxyethyl cellulose (1400 mg) was utilized to increase the viscosity of the dispersion medium. Incorporation of 2200 mg of TMP-001 was carried out with the help of the wetting agents glycerol (2200 mg) and polysorbate 80 (100 mg). Finally, the preparation was replenished with purified water and calcium chloride (2000 mg) was added for flocculation of TMP-001 particles.

2.5. Characterization and storage stability testing of the liquid oral suspension

To guarantee the chemical, physical and microbiological stability of the developed oral suspension throughout the shelf-life, stability testing was conducted according to ICH specifications Q1A and Q6A [10,12].

For this purpose, the suspension was stored in the final container (Aponorm medicine bottle 100 mL, WEPA Apothekenbedarf, Hillscheid, Germany) in a constant climate chamber (HPP 260 with Atmo Control Software Version 2.5.2.0, Memmert, Schwabach, Germany). Accelerated storage conditions according to ICH guideline Q1A were used [40 °C ± 2 °C and 75% relative humidity (RH) ± 5% RH (ICH, 2003)]. At predetermined time points, after 0, 30, 60 and 90 days, the containers were opened and the formulation was examined for resuspendibility, pH, uniformity of mass of the delivered dose, density, viscosity, particle size, and drug content.

The pH value of the suspensions was determined using a pH meter

(Calimatic 766, Knick, Berlin, Germany) which was calibrated in a pH range of 4 to 7 prior use. The uniformity of mass of the delivered doses from multidose containers was tested following the requirements defined by the European Pharmacopoeia. A number of 20 individual doses were randomly collected and the average mass was determined gravimetrically (analytical balance, New Classic MF, Mettler Toledo, Columbus, USA). The density of the formulation was measured using a pycnometer (Brand, Wertheim, Germany) at 20 °C ± 1 °C. The density (ρ) was calculated using the Eq. (1):

$$\rho(formulation) = \frac{m_2 - m_0}{m_1 - m_0} \cdot \rho_w \quad (1)$$

where m_0 was the weight of the empty pycnometer, m_1 and m_2 were weights of the pycnometer filled with water and formulation, respectively, to ρ_w the density of water (998.20 kg/m^3) at a temperature of 20 °C. To determine the viscosity of the formulation, a coaxial cylinder in a rotational viscometer (Physica Rheolab MC 1, Anton Paar, Ostfildern-Scharnhausen, Germany) was used. In total, 20 measuring points with a duration of 6 s and a shear rate of 100/s each were applied. Particle size distribution was determined using an Eyetech/Dipa 2000 system (Ankersmid, Nijverdal, Netherlands) after diluting samples 1:9 using purified water. The drug content was quantified by HPLC as described above.

2.6. Drug release testing of the liquid suspension

The drug release from the suspension was tested using the filtration method [13]. For testing the drug release properties of the developed formulation, a PTWS 820 dissolution apparatus (Pharma Test AG, Hainburg, Germany) was employed following the requirements of the United States Pharmacopoeia and the European Pharmacopoeia.

Prior to the *in vitro* release test, the filter adsorption of the employed syringe filters (Rezist™ 30/0.45 PTFE, 0.45 µm, lot A10011099, GE Healthcare, Buckinghamshire, UK) was tested using a saturated solution of TMP-001 at 37 °C. To investigate the drug release rate of the formulation, a standard dissolution vessel with a total volume of 500 mL of release medium at 37.0 °C ± 0.5 °C and a paddle configuration at a stirring rate set to 75 revolutions per minute were employed. When using FaSSGF, physiological conditions in the stomach in the fasted state were simulated, while FaSSIF-V2 represented the fasted state conditions in the upper intestine. Prior to all experiments, the release buffers were filtered through a filter membrane with a pore size of 0.45 µm (sterile membrane filters, cellulose nitrate, 47 d.mm, lot 60317100, Whatman Limited, Maidstone, England) and kept under vacuum for 30 min. All experiments were performed in triplicate. A volume of 30 mL of formulation, equivalent to a single dose of 600 mg of TMP-001, was added to the release medium. Before the first samples were taken from the vessels, the filters were preconditioned with 3 mL dissolution medium to avoid excessive adsorption of TMP-001 to the membrane. Samples were collected after 3, 5, 8, 10, 15, 30 and 60 min. For this purpose, samples of 1.5 mL were taken and approximately 1.3 mL were refiltered into the vessel to rinse the dead volume of the filtration units. The remaining 0.2 mL were filtered and diluted with mobile phase in ratio 1:1 and 1:69 for FaSSGF and FaSSIF-V2, respectively for quantification using HPLC (see Section 2.2).

