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Research paper

Powder for reconstitution of the anti-HIV-1 drug TMC278 – Formulation development, stability and animal studies

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ABSTRACT

Powders for reconstitution of the next-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) TMC278 with low water solubility were developed by using a spray-dry technology. Their flexible dosing ability makes them suitable for patients looking for a different approach for antiretroviral (ARV) therapy. The selection of formulation excipients was based on their potential to create and maintain supersaturation solubility of TMC278 in 0.01 M HCl. Suitable water-soluble carriers for TMC278 were selected by a supersaturation screening to formulate powders for reconstitution by spray-drying. The selected powders for reconstitution were compared to clinical tablets of TMC278.HCl, *in vitro* using dissolution and stability testing, and *in vivo* through administration to beagle dogs, fed immediately after dosing. The spray-dried powders for reconstitution made up of TMC278/PVP-VA 64 1:9 (w/w) and TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) showed ease of suspendability, nearly complete dissolution of the drug and acceptable stability after one month storage at 25 and 40 °C. In dogs, TMC278 was more slowly absorbed from tablets than from the suspended powders for reconstitution. Compared to the tablet, the relative bioavailability obtained with the powders ranged between 69% and 89% for TMC278/PVP-VA 64 1:9 (w/w) and between 85% and 157% for TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w). The absence of differences *in vivo* and *in vitro* between the powders made an eventual choice very difficult, yet their advantageous *in vivo* behaviour and flexible dosing possibility may provide a starting point for paediatric formulations.

1. Introduction

TMC278 (R278474) (rilpivirine or 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile) is a diarylpyrimidine (DAPY) NNRTI (Fig. 1) [1,2]. As with other NNRTIs, TMC278 interacts directly with a hydrophobic allosteric binding site at the HIV-1 reverse transcriptase (RT) enzyme, located at about 15 Å from the catalytic site, and therefore interferes in a non-competitive fashion with the binding of the normal substrates [3,4]. The DAPYs share a common horseshoe conformation with a central polar part (pyrimidine ring) [5] and two lateral hydrophobic wings [3]. TMC278 is an extremely potent inhibitor of HIV replication [1,2]; De Clercq (2005 [3]) described TMC278 as one of the most potent NNRTIs ever, with an EC_{50} of 0.1 nM. Its discovery was the result of continuous efforts of lead optimization over a 16 year time period, and serves as an example of modern drug design through classical medicinal chemistry [6]. It has a number of attractive attributes for development, which (1) is highly active against wild-type and various single and double mutant strains of HIV-1 conferring high resistance to NNRTIs [1,2,5,6], (2) has high oral bioavailability and a long elimination half-life, allowing once-daily oral treatment at low doses, (3) has a favourable adverse effect profile, and (4) is easy to synthesize and formulate [6]. It is suitable for high compliance oral treatment of HIV-1 infection [6]. Since NNRTIs are generally hydrophobic, obtaining

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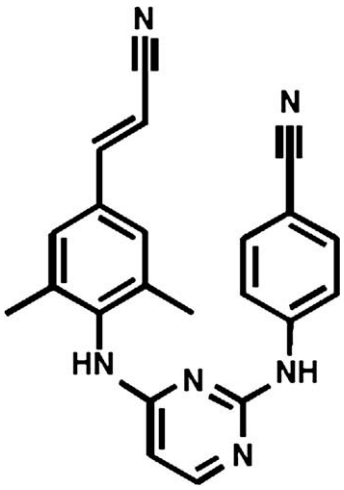

Fig. 1. Structure of TMC278.

solubility is expected to be more cumbersome than cell permeability [7–9]. Accordingly, TMC278 was predicted and reported to have low aqueous solubility and high serum-protein binding [6,10].

An increasing number of infants and children become infected with the Human Immunodeficiency Virus (HIV), most commonly through mother-to-child transmission of the virus, especially in developing countries [11,12]. Paediatric medication is always challenging as dose regimens might need to be adjusted according to age, weight or other parameters which change with growth. Therefore, dosage forms need to offer flexibility in dosing. In this case, there is a need for a more flexible dosing form than a tablet for paediatric patients. At a young age, a preference exists for drinkable formulations, i.e. syrups, or dry formulations that are converted to the liquid form through addition of water, or that can be sprinkled on food. Similarly, because of increasing renal dysfunction related to ageing, flexible dosage forms of some drugs are also advantageous for geriatric application.

Powders for reconstitution are attractive dry formulations because of both their compactness compared to liquid oral dosage forms and their advantages in storage and transport. Powders for reconstitution allow flexible application in small size, and are therefore fit for paediatric and geriatric drug administration. TMC278 is practically insoluble in water (<0.0001 mg/ml), and has a log*P*-value of 4.88 (internal report). Its solubility can be increased by converting it into a salt, of which only salt forms with strong acids (e.g. hydrochloric acid) show adequate solubility profiles.

The first part of this paper describes the formulation process for powders for reconstitution. TMC278 is formulated as a solid dispersion using one or more water-soluble carriers. Upon addition of water, the powder is converted into a supersaturated drug solution for oral administration. The selection of suitable carriers was based on a supersaturation screening study.

The second part of this paper reports on a bioavailability study in beagle dogs, in which the plasma levels of TMC278 from the powder for reconstitution are compared to those from tablets.

