FORMULATION OF ITRACONAZOLE NANOCOCRYSTALS AND EVALUATION OF THEIR BIOAVAILABILITY IN DOGS

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ABSTRACT

The aim of the study was to increase the bioavailability of itraconazole (ITRA) using nanosized cocrystals prepared via wet milling of ITRA in combination with dicarboxylic acids. Wet milling was used in order to create a nanosuspension of ITRA in combination with dicarboxylic acids. After spray-drying and bead layering, solid state was characterized by MDSC, XRD, Raman and FT-IR. The release profiles and bioavailability of the nanococrystalline suspension, the spray-dried and bead layered formulation were evaluated. A monodispers nanosuspension (549 ± 51 nm) of ITRA was developed using adipic acid and Tween®80. Solid state characterization indicated the formation of nanococrystals by hydrogen bounds between the triazole group of ITRA and the carboxyl group of adipic acid. A bioavailability study was performed in dogs. The faster drug release from the nanocrystal-based formulation was reflected in the in-vivo results since T_{max} of mean profile after administration of the formulations was observed 3h after administration, while T_{max} of mean profile after administration of the reference formulation was observed only 6h after administration. This fast release of ITRA was obtained by a dual concept: manufacturing of nanosized cocrystals of ITRA and adipic acid via wet milling. Formation of stable nanosized cocrystals via this approach seems a good alternative for amorphous systems to increase the solubility and obtain a fast drug release of BCS class II drugs.

KEYWORDS : Itraconazole, wet milling, cocrystals, nanosuspension, dicarboxylic acids

1. INTRODUCTION

The number of poorly soluble active pharmaceutical ingredients (APIs) has increased in the last 10 years as the majority of innovative drugs in the development pipeline belong to the class II and IV of the biopharmaceutics classification system (BCS) (i.e. they are classified into the two low solubility categories). Since their low solubility and dissolution rate limits the bioavailability after oral administration, formulation development of these compounds is a major challenge [1, 2]. Strategies that have been used to increase the solubility of BCS II and IV drugs include the use of inclusion complexes, salts, solid dispersions/solutions, cocrystals, and particle size reduction [3].

Itraconazole (ITRA), a broad-spectrum triazole antifungal agent belonging to BSC class II (aqueous solubility: 1 ng/ml at pH 7 and 5 µg/ml at pH 1) [4], was for more than two decades used in pharmaceutical research as a model drug to illustrate the enhancement of its solubility and bioavailability using different approaches. While the commercially available formulation (Sporanox®) was developed as an amorphous solid dispersion of ITRA in a hydroxypropylmethylcellulose (HPMC) matrix (layered on sugar beads) to enhance its solubility [5], amorphous solid dispersions of ITRA and HPMC were also manufactured by hot melt extrusion [6, 7].

Another strategy to increase the solubility of ITRA is by the formation of cocrystals. Pharmaceutical cocrystals are defined as crystalline solids in which at least one of the molecular components is an API combined with another pharmaceutical acceptable molecule, which interact via hydrogen bonding and other weak forces [8, 9]. ITRA cocrystals have been identified in combination with dicarboxylic acids (fumaric, succinic, malic and tartaric acid), where the carboxylic acid interacts with the triazole group of ITRA via hydrogen bonds [9]. These cocrystals enhanced the solubility of ITRA to a level comparable to the solubility of the amorphous form. There is a thin line between the definition of a salt and a cocrystal and currently discussions are ongoing in the literature about the definition of a cocrystal. The acid ionization constant, pKa, is a commonly employed tool for predicting solid form molecular ionization states. When ΔpKa ($pKa_{base} - pKa_{acid}$) is lower than 1, there