2.7. Description of the PBPK-model

The physiologically based pharmacokinetic (PBPK) model employed, was an improved version of that published previously [13]. In the current model, gastric emptying rate was adjusted (3.2 h^{-1}) to comply with latest research on physiological conditions in the stomach [14]. Additionally, the dissolution behavior in stomach and intestine was assessed more accurately by applying the following equations with time (t) as the independent variable and m, b and c as the release parameters:

$$f = \frac{t^b}{t^b + m} \cdot c \quad (2)$$

$$f = \frac{t^b}{t^b + m^{-t}} \cdot c \quad (3)$$

The reciprocal powered time model (RPT) has been applied to a wide range of release profiles [13,15]. To broaden the spectrum of release data described by the equation, the variable c was introduced [see Eqs. (2) and (3)].

Furthermore, in a derivation of this model, an exponential term reduces the impact of the variable m at the later time points and places more focus on the initial drug release [see Eq. (3)]. This equation was termed the reciprocal powered time exponent model (RPTE). Both equations were applied to fit the *in vitro* release data obtained with each release medium.

In the present study, *in vitro* release kinetics and *in vivo* pharmacokinetic data were combined to estimate the *in vivo* performance of the new formulation. The new liquid formulation was designed to meet the special requirements of MS patients. Pharmacokinetic data for the model prediction were extracted from a clinical trial conducted with an early capsule formulation of TMP-001. Input parameters of the PBPK model are presented in Table 1.

To compare the simulated profiles with the *in vivo* profile, the absolute average fold error (AAFE) was calculated according to the following Eq. (4) [19]:

$$AAFE = 10^{\frac{1}{n} \cdot \sum \left| \log \frac{predicted_t}{observed_t} \right|} \quad (4)$$

2.8. Partial parameter sensitivity analysis of the in silico model

A PPSA was conducted by investigating the impact of gastric emptying as well as elimination and distribution parameters on the pharmacokinetic profile. The elimination and distribution parameters were varied in the range described in the clinical trials (see Table 2).

2.9. Evaluation of the effects of gastric emptying patterns in silico

To simulate the physiological patterns underlying gastric emptying, three different models were evaluated. Initially, the gastric emptying rate was varied in a range between 0.5 h^{-1} and 6 h^{-1} assuming first-

Table 1
Input parameters of the PBPK model.

Parameter	Value	Description	Reference
FaSSGF m	0.9190	Release parameters	See section
FaSSGF b	3.6644*10^{-10}		3.3
FaSSGFc	4.9213		
FaSSIF-V2 m	1.1589*10^{-9}	Release parameters	See section
FaSSIF-V2 b	5.8284		3.3
FaSSIF-V2 c	97.3540		
Sine amplitude	0.5–6 h^{-1}	Emptying parameter	See section 2.9
Sine periode	0.5–3 h	Emptying parameter	See section 2.9
Onset MMC III	0.5–12 h	Onset of the housekeeper wave	See section 2.9
Intestinal volume	105 mL	Release parameter	[16]
MRT	3 h	Absorption time frame	[17]
Gastric emptying rate	3.2 h^{-1}	1st order gastric emptying	[14]
V_D	6190.13 mL	Volume of distribution	[18]
k_{10}	0.17712 h^{-1}	Elimination rate constant	[18]
k_{12}	0.0927448 h^{-1}	Distribution rate constant	[18]
k_{21}	0.229005 h^{-1}	Distribution rate constant	[18]

Table 2
Range of elimination and distribution parameters tested in the PPSA.

Parameter	Lower limit	Upper limit
k_{10}	0.177120 h^{-1}	0.178830 h^{-1}
k_{12}	0.072700 h^{-1}	0.092745 h^{-1}
k_{21}	0.089549 h^{-1}	0.229005 h^{-1}

order kinetics. Similar approaches have been used in previous simulations [14,20]. A broad range was tested to cover even an unexpectedly high clinical variability.

In a second step, different patterns were modelled using a stochastic model of gastric emptying in the above-mentioned range (see Fig. 1). A delay in gastric emptying (due to a closed pylorus) was assumed to occur in an unpredictable manner. Since this delay also affects the t_{max} value, a parameter optimization was conducted using the optimization module of Stella® Architect selecting a constant empying rate of 3.2 h^{-1} and 1.875 h as the highest acceptable t_{max} value (see Fig. 1, left). This corresponds to the upper limit defined by the bioequivalence criteria when applied to the t_{max} value of the liquid formultion. This was to determine probability of such events, taking the t_{max} value into account as a limiting parameter. Later simulations were conducted using this propability and a random emptying rate varying between 0.5 h^{-1} and 6 h^{-1} (see Fig. 1, right).