2. Materials and methods

2.1. Materials

TMC278. (Tibotec, Mechelen, Belgium), HPMC 2910 5 mPa s (Dow Chemical Company, Midland, MI, USA), PEG 6000 (Clariant, Gendorf, Germany), PVP-VA 64, PVP K25 and Kollicoat IR (all BASF, Ludwigshafen, Germany), Tween 20 (Uniqema, Everberg, Belgium), Cremophor EL (Federa, Brussels, Belgium), d-alpha tocopheryl polyethylene glycol 1000 succinate (Vit E TPGS; Peboc Division of Eastman, Anglesey, UK), L-(+)-tartaric acid, 99+% (Acros Organics, Geel, Belgium), citric acid-1-hydrate gritty, extra pure (Riedel-de Haën, Seelze, Germany), hydrochloric acid min. 37% Extra pure Ph. Eur. and hydrochloric acid 1 mol/l 1 N, Titrinorm ready to use (VWRInternational, Fontenay sous Bois, France), Milli-Q water obtained with a Veolia purification system (Veolia Water systems, High Wycombe, UK), dimethylformamide, Normapur analytical reagent (DMF; VWRInternational) and dimethylformamide, pure (Acros Organics) dimethylsulfoxide, p.a. (DMSO; Acros Organics), acetonitrile far UV, HPLC gradient grade (ACN; Fisher Scientific, Leicestershire, UK), potassium dihydrogen phosphate Analytical Reagent (Riedel-de Haën), dichloromethane analytical reagent grade (Fisher Scientific), methanol for HPLC (Acros Organics), methanol spectrophotometric grade, acetic acid, ammonium formate, formic acid, all of analytical grade (Merck, Darmstadt, Germany), NH_4OH 25%, analytical grade (Fisher Scientific, Leicestershire, UK), and liquid nitrogen (Air Liquide Medical, Machelen, Belgium) were used as received.

2.2. Methods

2.2.1. Supersaturation screening in HCl 0.01 M in the presence of polymers, surfactants and acids

Solutions of the polymers HPMC 2910 5 mPa s, PEG 6000, PVP-VA 64, PVP K25 and Kollicoat IR were prepared in concentrations of 8%, 4%, 1%, 0.1%, 0.05% and 0.01% (w/v), while those of the surfactants Tween 20, Cremophor EL and Vit E TPGS were prepared in concentrations of 2%, 1%, 0.1%, 0.05% and 0.01% (w/v), each in HCl 0.01 M. Solutions of citric acid and tartaric acid were prepared in concentrations of 5%, 3%, 1%, 0.1%, 0.05% and 0.01% (w/v), once in HCl 0.01 M and once in water.

Two-hundred microliters of a 15 mg/ml TMC278 stock solution in DMF was mixed with 10 ml of solutions of polymer, surfactant or acid in HCl 0.01 M, or of the acid in water, in glass test tubes (=time 0), and stirred in a Model L 26 rotary-mixer (Labinco BV, Breda, The Netherlands) at 26 rpm. An 800 μl sample was taken from the test tubes at time points 5, 30, 60 and 120 min, and was filtered through a 0.45 μm polytetrafluoroethylene filter (Macherey Nagel, Düren, Germany). No substitution of volume in the test tubes was done. The samples were gathered at room temperature. Prior to HPLC-analysis, the samples were 1:5 diluted with DMF and vortexed.

2.2.2. Supersaturation screening in water in the presence of polymers, surfactants and acids

In a second step, the experimental setup simulates more closely the eventual use of the formulation, involving the prior dissolution/suspension under stirring of a dose of the powder for reconstitution in a glass of water, which upon drinking comes quantitatively into the acidic environment of the stomach.

Therefore, the solutions of the polymers HPMC 2910 5 mPa s, PEG 6000, PVP-VA 64, PVP K25 and Kollicoat IR were prepared in concentrations of 8%, 4%, 1%, 0.1%, 0.05% and 0.01% (w/v), whereas solutions of the surfactants in concentrations of 2%, 1%, 0.1%, 0.05% and 0.01% (w/v), and the acids citric acid and tartaric acid were prepared in concentrations of 5%, 3%, 1%, 0.1%, 0.05% and 0.01% (w/v), this time each in water.

Twenty microliters of a 15 mg/ml TMC278 solution in DMF was added to 1.0 ml of the aqueous media, and after 5 min stirring in the rotary-mixer at 26 rpm 10 ml of HCl 0.01 M was added (=time 0).

The rest of the conditions remain the same as those discussed in section 2.2.1.

2.2.3. Preparation of solutions for spray-drying

TMC278, HPMC 2910 5 mPa s, PVP-VA 64 and Cremophor EL were weighed on an analytical balance XS105 (Mettler Toledo, Schwerzenbach, Switzerland), and were dissolved in dichloromethane/methanol 1:1 (v/v) using magnetic stirring. Six solutions differing in mass ratios and composing substances were prepared: TMC278/HPMC 2910 5 mPa s 1:4 and 1:9 (w/w), and TMC278/HPMC 2910 5 mPa s/Cremophor EL 1:8.5:0.5 (w/w/w), each combination dissolved so that the solid substance/solvent ratio is 10:90 (w/v), and TMC278/PVP-VA 64 1:4 and 1:9 (w/w), and TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w), each with solid substance/solvent ratio of 5:95 (w/v).

2.2.4. Preparation of solid dispersions by spray-drying

The solutions were spray-dried with a Büchi Mini Spray Dryer B-191 (Büchi, Flawil, Switzerland), applying an inlet temperature of 80 °C, a flow control of 800 l N/h pressurized air, an aspirator setting of 100%, and a peristaltic pump setting at 40% (6.8 ml solution/min). Afterwards, the resulting powders were stored in either a Vuototest vacuum dryer (Mazzali Systems, Barlassina, Italy) with a KNF Laboport vacuum pump (Neuberger, Freiburg, Germany) at room temperature for five days (thereby regularly interrupting the vacuum to enable the dynamic process of solvent evaporation), or in a Christ Alpha (Medizinischer Apparatebau, Osterode/Harz, Germany) vacuum dryer, from which the solvent was continuously removed.

2.2.5. Dissolution

The setup of the dissolution study was to first disperse an amount of powder for reconstitution that equals a dose of 25 mg TMC278 in 50 ml water, and afterwards to put this dispersion in 0.5 l of dissolution medium (HCl 0.01 M).