will be substantially less proton transfer. If this requirement is fulfilled, the API-excipient should be classified as cocrystal. But exceptions on this rule are already noticed as some API-excipient interactions are classified as a cocrystal, although the ΔpKa lays between 1-3 [10]. Another definition is that cocrystals are in neutral state and interact via non-ionic interactions [10]. These non-ionic interactions such as hydrogen bounds are very robust, so cocyrstal forms exhibit a high thermal stability and they are also very stable at high relative humidity, where salts are known to be unstable [11]. Although cocrystals can be formed by various methods such as solution crystallization [12, 13], slurry conversions [14], ultrasonication [15, 16] and melt processes [17, 18], (wet or dry) grinding methods [19-21] are often preferred preparation methods based on their simplicity, eco-friendliness and high productivity [22]. Since wet-milling (i.e. mechanical grinding using milling pearls in a liquid medium) is also a typical top-down method to reduce the particle size of poorly water soluble drugs below 1 µm [2], this technique is used in this work to prepare nanosized cocrystals of ITRA, allowing in a single step to nanosize the ITRA particles and simultaneously form ITRA cocrystals to increase its solubility and bioavailability. Therefore, ITRA was milled in combination with dicarboxylic acids (maleic, adipic, glutaric and succinic acid) and with a surfactant (Tween 80®) to form a stable nanosuspenion. As further transformation of the nanosuspension into a solid dosage form is required to enhance its physical stability and to improve patient convenience [23], the ITRA nanosuspensions were transformed in a solid dosage form by spray-drying and bead coating.

2. MATERIALS AND METHODS

2.1 Materials

Adipic, maleic, glutaric and succinic acid were purchased from Sigma (Bornem, Belgium). Polysorbate 80 (Tween® 80) was obtained from Mosselman (Ghlin, Belgium). Itraconazole (ITRA) was kindly donated by Janssen Pharmaceutica (Beerse, Belgium).

2.2 Preparation and characterization of the ITRA nanosuspension

The cocrystalline ITRA nanosuspensions were prepared by a wet milling technique using Tween® 80 as stabilizer. After dissolving the stabilizer in a 20 ml vial containing 5 ml of demineralized water, ITRA (250 mg) was dispersed in this aqueous phase. Different dicarboxylic acids (maleic, adipic, glutaric and succinic acid) were dissolved in different concentrations in the suspension (Table 1). Zirconium oxide beads (amount 30 g, diameter 0.5 mm) were added to the suspension as milling pearls. The vials were placed on a roller-mill (Peira, Beerse, Belgium) and grinding was performed at 150 rpm for 60 h. After milling the nanoparticles were separated from the grinding pearls by sieving.

The mean particle size and polydispersity index (PDI) of the nanosuspensions was determined by photon correlation spectroscopy, using a Zetasizer 3000 (Malvern Instruments, Worcestershire, UK). Prior to analysis, nanosuspensions were diluted with distilled water.

2.3 Conversion of the nanosuspension into solid dosage forms

2.3.1 Spray-drying

A nanosuspension containing 5% ITRA, 8% adipic acid and 1.25% (w/v) Tween® 80 was selected for further processing via spray-drying. Prior to spray-drying mannitol was added to the nanosuspension in equal amounts compared to the solid fraction, yielding an ITRA concentration of 17.6% (w/w) in the final powder (ITRA-SD2). The nanosuspension, under constant stirring during processing, was spray-dried in a Büchi mini spray-dryer (Model B295, Büchi, Flawil, Switzerland) using a two-fluid

nozzle with a 0.7 mm diameter and nitrogen (at 4 bar) as drying medium. The following process conditions were used: liquid feed rate of 3.4 ml/min; inlet air temperature of 120 °C; aspiration level of 80%. The measured outlet temperature was 56 °C. The yield of the spray-drying process was calculated by dividing the obtained powder weight by the theoretical weight of the powder.