In the third approach, gastric emptying was simulated using a sine function in combination with a "housekeeper wave". This is defined as phase III of the migrating myoelectric complex (MMC III) also known as migrating motor complex, and leads to a complete emptying of the stomach [21]. Periodic emptying in the sine phase followed Eq. (5). The overall transport rate was set to non-negative interpreting all negative values as 'no transport' while period and amplitude were varied using Stella® Architect in sensitivity mode.

$$k_{GE} = amplitude \cdot \sin\left(\frac{2 \cdot t}{period}\right) \quad (5)$$

An amplitude of 0.5 h^{-1} to 6 h^{-1} and a period of 0.5 h to 3 h defined the emptying pattern. The onset of the housekeeper wave was scheduled between 0.5 h and 12 h. Within these ranges, all parameters were varied using random sampling mode and incremental distribution.

2.10. Evaluation of the effects of swallowing disorder in silico

Since dysphagia in MS patients could impede the application and consequently the success of drug therapy, the influence of swallowing disorders was evaluated *in silico*. For this, a non-periodic randomized partial administration of the dose was simulated using a stochastic model whereby the remaining dose was limited to a total amount of 0.1% (RD) after 20 min (P). The total volume of a single dose was set to 30 mL as defined by the capacity of the mouth cavity [22]. The swallowing process itself was considered a first order process [see Eq. (6)] [23,24] calculating the rate constant (k_{sw}) from Eq. (7) with the predefined parameters P and RD, an initial dose of 600 mg TMP-001 ($Dose_{t0}$) and the remaining amount of drug ($Dose_{tP}$) set to 0.1% of the initial dose.

$$[Dose]_t = [Dose]_0 * e^{-k_{sw} * t} \quad (6)$$

$$k_{sw} = -\frac{\ln\left(\frac{Dose_{t0} * RD}{Dose_{t0}}\right)}{P}, \quad (7)$$

The resulting swallowing rate was randomly altered between 0 and 2-fold k_{sw} using sensitivity mode.

2.11. Statistical and graphical analysis

All data are expressed as the mean value ± standard deviation (SD), which were calculated and plotted using Microsoft Excel (Microsoft, Redmond, Washington, USA) and SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany), respectively. All experiments were carried out in triplicate. Analysis of pharmacokinetic data were carried out using Phoenix 64 WinNonlin® (Certara, Princeton, New Jersey, United States). After reaching c_{max} *in vivo* all values below the lower limit of quantification (LLOQ) were replaced with LLOQ/2 [25].The prediction of *in vivo* pharmacokinetics was calculated using Stella® Architect (isee systems, Lebanon, New Hampshire, USA).

3. Results and discussion

Over many years, release testing has been applied to predict the *in vivo* performance of oral dosage forms [26]. In the present approach, a novel formulation of the drug candidate TMP-001 was developed. Biorelevant dissolution methods were employed to predict the *in vivo* dissolution rate in stomach and intestine.

However, the pharmacokinetic profiles observed during a phase I clinical trial for TMP-001 could not be sufficiently explained by current *in silico* simulations. Therefore, the impact of individual physiological patterns, including gastric emptying and swallowing disorders, on human pharmacokinetics was investigated as part of the sensitivity analysis. A novel, optimized PBPK model for the prediction of pharmacokinetics was developed and the error range was systematically explored.

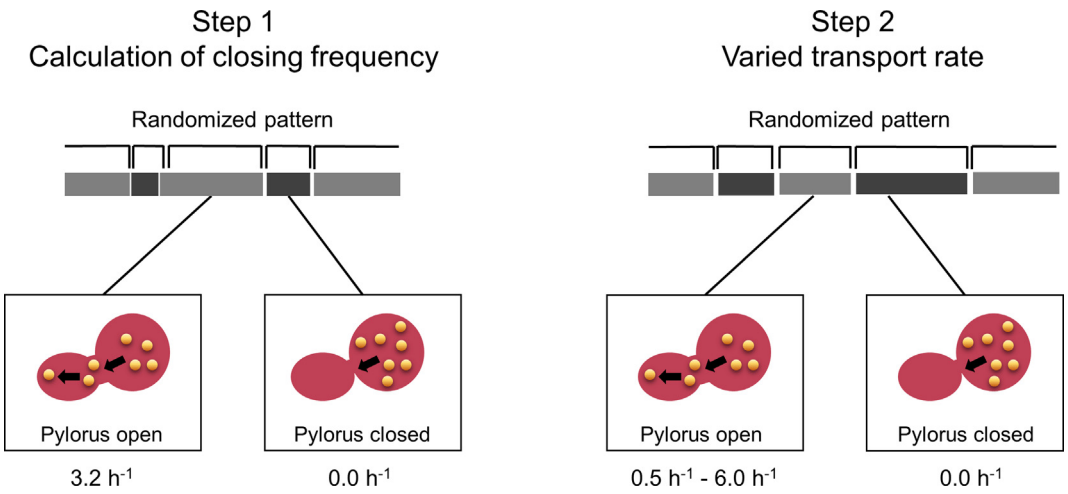

Fig. 1. Illustration of the stochastic model applied to simulate gastric emptying during model optimization (left) and *in silico* prediction (right).