The dissolution was performed according to the paddle method at 37 °C as described in USP 29, method 2 [13]. The stirring rate was set at 100 rpm, and the experiments were executed using the Hanson SR8PLUS Dissolution Test Station (Hanson Virtual Instruments, Hanson Research, CA, USA). A sample of 5 ml was isolated from the medium at time points 5, 15, 30, 45, 60, 90 and 120 min and filtered through a 0.45 μm polytetrafluoroethylene filter (Macherey Nagel, Düren, Germany) in a glass test tube. At each sampling point, a volume of blank dissolution medium equal to the amount of sample isolated was added to the dissolution vessel for substitution. All six solid dispersions were analyzed in triplicate; the preferred two were re-examined in sixfold.

The release of TMC278 from the solid dispersions was quantified using HPLC (cfr. Section 2.2.7), and was expressed as percentage of the added amount TMC278, corrected for substitution.

2.2.6. Stability of the solid dispersions

The stability of the eventually selected solid dispersions was examined by dissolution testing as described in Section 2.2.5. The spray-dried powders (six tests per powder) were investigated after 1 month storage at 25 and 40 °C. Their stability was expressed as the percentage TMC278 dissolved at 120 min dissolution considering the percentage TMC278 dissolved immediately after production of the spray-dried powder (Section 2.2.5) as 100%.

Table 1
Gradient program for the dissolution quantification of TMC278 powder for reconstitution

Time (min)	% B (v/v)
0.0 → 2.0	0 → 100
2.0 → 6.0	100
6.0 → 6.5	100 → 0
6.5 → 8.0	0

2.2.7. Dissolution quantification of TMC278 with HPLC

The HPLC-instrument consisted of a P680 HPLC pump, an ASI-100 automated sample injector and a UVD 170 U detector (Dionex Corporation, Sunnyvale, CA, USA); a Julabo EM heating circulator was used to reach a fixed column temperature. The data were gathered and treated with Chromeleon version 6.60 (Dionex) HPLC software.

The stationary phase used was Hypersil BDS C18 (250 mm × 4.6 mm, 5 μm; Thermo-Hypersil-Keystone, Cheshire, UK).

During the supersaturation screening, a rapid HPLC method was developed, using ACN/0.2 M potassium dihydrogen phosphate/water 70:5:25 (v/v/v), applying a flow rate of 2.0 ml/min, a detection wavelength of 270 nm, an injection volume of 20 μl, a column temperature of 30 °C, and a run time of 2.5 min. Every standard was measured in fourfold, and every sample in duplicate.

During the dissolution tests, a gradient program was run (Table 1) between mobile phase A (ACN/0.2 M potassium dihydrogen phosphate/water 10:20:70 (v/v/v)) and B (ACN/0.2 M potassium dihydrogen phosphate/water 50:20:30 (v/v/v)), at a flow rate of 2.0 ml/min; 20 μl was injected, the column temperature was set at 40 °C, and the detection wavelength at 270 nm. Every sample was injected in triplicate.

2.2.8. Animal study

Six male beagle dogs (Marshall Farms, Green Hill 2001, Italy), approximately 1–3 years old and weighing between 8 and 14 kg at the start of the experimental phase, were used. Animals were treated in accordance with the following legislation: the provisions of the Belgian law of 18 October 1991 on the approval of the European convention on the protection of vertebrates that are used for experimental and other scientific purposes, and also of annexes A and B, drawn up in Strasbourg on 18 March 1986; the Royal Decree of 14 November 1993 on the protection of laboratory animals. The study was approved by the local ethics committee on animal experiments, and was performed in an AAALAC-accredited laboratory, and complied with European and Belgian regulations for animal experiments. The dogs were given free and continuous access to water. Each dosing day, the dogs were deprived of food approximately 18 h before dosing, and were fed immediately after dosing with free access to the food. Dosing in the first phase of the study was done by oral gavage of 40 ml of freshly prepared aqueous suspensions of TMC278 (TMC278/PVP-VA 64 1:9 (w/w) and TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w)). Each of the two suspensions was administered to 3 dogs. Per dog, two vials each containing 25 mg of TMC278, formulated as TMC278/PVP-VA 64 1:9 (w/w) powder for reconstitution (dogs 1, 2, 3), or two vials each containing 25 mg of TMC278, formulated as TMC28/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) powder for reconstitution (dogs 4, 5, 6), were applied. After a washout period of 14 days, all 6 dogs were dosed by administration of a tablet for clinical use of TMC278.HCl (Lot PD1944, Sample 88348), providing a dose of 50 mg TMC278 free base per animal.

Blood samples (1 ml on EDTA, EDTA Vacuette Greiner, Cat. No. 454034, Greiner Labortechnik, Kremsmuenster, Austria) were taken from a jugular vein from the dogs at time 0 (=predose) and at 0.5 (30 min), 1, 2, 4, 8, 24, 32 and 48 h after dose administration. At all times, blood and plasma samples were protected from light, including storage and handling. The samples were centrifuged within 2 h of blood sampling at about 1900g for approximately 10 min at room temperature in a Hettich Rotixa 50 R centrifuge (Hettich, Bäch, Switzerland) to allow plasma separation. Immediately thereafter, plasma was separated, transferred into a second tube and stored in the freezer at approximately −20 °C within 2 h after the start of centrifugation. Plasma samples were transferred to the Bioanalytical Department for further storage in a freezer (approximately −20 °C) until analysis. The plasma samples

were analyzed using LC-MS/MS in accordance with Bioanalysis departmental SOPs. Plasma concentrations of TMC278 were determined after proper sample clean-up. 0.1 ml aliquots of plasma were extracted using a Bond Elut Certify solid phase column, 130 mg for solid phase extraction (Varian, Harbor City, CA, USA). The stable isotope labeled TMC278 was used as internal standard. The SPE column was conditioned with 3 ml methanol, 3 ml water and 1 ml acetic acid of 1 M.