2.3.2 Bead-layering

120 ml of a diluted nanosuspension containing 4.2% ITRA, 6.7% adipic acid and 1.0 % Tween® 80 (w/v) was layered on 50 g microcrystalline cellulose (MCC) beads (Cellets®500, Pharmtrans Sanac, Switzerland) using an Oystar Hüttlin Mycrolab (Hüttlin, Schopfheim, Germany). The instrument parameters were set as follow: inlet temperature of 66 °C, atomizing air pressure of 0.8 bar, spray rate of 3 g/min. Prior to the bead layering process mannitol was added in equal amounts compared to the solid fraction of the nanosuspension, yielding an ITRA concentration of 17.6% in the layer and 6.4% overall in the beads (ITRA-BL1).

2.4 Characterization

2.4.1 Thermal analysis

The thermal properties of pure compounds, physical mixtures and corresponding spray-dried formulations were analyzed using a differential scanning calorimeter Q2000 (TA instruments, Zellik, Belgium) equipped with a refrigerated cooling system. Samples (± 5 mg) were run in Tzero pans (TA instruments, Zellik, Belgium) with an underlying heating rate of 2 °C/min. The modulation period and amplitude were set at 60 s and 0.318 °C, respectively. Dry nitrogen at a flow rate of 50 ml/min was used to purge the DSC cell. The samples were evaluated according to the three cycle analysis (heating, cooling and heating) from 0 to 180 °C. Modulated differential scanning calorimetry (MDSC) data were analyzed using the Universal Analysis 2000 software (TA Instruments, Zellik, Belgium).

2.4.2 X-ray diffraction

The crystallinity of pure compounds, physical mixtures and corresponding spray-dried formulations was determined via X-ray diffraction using a D5000 Cu Kα diffractor (λ = 0.154 nm) (Siemens, Karlsruhe, Germany) with a voltage of 40 mV in the angular range of 10° < 2ϑ < 60° using a step scan mode (step width = 0.02°, counting time = 1 s/step).

2.4.3 Raman spectroscopy

A Rxn1 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA), equipped with an air-cooled CCD detector (back-illuminated deep depletion design) was used to collect the Raman spectra. The laser wavelength was the 785 nm line from a 785 nm Invictus NIR diode laser. Spectra were recorded every 15 sec with a resolution of 4 cm^{-1} and an exposure time of 2 sec, using a laser power of 400 mW. Data collection and data transfer were automated using HoloGRAMS™ data collection software, HoloREACT™ reaction analysis and profiling software and Matlab software (version 7.1, The MathWorks Inc., Natick, MA). Data analysis was performed using SIMCA P+ (Version 12.0.1.0, Umetrics, Umeå, Sweden). The region of 800-860 cm^{-1} was thoroughly examined, since this region contains valuable information about the triazole group of itraconazole [9]. The peak at 1000 cm^{-1} corresponding to the C-N stretch in the triazole ring was also analysed.

2.4.4 Fourier transform infrared (FT-IR) spectroscopy

Attenuated total reflection Fourier transform infrared (ATR FT-IR) spectroscopy was performed on pure compounds, physical mixtures and spray-dried formulations to identify interactions between ITRA and adipic acid. Spectra were recorded using a Bruker Vertex 70 FT-IR spectrometer equipped with a DTGS detector and a PIKE accessory, equipped with a diamond ATR crystal (4 cm^{-1} resolution, 32 scans).

2.5 Dissolution of cocrystals

Dissolution was performed using USP dissolution Apparatus 1 (baskets). The equipment consisting of a VanKel VK7010 dissolution tester combined with a VK 8000 automatic sampling station (VanKel Industries, NJ, USA). The release of ITRA from nanosuspensions, capsules and Sporanox® (all containing 75 mg ITRA) was tested in pH 1 (0.1 N HCL) and pH 3 (206 ml 0.2 M Na_2HPO_4 and 794 ml 0.1 M Citric acid) (total volume: 900 mL, at a temperature of 37 ± 0.5 °C) using a basket speed set at 100 rpm. Samples of 5 mL were withdrawn after 5, 10, 15, 30, 45, 60, 120, 180 and 240 min without media replacement. Each formulation was tested in triplicate. Samples were filtered through a 0.45 µm filter (Millipore SLHV R04) in order to avoid the presence of unsolved nanoparticles.