3.1. Solubility and filter adsorption of TMP-001

Solubility of the compound was determined in all biorelevant media. As expected, the weak acid TMP-001 exhibited lower solubility in the acidic medium. In FaSSGF (pH = 1.6), a solubility of 20.00 µg/mL ± 0.68 µg/mL was determined. In FaSSIF-V2, solubility reached a final value of 1610.97 µg/mL ± 46.23 µg/mL. The loss of drug substance after filter preconditioning was determined to be 5.89% ± 1.36% in FaSSGF and 3.08% ± 0.59% in FaSSIF-V2.

3.2. Formulation and storage stability of the liquid oral suspension

All quality parameters as defined by the investigational medicinal product dossier were monitored over a time period of 90 days under accelerated storage conditions following ICH guidelines Q1A and Q6A [10,12].

An average pH value of 4.06 ± 0.18, a density of 1.1395 g/cm^3 ± 0.0122 g/cm^3 as well as a D90 value of 31.98 µm ± 6.20 µm were observed. The suspension had an average viscosity of 156.9 mPa·s ± 6.7 mPa·s and a drug content of 104.66% [m/m] ± 2.61% [m/m]. Throughout the entire shelf-life, the formulation exhibited sufficient chemical, physical and microbial stability to serve as an investigational medicinal product.

3.3. In vitro drug release of liquid oral dosage form

The drug release of TMP-001 from the suspension was tested *in vitro* under biorelevant conditions to simulate the physiological environment. The commercially available dissolution media FaSSGF and FaSSIF-V2 were employed (see Fig. 2).

A rapid increase in the dissolution profile was observed in both media. In FaSSGF, the solubility of TMP-001 was exceeded during the plateau phase. At later time points, an average drug concentration of approximately 29 µg/mL was achieved (2.38% ± 0.11%). This effect can be attributed to the presence of polysorbate 80 in the formulation, acting as a solubilizing agent in the dissolution test.

In FaSSIF-V2, the plateau phase was reached at 96.91% ± 1.00%. This corresponds to a loss of drug substance of approximately 3% observed during the filter adsorption studies. The optimal curve fit was obtained with the RPTE model when using FaSSGF (R = 0.9994) and the RPT model for release profiles measured in FaSSIF-V2 (R = 0.9999).

Table 3
Predicted and observed AUC, c_{max} and t_{max} of a capsule formulation used for validation of the PBPK model.

	Predicted	Observed	Ratio
$AUC_{0\text{-}24h}$	508.52 µg/mL*h^{-1}	521.04 µg/mL*h^{-1}	0.98
C_{max}	70.07 µg/mL	65.23 µg/mL	1.07
T_{max}	1.67 h	1.5 h	1.11

3.4. In silico prediction of human pharmacokinetics

Prior to the *in silico* predictions, a model validation was conducted using *in vitro* release and human *in vivo* data from a capsule formulation. The predictions accurately reflected human pharmacokinetics of the drug candidate (see Table 3).

Human pharmacokinetic data of the liquid oral suspension was determined in the course of a phase IIa clinical trial (US National Library of Medicine 2017, NCT02686788). A dose of 600 mg was administered to six healthy volunteers (manuscript in preparation) [18]. Individual pharmacokinetic profiles obtained were further evaluated by non-compartmental analysis using WinNonlin®. Hence, an area under the curve$_{0\rightarrow24}$ ($AUC_{0\rightarrow24}$) of 349.34 µg · h/mL ± 105.62 µg·h/mL and a median t_{max} value of 1.5 h with a mean deviation from median of ± 0.5 h were observed.

The mean c_{max} value calculated from single profiles was 61.61 µg/mL ± 13.11 µg/mL, whereas the value in the mean profile of all subjects was 53.30 µg/mL. This difference can be attributed to the wide range of observed t_{max} values drawn from the single pharmacokinetic profiles of individual subjects.

Remarkably, two out of six plasma profiles exhibited a double peak and two further profiles showed shouldering behavior leading to a double peak in the mean plasma profile. The early *in silico* model was not able to simulate this behavior (see Fig. 3).

However, the *in silico* predictions still reflected human pharmacokinetics and met bioequivalence criteria (ratios of 0.8 to 1.25) for c_{max} (70.77 µg/mL) and t_{max} resulting in ratios of 1.15 and 1.17, respectively. For the $AUC_{0\rightarrow24}$ a slight overprediction with a ratio of 1.45 was observed. An AAFE of 1.30 was calculated indicating comparable *in silico* and *in vivo* data [19].