After addition of 3 ml acetic acid to 0.1 ml aliquots of plasma the samples were extracted on the column followed by washing the column with 3 ml water, 1 ml acetic acid of 1 M and 3 ml methanol. The column was eluted with 3 ml methanol/NH_4OH 25% 98:2 (v/v). The extract was evaporated to dryness, and was reconstituted in 150 μl of ammonium formate 0.01 M (adjusted to pH 4 with formic acid)/methanol 50/50 (v/v). Twenty microliters of aliquots were injected onto a Polaris C18-A reversed phase LC-column (5 cm × 4.6 mm; 3 μm; Varian, Palo Alto, CA, USA) operating at ambient temperature at a flow rate of 1 ml/min before splitting. The elution mixture was ammonium formate 0.01 M (adjusted to pH 4 with formic acid)/methanol 35:65 (v/v). LC-MS/MS analysis, applying multiple reaction monitoring (MRM), was carried out on an API-3000 system (Applied Biosystems/Sciex, Foster City, CA, USA), which was coupled to an HPLC system (Agilent, Palo Alto, CA, USA).

The MRM transitions used for the quantification were *m/z* 367.2 → 224.0 and *m/z* 372.2 → 225.0 for TMC278 and its internal standard, respectively. The calibration range was 1–2000 ng/ml. The lower limit of quantification (LLOQ) of 1.0 ng/ml was attained.

3. Results and discussion

The formulation of the free base of TMC278 as a powder for reconstitution was optimized in consecutive steps.

3.1. Supersaturation screening

When a solid dispersion made up of a carrier and a drug with low water solubility is dissolved, a carrier suitable for supersaturation maintenance will create a microenvironment that can keep the active substance in solution, thus enhancing and sustaining its dissolution and oral bioavailability [14]. The selection of suitable carriers was based on a supersaturation screening approach.

The screening for appropriate carriers was obtained by predissolving the single carriers (polymers: HPMC 2910 5 mPa s, PEG 6000, PVP-VA 64, PVP K25 and Kollicoat IR, and surfactants: Tween 20, Cremophor EL and Vit E TPGS) in different concentrations in an appropriate dissolution medium (HCl 0.01 M), after which TMC278, dissolved in DMF, was added. Thereby, the drug concentration in its solvent was maximized (a) to minimize the influence of this solvent on the supersaturation results and (b) to enable discrimination of the supersaturation potential of the different carriers and their concentrations. Using a solution of TMC278 was preferred over adding the solid substance to the test tubes because the former allows to add a constant amount of drug to the medium and enables evaluating whether the carrier and its concentration can sustain the initial supersaturation. Samples were taken at standard time intervals, and the supersaturation experiments were conducted for 2 h. Hence, it could quickly be checked whether the carrier can create a microenvironment able to sustain the supersaturation of TMC278 with low water solubility. In fact, this already simulates how the compound will remain dissolved in the stomach (HCl 0.01 M) in the presence of these carriers. Moreover, the influence of acid on the supersaturation was examined by predissolving different concentrations of citric acid and L-(+)-tartaric acid in HCl 0.01 M and in water, the latter media prepared to estimate the pure effect of the predissolved acid.

The use of the powder for reconstitution consists of dissolving/suspending the powder in a glass of water, prior to administration. Subsequently, the drug comes into the acidic environment of the stomach, and the salt is formed in situ in an effective therapeutic concentration.

To simulate the dissolution/suspension of the powder for reconstitution into a glass of water that is afterwards quantitatively administered, the above-described screening was additionally executed in an adapted way, that is, adding the concentrated drug solution to water in which the different carriers were dissolved in different concentrations. After stirring, the dissolution medium was added quantitatively. Also here, the influence of acids on the supersaturation of the active ingredient was assessed.

Fig. 2 visualizes the successful supersaturation profiles of TMC278 in the different concentrations of (a) HPMC 2910 5 mPa s, (b) PVP-VA 64, (c) PVP K25, (d) Cremophor EL, (e) Vit E TPGS, and (f) citric acid dissolved in HCl 0.01 M, with sampling points at 5, 30, 60 and 120 min. The profiles express the concentration of TMC278, with 100% equal to the 15 mg/ml concentration of stock solution added to the supersaturation media.

The polymers HPMC 2910 5 mPa s, PVP-VA 64 and PVP K25 are able to sustain the initial supersaturated condition of TMC278 for the complete duration of the experiment, and independent of their concentration in the medium. For Vit E TPGS and Cremophor EL, only their 2% and 1% concentrations were able to keep the 100% supersaturation level, while the lower concentrations of surfactants merely sustain this condition in the first 60 and 30 min, respectively, after which precipitation was seen. All percentages of citric acid keep up the 100% TMC278 level in solution except for the 0.01% citric acid medium, which only guarantees 100% supersaturation during the first hour.

Fig. 3 visualizes the supersaturation profiles of TMC278 in the different concentrations of (a) HPMC 2910 5 mPa s, (b) PVP-VA 64, (c) Cremophor EL, and (d) Vit E TPGS dissolved in water, after which this medium was quantitatively mixed with HCl 0.01 M, with sampling points at 5, 30, 60 and 120 min, expressed as concentration of TMC278 stock solution dissolved (100% = 15 mg/ml).

It can be concluded that the polymers HPMC 2910 5 mPa s and PVP-VA 64 in general sustain the supersaturation of TMC278 even if the carrier is first dissolved in water and afterwards 1:10 diluted in HCl 0.01 M, as would be the case with prior suspension of the powder for reconstitution in a glass of water followed by oral administration. They guarantee supersaturation during the complete experiment and independent of their concentration. The 2% and 1% solutions of the surfactants Vit E TPGS and Cremophor EL can also keep up the 100% supersaturation level under these new conditions. The lower concentrations show a small decrease in supersaturation, however, keeping TMC278 in solution at a constant concentration during the complete experiment, with slightly higher TMC278 levels for Cremophor EL when comparing the same percentages of surfactants. The lower concentrations of PVP K25 and all examined percentages of citric acid dissolved in water immediately showed a small decrease of the concentration TMC278 (figures not shown).

From Figs. 2 and 3, it can be concluded that HPMC 2910 5 mPa s and PVP-VA 64 are the best performing polymers with respect to their supersaturation potential towards TMC278, whereas Cremophor EL is the best surfactant for this purpose. It was decided not to include the acid in the powder for reconstitution because some conditions and concentrations do not support keeping TMC278 supersaturated.