Consequently, a PPSA was conducted in order to explain the double peak and shouldering behavior as well as to include more parameters that could influence future simulations.

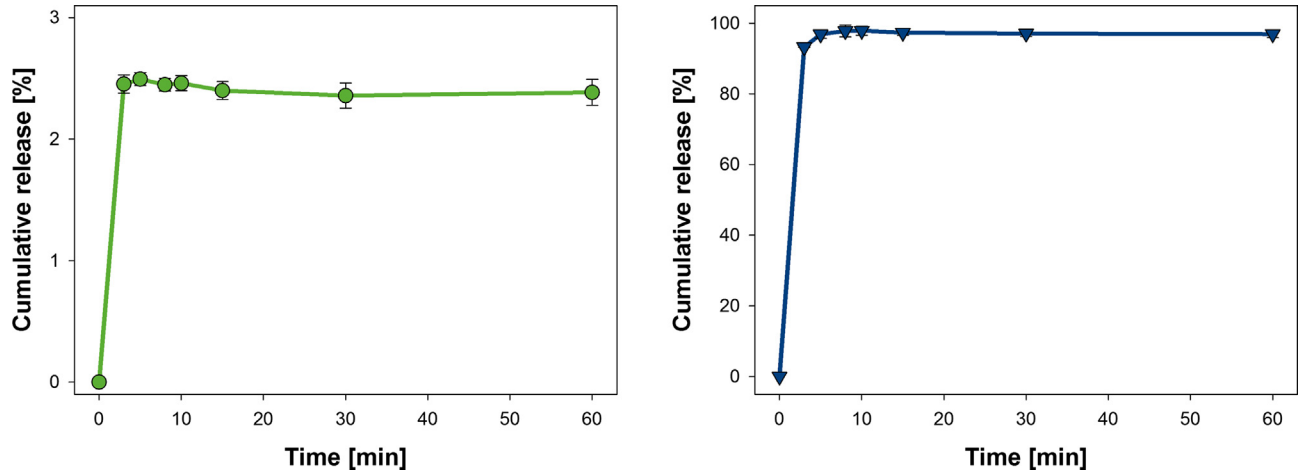

Fig. 2. *In vitro* drug release test using biorelevant media. The stomach compartment is simulated using FaSSGF (left) and the upper intestine by using FaSSIF-V2 (right).

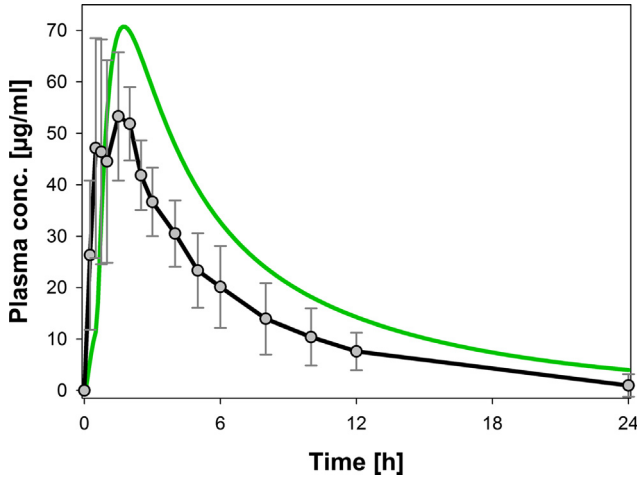

Fig. 3. Simulated profile (green line) and *in vivo* mean profile of the TMP-001 oral suspension (grey dots and black line).

3.5. Partial parameter sensitivity analysis of the in silico model

The advanced PPSA was used to explain *in vivo* pharmacokinetics of TMP-001 based on physiologically relevant mechanisms and to improve our predictions for future simulations. Initially, the *in vivo* parameters k_{10}, k_{12} and k_{21} were varied over a range relevant for the *in vivo* situation. The influence on $AUC_{0\rightarrow24}$, c_{max} and t_{max} was negligible for all simulations (see Fig. 4) suggesting a high reliability of the model with regard to these parameters.

4. Evaluation of the effects of gastric emptying patterns *in silico*

In the mechanistic *in silico* analysis, the impact of gastric emptying on the pharmacokinetic profile was systematically investigated. Following a more conventional approach, a constant gastric emptying rate was varied between different simulations assuming first-order kinetics. The changes in mass transport per time (see Fig. 5A) resulted in a shift of the c_{max} and t_{max} value without changing the shape of the profile (see Fig. 5B). Therefore, the occurrence of a specific non-continous physiological transport pattern was assumed. For solid dosage forms with a similar compound, the double peak manifestation was described earlier to be a result of a delayed gastric emptying [27].

In a stochastic approach, a randomized normally distributed emptying pattern with repeated non-predictable transport and lag phases

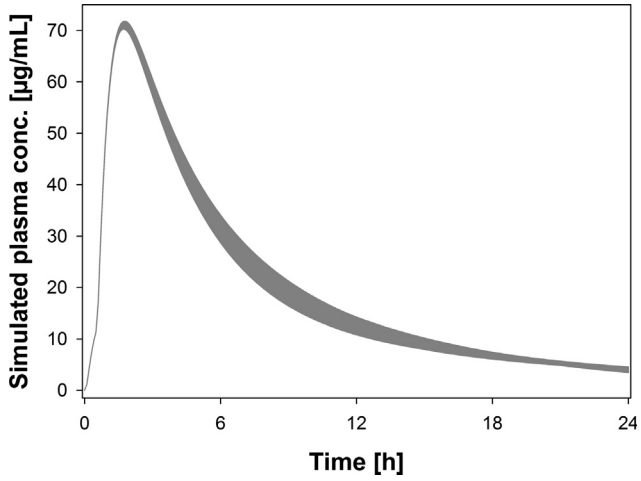

Fig. 4. PPSA of the *in silico* model applying incremental changes to distribution (k_{12}, k_{21}) and elimination (k_{10}) parameters.

was used. In a first step, the probability of the occurrence of a lag phase was calculated with a model optimization, assuming a constant transport rate of $3.2\,h^{-1}$ and resulted in a probability value of 21.8%.

Thereafter, the emptying rate was randomly selected over a predefined range ($0.5\,h^{-1}$ to $6\,h^{-1}$) with changes in each iteration of the model (see Fig. 5C). The maximum t_{max} value of 1.875 h was calculated applying bioequivalence criteria to the *in vivo* t_{max} value of 1.5 h. However, the high variability of this emptying process did not lead to a double peak behavior but an almost constant drug transport (see Fig. 5C and D).

The sine model simulates a periodic emptying pattern in combination with a housekeeper wave. During the emptying phases, gastric emptying follows a sine function which gradually releases the drug into the intestine and is accompanied by constantly repeated emptying and lag phases. The onset of the housekeeper wave leads to a change in the empyting pattern and consequently to a quicker emptying of the stomach. The transport rate (amplitude), the duration of a lag phase (half the period) as well as the time point after which a housekeeper wave occurred was altered with each run (Fig. 5E). This led to a number of double peaks in the simulated profiles (see Fig. 5F).

Double peak behavior has been studied for several compounds including cimetidine [20], diclofenac [28] and flurbiprofen [27]. The sine model is in line with our current understanding of gastrointestinal physiology [29–31] and predicts the phenomenon accurately. To simulate gastric emptying patterns, the overwhelming majority of *in silico* models are based on exponential functions [32–35]. In many cases, more complex simulations are designed for the fed state only [36–38]. Commercial software commonly uses zero or first order functions to simulate gastric emptying [39–41] which limits the predictive power in terms of clinically relevant profile shapes.

Pioneering this area, Oberle and Amidon designed an *in silico* model to explain the influence of gastric emptying on the pharmacokinetic profile [20]. The approach was based on a three-phase model using three constant flow rates to simulate the emptying process. Two pharmacokinetic studies were used to validate the *in silico* predictions [42,43] in a statistical evaluation of absence or presence of double peaks [20]. The model characteristics of a non-gradual multiphase emptying pattern reflect the dosage forms used during model validation. Only tablet formulations likely to result in a high amount of undissolved and non-dispersed drug were included.

Over the years, double peak behavior has been described for a number of liquids. The present study simulates pharmacokinetics of a liquid dosage form of the drug candidate TMP-001. In the *in vitro* setting, the suspension remained stable even after addition of FaSSGF indicating a stable dispersion. The sine model combines relevant characteristics of solid and liquid emptying by simulating a gradually changing flow rate undergoing periodic changes. Rather than detecting the absence or presence of double peaks, the algorithm was able to create similarly shaped pharmacokinetic profiles as observed in the *in vivo* study (see Fig. 5F).

A detailed single parameter analysis showed that an early housekeeper wave after less than 1.65 h led to a single-peak profile which explains 33% of the individual plasma profiles observed in the clinical trial. The plasma profiles of four out of six volunteers showed double peak or shouldering. A transport rate of less than $4\,h^{-1}$ resulted in appearance of a shoulder in the plasma profiles. The extent to which this phenomenon occurred correlated with decreasing transport rates while higher rates resulted in single peak profiles. This effect was independent of the lag time and the onset of the housekeeper wave. It should be noted that all of these parameters could be responsible for the shouldering while only a change in the lag phase led to double peak profiles. These occured when the sine period was slightly lower than the onset of a housekeeper wave which then interrupted the lag phase.