3.2. Dissolution testing of six selected TMC278/carrier mixtures

The next step involved the formulation of six powders for reconstitution (PFR) selected as representing the best conditions

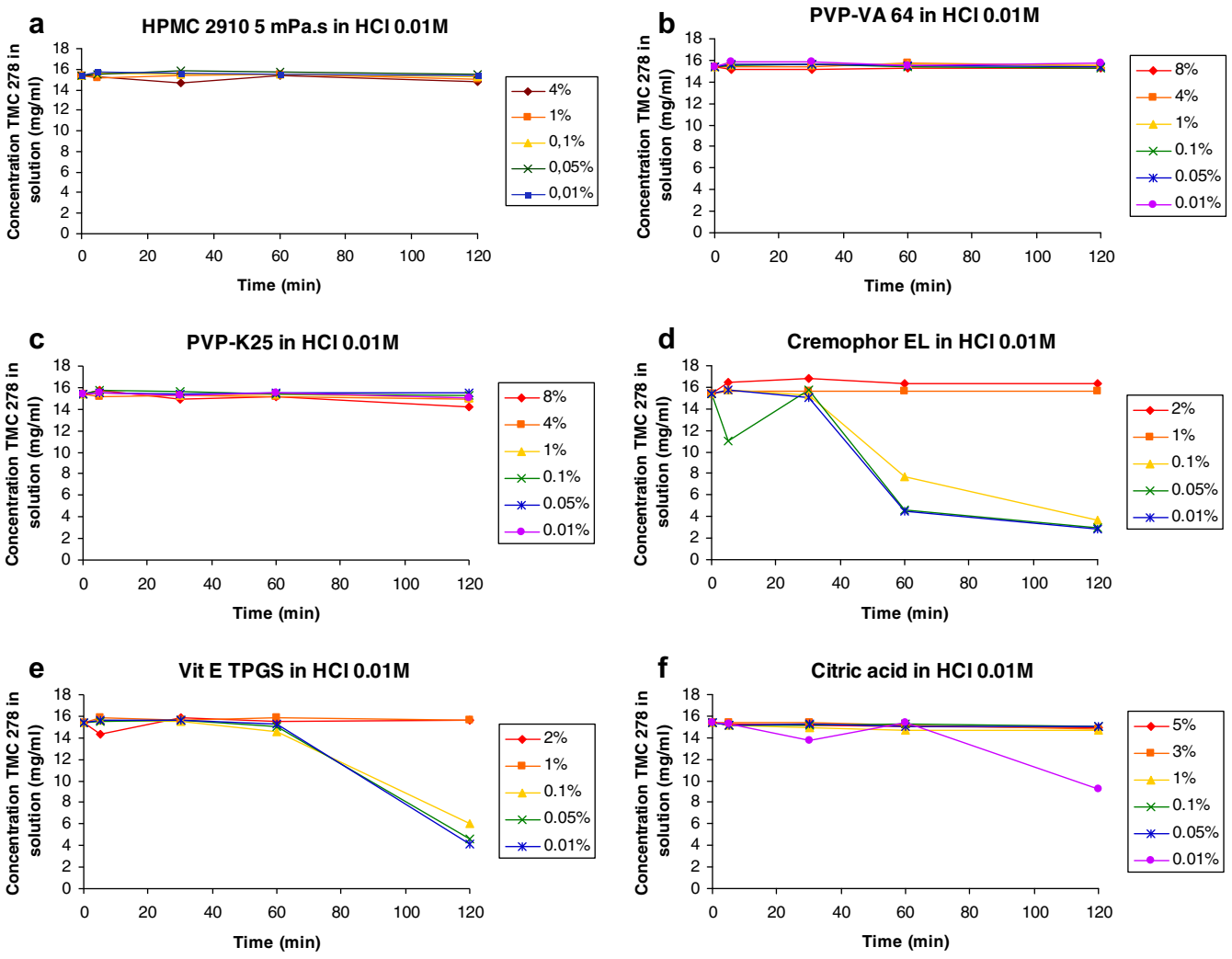

Fig. 2. Supersaturation profiles of TMC278 in the different concentrations of (a) HPMC 2910 5 mPa s, (b) PVP-VA 64, (c) PVP K25, (d) Cremophor EL, (e) Vit E TPGS, and (f) citric acid dissolved in HCl 0.01 M, with sampling points at 5, 30, 60 and 120 min, expressed as concentration of TMC278 stock solution in solution (100% = 15 mg/ml).

based on the supersaturation screening results and using spray-drying. The six powder formulations are described in Table 2.

All six formulations were as easy to process via spray-drying: the same instrumental parameters could be used, and the solutions to be spray-dried smoothly ran over the peristaltic pump and through the nozzle of the apparatus. Yet, each time some of the spray-dried material stuck to the spray cilinder, the separator and the product collector, and was sucked into the filtering bag. The yield of the process was ca. 50% for PFR1, ca. 60% for PFR2, ca. 45% for PFR3, ca. 74% for PFR4, ca. 47% for PFR5, and ca. 63% for PFR6. The flow properties of the spray-dried material were sufficient to fill them into capsules.

The six powders were investigated by dissolution. The dose of TMC278 in 250 mg of PFR1, PFR3, PFR5 and PFR6 and in 125 mg of PFR2 and PFR4 is 25 mg. Differences in the ease of applicability raised during their prior suspension in 50 ml of water: PFR4 did not suspend even after thorough stirring; PFR1 floated on the water surface while PFR2 was easier, yet slowly, suspendable; PFR3 and PFR5 suspended only if stirred thoroughly, and PFR6 suspended best after stirring.