Assuming the shouldering or double peak behavior originated from a housekeeper wave in the lag phase, combined with a medium to slow transport rate, the *in vivo* lag time in subjects ranged from 0.55 h to

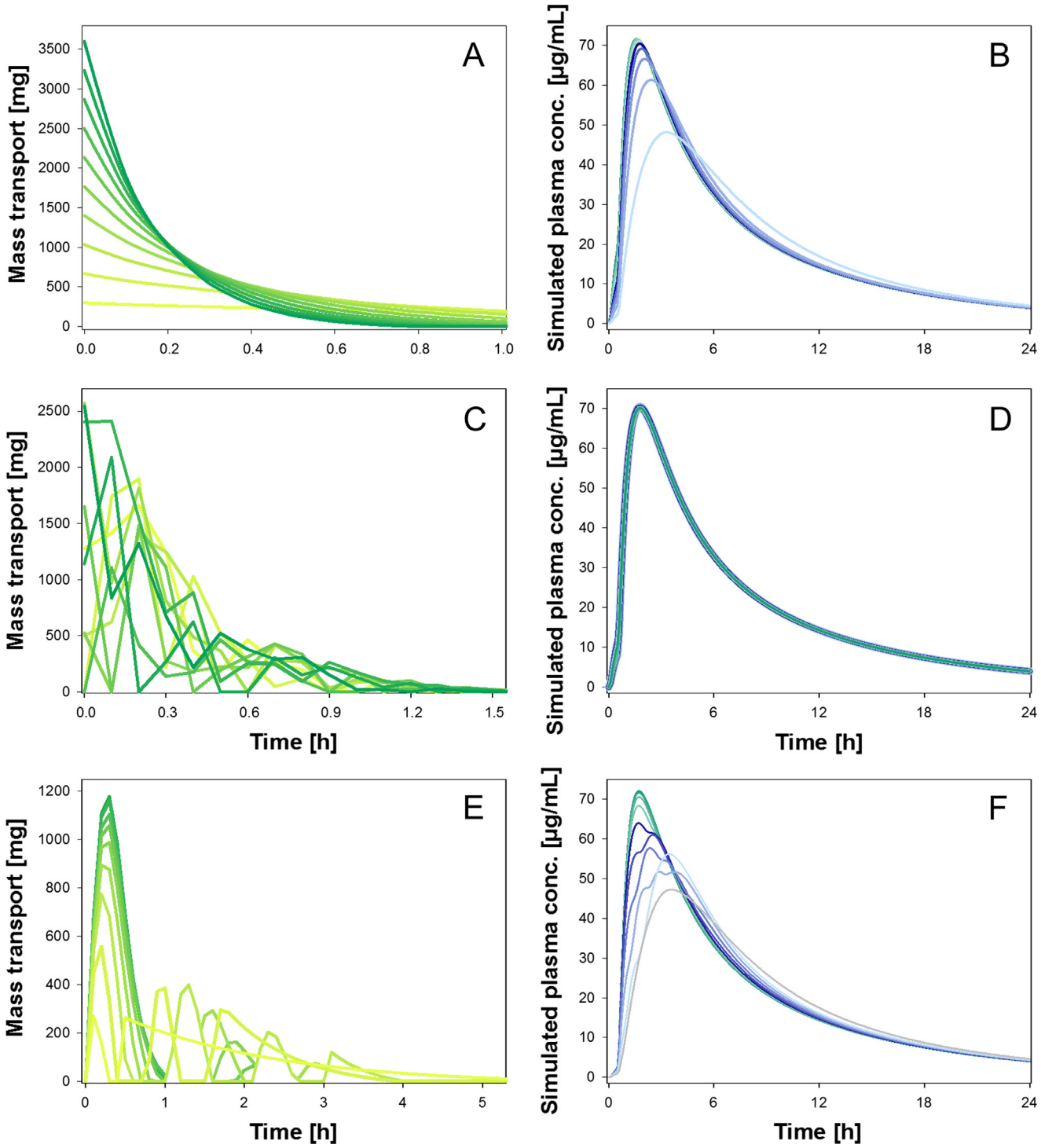

Fig. 5. PPSA of gastric emptying with illustrations of mass transport (left) and the resulting plasma profiles (right). Mass transport following first order kinetics (A and B), a stochastic transport model (C and D) and a periodic sinusoidal model (E and F) are presented. For each model ten runs were conducted using values within the defined range.

1.2 h in the fasted state.

The advanced *in silico* model confirmed the influence of a periodic emptying pattern on plasma pharmacokinetics and provided new insights into the physiological range relevant for liquid dosage forms.

4.1. Evaluation of the effects of swallowing disorder in silico

Swallowing disorders are a common symptom of MS patients and have been assumed to alter the biopharmaceutical properties of a formulation. A delayed intake of up to 20 min was simulated using a stochastic model, resulting in a swallowing rate constant k_{sw} of approximately 20.7 h^{-1} (see Eq. (7)). A total number of ten simulations was calculated to illustrate the impact of randomized non-period emptying on drug transport (see Fig. 6A). The resulting plasma profiles are presented in Fig. 6B. A duration of 20 min for intake was regarded as worst case scenario based on non-public reports from clinical practice at Goethe University Hospital.

Simulations of healthy (blue dash) and impaired (red lines) subjects

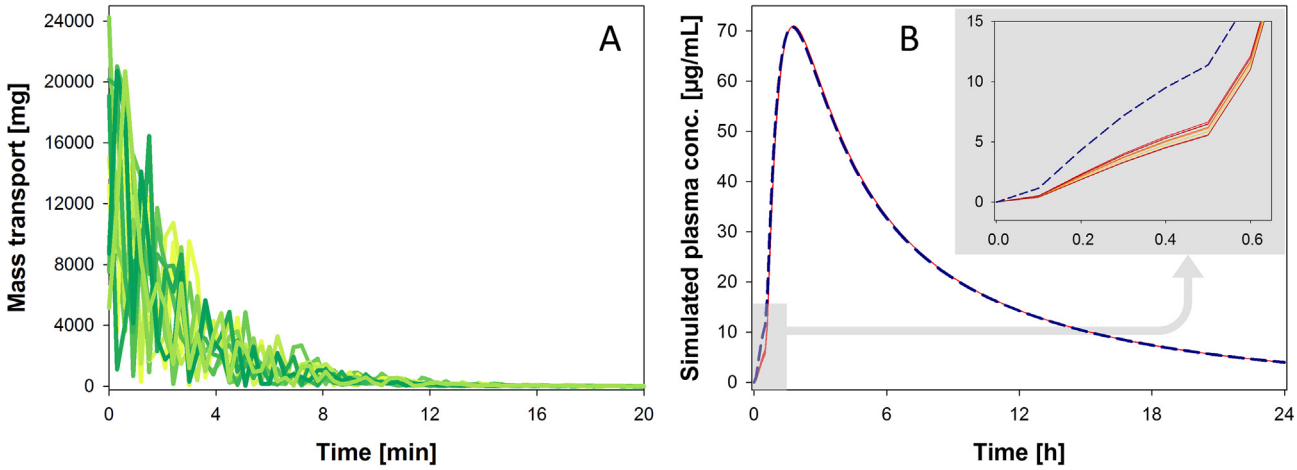

Fig. 6. Simulated intake for patients with swallowing disorder (A) and the resulting pharmacokinetic profiles (B, red lines) compared to healthy volunteers (B, blue dash).

are presented in Fig. 6B. From these simulations, we concluded that a swallowing disorder can be a major issue in clinical practice but has only minor influence on plasma pharmacokinetics for the developed liquid formulation. This was also confirmed by earlier observations defining gastric emptying to be rate-limiting for pharmacokinetics of compounds exhibiting double peak behavior. In this context, the stomach acts as a drug depot releasing the drug according to physiological emptying procedures. Therefore, only much slower intake would affect the pharmacokinetic profile.

5. Conclusion

The drug candidate TMP-001 is currently under investigation for the treatment of MS. In this study, an easy-to-swallow oral suspension of the compound was developed. An optimal physical, chemical and microbial stability was achieved and the formulation fulfilled all requirements for the clinical trials.

From the beginning, formulation development was supported by biorelevant *in vitro* release studies in combination with PBPK modeling to predict human pharmacokinetics. Despite the fact that c_{max} and t_{max} value were within the expected range, a strong impact of gastric transport on the plasma profiles was revealed.

In this context, the gastric emptying rate was identified to be responsible for this behavior. The shouldering and double peak profiles observed during the clinical trial were correlated to physiological variability of a periodic gastric emptying pattern.

A rapid dissolution of the formulation, as confirmed by the biorelevant release tests, was a prerequisite for this investigation. Under such conditions, the gastric emptying was rate-limiting to drug absorption. This pattern corresponded to a physiological gastric lag phase ranging from 0.5 to 1.2 h in the fasted state.

In a second approach, the effect of swallowing disorders on human pharmacokinetics was falsified using a stochastic *in silico* model to simulate the uncontrolled intake of the drug formulation.

In future, advanced simulations will enable a more accurate prediction of human pharmacokinetics based on biorelevant *in vitro* and *in silico* methods. Against this background, the impact of multiple physiological and formulation parameters on the *in vivo* performance can be investigated. This becomes even more important when considering TMP-001 as a drug candidate for sustained release formulations.

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