Afterwards, the dissolution of the above mixtures and suspensions was examined in triplicate, considering the theoretical amount of TMC278 present as 100%. All samples were transferred quantitatively to the dissolution medium. All powders for reconstitution reached a dissolution plateau after 5 min and sustained this supersaturation level during the complete duration of the experiment. However, PFR3 and PFR6 dissolved 99.4 ± 2.5% and 98.5 ± 1.0% of TMC278, respectively, whereas all other formulations' dissolution plateaus leveled somewhat lower, i.e. 95.3 ± 2.7% (PFR1), 96.2 ± 1.3% (PFR2), 96.7 ± 3.3% (PFR4) and 87.2 ± 2.8% (PFR5). Paired *t*-tests for averages calculated on the 5% significance level (two-sided) showed that the dissolution percentages of all powders for reconstitution differ significantly, except the comparison of PFR1-PFR2, PFR2-PFR4 and PFR3-PFR6. This allows to conclude that PFR3 and PFR6 can be preferred for their dissolution performances compared to the remaining powders for reconstitution, yet none of the two proves advantageous dissolution behaviour over the other.

Considering the similar ease of processing of the solutions to spray-dry, and the resemblant flow characteristics of the six powders for reconstitution, which otherwise could affect dissolution, no selection could be made between them based on these properties. For PFR6, the one but highest process yield was obtained. Although PFR3 is associated with a somewhat lower yield compared to the other powders for reconstitution, its beneficial applicability and dissolution properties were considered more important.

Based on the ease of applicability, i.e. suspendability in water, and dissolution results of the six powders for reconstitution, PFR3 and PFR6 were preferred, and a batch of each was produced for further *in vitro* and *in vivo* testing.

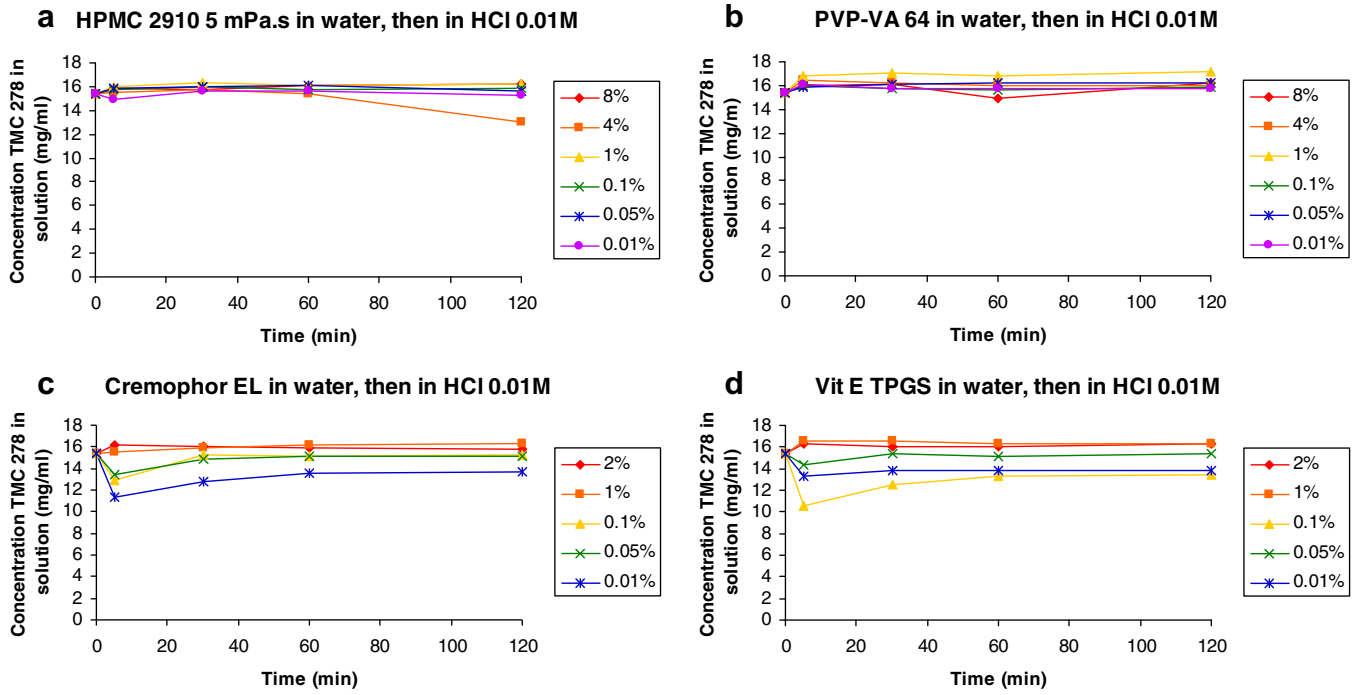

Fig. 3. Supersaturation profiles of TMC278 in the different concentrations of (a) HPMC 2910 5 mPa s, (b) PVP-VA 64, (c) Cremophor EL, and (d) Vit E TPGS dissolved in water, after which this medium is quantitatively mixed with HCl 0.01 M, with sampling points at 5, 30, 60 and 120 min, expressed as concentration of TMC278 stock solution in solution (100% = 15 mg/ml).

Table 2
Composition of six powders for reconstitution (PFR) selected as representing the best conditions based on the supersaturation screening results

Name	Composition
PFR1	TMC278/HPMC 2910 5 mPa s (1:9, w/w)
PFR2	TMC278/HPMC 2910 5 mPa s (1:4, w/w)
PFR3	TMC278/PVP-VA 64 (1:9, w/w)
PFR4	TMC278/PVP-VA 64 (1:4, w/w)
PFR5	TMC278/HPMC 2910 5 mPa s/Cremophor EL (1:8.5:0.5, w/w/w)
PFR6	TMC278/PVP-VA 64/Cremophor EL (1:8.5:0.5, w/w/w)

3.3. In vitro dissolution/stability study results of PFR3 and PFR6

Table 3 summarizes the mean dissolution percentages (±SD; $n = 6$) of TMC278 in HCl 0.01 M immediately after production of the spray-dried powders and after 1 month storage at 25 °C and 40 °C for solid dispersions (a) TMC278/PVP-VA 64 1:9 (w/w) (PFR3) and (b) TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) (PFR6), expressed as a percentage of the theoretical amount of TMC278 present in the dispersions.

Table 3
Mean dissolution percentages (±SD; $n = 6$) at sampling points 5 and 120 min, and averaged over the complete dissolution plateau 5–120 min of TMC278 in HCl 0.01 M immediately after the production of the spray-dried powders and after 1 month storage at 25 and 40 °C for solid dispersions (a) TMC278/PVP-VA 64 1:9 (w/w) (PFR3) and (b) TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) (PFR6), expressed as a percentage of the theoretical amount of TMC278 present in the dispersions

	Mean dissolution percentages		
	At 5 min	At 120 min	Plateau 5–120 min
Freshly prepared PFR3	94.5 ± 1.5%	93.7 ± 0.7%	93.9 ± 1.5%
PFR3, 1 month at 25 °C	93.7 ± 1.4%	93.7 ± 0.7%	93.7 ± 1.3%
PFR3, 1 month at 40 °C	92.5 ± 1.7%	93.4 ± 1.0%	93.3 ± 1.5%
Freshly prepared PFR6	93.3 ± 1.2%	93.1 ± 0.8%	93.2 ± 1.4%
PFR6, 1 month at 25 °C	90.9 ± 1.4%	92.4 ± 0.3%	91.3 ± 1.4%
PFR6, 1 month at 40 °C	93.1 ± 0.9%	92.5 ± 0.9%	92.4 ± 0.8%

For all six dissolution replicates of the freshly prepared batches of both PFR3 and PFR6 and after 1 month storage at 25 and 40 °C, TMC278 reached a steady state plateau after 5 min, as the mean dissolution percentages resemble those at 120 min and between 5 and 120 min (Table 3). This may be due to rapid dissolution of the powder for reconstitution when suspended in water.

For the freshly prepared batches, a paired *t*-test for averages calculated on the 5% significance level (two-sided) showed no significant difference between the mean percentages of TMC278 released from PFR3 and PFR6. Thus it was decided to consider both of them further for stability study.

Considering the mean dissolution percentages of TMC278 from the freshly prepared batches as 100%, the release of the drug at 120 min was 100.0% and 99.2% after storing PFR3 and PFR6 for 1 month at 25 °C, and 99.7% and 99.4% after 1 month storage at 40 °C.

To conclude, very rapid and almost complete dissolution of TMC278 was reached and maintained over the complete duration of the dissolution experiment, for both the freshly prepared batches of PFR3 and PFR6 and after 1 month storage at 25 and 40 °C, justifying the eventual selection of powders for reconstitution. Since PFR3 and PFR6 give rise to very similar dissolution profiles and release percentages no advantage was detected for one powder over the other.

After the storage period, both formulations seem stable as their content exceeds the conventionally accepted 95% threshold (considering the content of drug released from the freshly prepared batches as 100%), and moreover approaches or equals 100%. Since no difference could be detected in their dissolution profiles and 1 month stability data at 25 and 40 °C, both PFR3 and PFR6 were further considered for the *in vivo* study.

3.4. In vivo study of PFR3 and PFR6

Individual plasma concentration versus time profiles were subjected to a non-compartmental pharmacokinetic analysis using validated WinNonlin software v4.0.1a (Pharsight Corporation,

Mountain View, CA, USA). Peak concentrations (C_{max}), corresponding peak times (T_{max}), half-lives ($t_{1/2}$) and AUC-values were determined for TMC278. Mean ($n = 3$ or 6, ±SD) pharmacokinetic parameters per formulation were calculated. This experiment in two groups of 3 dogs aimed to obtain a general impression on the comparative pharmacokinetics for the two powders for reconstitution and for the clinical tablet. It was not powered for formal bioequivalence statistics. Descriptive statistics were calculated for all dose groups. The dose of TMC278 in the powder for reconstitution was 50 mg, expressed in TMC278-base equivalents, to enable comparison with the clinical TMC278.HCl tablet. Actual doses in mg per kg body weight were calculated using individual dog weights. Therefore, the relative bioavailability (F_{rel}) of TMC278 dosed with the powders for reconstitution versus the clinical tablet was estimated individually.

Individual and mean ($n = 3$ or 6, ±SD) pharmacokinetic parameters of TMC278 after single oral dosing of a suspension of the powder for reconstitution or a tablet of TMC278 are reported in Table 4, as are the individual relative bioavailability values. Mean plasma concentration versus time profiles of TMC278 are depicted in Fig. 4.

After oral administration of a suspension of TMC278/PVP-VA 64 1:9 (w/w) powder for reconstitution 3 (treatment A) and of a suspension of TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) powder for reconstitution 6 (treatment B), both at 50 mg of TMC278 (actual dose range: approximately 5 mg/kg), high plasma concentrations were already observed at 0.5 h after dosing, demonstrating the absence of an absorption lag time. Peak concentrations were attained more rapidly with treatment A than with treatment B. However, both powders for reconstitution yielded a faster absorption than the clinical tablet (Treatment C), for which C_{max} was attained only after 2 to 24 h post-dose. Beyond C_{max}, plasma levels declined slowly for the powders for reconstitution and for the clinical tablet, with similar ranges for the half-life-values (>30 h), as evaluated between 32 and 48 h ($t_{1/2,\ 32-48h}$). As the half-lives were long, the $AUC_{0-\infty}$-values could not be calculated adequately, with over 30% of the AUC extrapolated. Therefore the overall exposure to TMC278 was expressed by AUC_{0-48h}-values. It needs to be mentioned that dog 1 shows an aberrant T_{max}-value leading to a distorted average and standard deviation. Removal of the outlier ($n = 5$) leads to a mean (±SD) T_{max}-value of 4 ± 4 h.

The relative bioavailability from the powder for reconstitution 3 containing TMC278/PVP-VA 64 1:9 (w/w) versus the clinical tablet was on average 82%, ranging between 69% and 89% (details in Table 4). The relative bioavailability of the formulation PFR6 containing TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) averaged 125%, ranging from 85% to 157%.

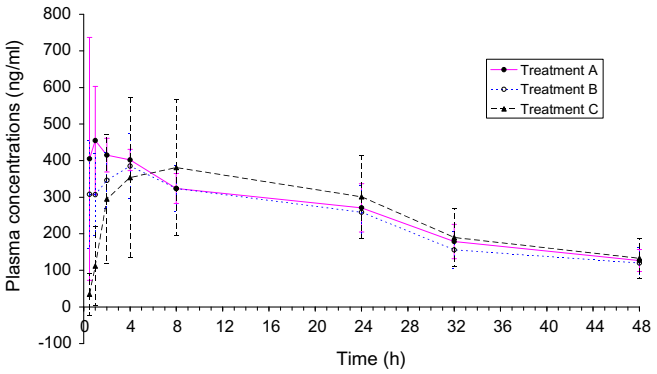

Fig. 4. Mean plasma concentration (ng/ml) versus time profiles and standard deviation of TMC278 in fasted male beagle dog after single oral administration of 3 different formulations at a dose of 50 mg equiv TMC278 (treatment A (●):TMC278/PVP-VA 64 1:9 (w/w) powder (PFR3); treatment B (o): TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) powder (PFR6); treatment C (▲): a tablet of TMC278).

Taking into account the variability, as evidences through the individual data and the standard deviation (SD) on the different pharmacokinetic parameters and F_{rel} (Table 4), it can be concluded that PFR3 and PFR6 do not differ from each other and from the clinical tablet in a clinically relevant way. Taking this variability into account, the comparative mean plasma concentration time plots (Fig. 4) also indicate no relevant differences in plasma concentrations following intake of PFR3 and PFR6, in spite of apparently faster absorption with PFR3.

The above results prove the equivalence or potentially advantageous *in vivo* behaviour of PFR3 and PFR6 with reference to the clinical tablet. The powders' fast, almost complete and maintained *in vitro* dissolution is translated *in vivo* with a fast release and high mean plasma concentrations within the first 4 h. The clinical tablet's release appears slower as it shows a delayed maximum mean plasma concentration compared to the powders' for reconstitution. With the limited number of animals involved, no relevant differences in *in vivo* performance can be claimed for either PFR3 or

Table 4
Individual and mean pharmacokinetic parameters of TMC278 after single oral administration of 50 mg equiv TMC278 of (A) a powder for reconstitution containing TMC278/PVP-VA 64 1:9 (w/w) (PRR3; $n = 3$, mean ± SD), (B) a powder for reconstitution containing TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) (PFR6; $n = 3$, mean ± SD), and (C) a clinical tablet of 50 mg equiv TMC278.HCl ($n = 6$, mean ± SD) in fasted male beagle dogs, and individual relative bioavailability (F_{rel}) of TMC278 as two powders for reconstitution (A and B) as compared to the clinical tablet (C)

	Dog	1	2	3	4	5	6	Mean	SD
Treatment A	Actual dose (mg equiv/kg)	4.83	4.31	5.75					
	C_{max} (ng/ml)	428	427	778				544	202
	T_{max} (h)	1	4	0.5				2	2
	$t_{1/2,\ 32-48h}$ (h)	33.8	44.5	25.2				34.5	9.7
	AUC_{0-48h} (ng h/ml)	9910	12300	13600				11900	1870
Treatment B	Actual dose (mg equiv/kg)				3.83	6.06	5.26		
	C_{max} (ng/ml)				485	379	466	443	57
	T_{max} (h)				4	8	0.5	4	4
	$t_{1/2,\ 32-48h}$ (h)				76.1	31.7	32.6	46.8	25.4
	AUC_{0-48h} (ng h/ml)				12600	12400	8520	11200	2290
Treatment C	Actual dose (mg equiv/kg)	4.90	4.55	5.95	3.88	6.25	5.15		
	C_{max} (ng/ml)	370	647	581	536	518	351	501	117
	T_{max} (h)	24	4	8	2	4	2	7	9
	$t_{1/2,\ 32-48h}$ (h)	24.0	37.1	29.9	133.0	22.7	27.7	45.7	43.1
	AUC_{0-48h} (ng h/ml)	11100	17900	15200	9530	14600	5440	12300	4480
	F_{rel} (%)	89%	69%	89%	132%	85%	157%		

PFR6. Therefore, it was not possible to choose which of the two formulations is to be preferred. The advantageous *in vitro* and *in vivo* results of both powders for reconstitution, augmented with their possibility of flexible dosing, make PFR3 and PFR6 good alternatives for the clinical tablet and reasonable starting points for the development of paediatric formulations.

4. Conclusions

Powders for reconstitution of TMC278 were developed to provide potential dry formulations offering flexible dosage regimens combined with small dosage size, convenient for patients who are looking for a different approach for antiretroviral (ARV) therapy. Upon addition of water, the powders are converted into a supersaturated solution for oral administration. A screening procedure was developed to simulate real-life predictions for formulating solid dispersions with carriers that can sustain supersaturation and thus enhance oral bioavailability. After the initial development of six selected powders for reconstitution, two formulations were preferred based on the results of ease of applicability and dissolution: TMC278/PVP-VA 64 1:9 (w/w) (PFR3) and TMC278/PVP-VA 64/Cremophor EL 1:8.5:0.5 (w/w/w) (PFR6). Both powders showed ease of suspension in water prior to dissolution. A steady state dissolution level around 93% was reached at the first sampling point, and stability after 1 month storage at 25 and 40 °C was at least 99.7% and 99.2% for PFR3 and PFR6, respectively. Beagle dog studies comparing the suspended powders for reconstitution with the clinical TMC278.HCl tablet showed a slower absorption of the latter, and relative biovailabilities slightly lower than the clinical tablet for PFR3 (mean F_{rel} of 82%) and somewhat higher relative to the clinical tablet for PFR6 (mean F_{rel} of 125%). Taking into account the variability of the *in vivo* parameters, no difference was observed for PFR3 and PFR6. Yet the equivalent or slightly advantageous behaviour of both powders compared to the tablet and their possibility of flexible dosing mean that they could extend the clinical formulations of TMC278 in the future.

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