The samples were analyzed by high performance liquid chromatography (HPLC). The HPLC-system (Merck Hitachi, Tokyo, Japan) consisted of a pump (L-6000), an integrator (D-2000), an autosampler (L-7200) with a 25 µL loop and a UV/VIS detector (L-4200). Detection was performed at 260 nm. To achieve chromatographic separation a guard column (Licrospher®100 100-RP-18, 4*4 mm (5 µm), Merck, Darmstadt, Germany) and an analytical column (Lichrospher® 100-RP-18, 125*4 mm (5 µm), Merck, Darmstadt, Germany) were used. The mobile phase consisting of acetonitrile (Biosolve, Valkenswaard, The Netherlands) and a 0.01N solution of tetrabutyl ammonium hydrogen sulfate (Sigma, Bornem, Belgium) (55/45, v/v) was used at a flow rate of 1.0 ml/min. The method validation indicated a linear relationship between the ITRA concentration and the response (range: 0 – 110 µg/ml and R^2= 0.9991 ± 0.0006). The limit of detection and quantification were 4.2 µg/ml and 14.0 µg/ml, respectively.

2.6 In vivo experiments

All procedures were performed in accordance with the guidelines and after approval by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (Merelbeke, Belgium). To evaluate the bioavailability of ITRA after oral administration, the following formulations (containing 200 mg ITRA) were administrated to 6 male mixed-breed dogs (weight: 20-40 kg) in a cross-over study with a wash-out period of at least 10 days: nanosuspension containing 5% ITRA, 8% adipic acid

and 1.25% Tween®80 (w/v) (formulation F1); capsules containing ITRA-SD2 (formulation F2); capsules containing ITRA-BL1 (formulation F3); Sporanox® (Janssen Cilag, Berchem, Belgium) as reference formulation (formulation F4).

Dogs were fasted 12 h prior to administration of the formulations; however water was ad libitum available during the experiment. Blood samples were taken in dry heparinized tubes at 0.5, 1, 2, 4, 6, 8, 12 and 24 h after administration. Blood samples were centrifuged immediately and separated plasma was stored at -20 °C until analysis. The samples were analyzed using high performance liquid chromatography (LC) with tandem mass spectrometric detection (MS/MS) in the Multiple Reaction Monitoring (MRM) mode. The LC-MS system consisted of a pump (Kontron type 325 pump, Kontron Instruments, Milano, Italy), an autosampler (Kontron 460) with a 25 µl loop and a Micromass Quattro II triple quadrupole MS detector (Micromass, Manchester, UK) in the electrospray mode. Time-dependent MRM detection was performed at m/z 705.5 to 392.3 (quantifier) and m/z 705.5 to 335.1 (qualifier) with a dwell time of 0.5 s and collision energy of 25 mV for ITRA; and m/z 416.0 to 159.0 with a dwell time of 0.5 s and collision energy of 25 mV for miconazole (internal standard). Argon was used as collision gas, and a cone voltage of 25 mV and capillary voltage of 3.5 kV was applied for both compounds. Data analysis was performed using Masslynx 4.0 and Quanlynx software (Micromass, Manchester, UK).

To achieve chromatographic separation, a Zorbax SB-C18 (2.1 x 30 mm, 2.5 µm particles) Rapid Resolution Cartridge column (Agilent Technologies, Waldbronn, Germany) was used. The mobile phase consisted of ammonium acetate 10 mM in ultrapure water, pH 3.5 and acetonitrile (Biosolve, Valkenswaard, The Netherlands) 40/60, v/v. Using 20 µl injections, isocratic elution at 0.2 ml/min afforded good separation between ITRA (Tr. 2.70) and the internal standard (Tr. 3.3) within a 5 min runtime.

Frozen plasma samples, calibrator samples and QC samples were allowed to thaw at room temperature. For samples within the assay range, a volume of 500 µl was transferred into an empty

test tube. Other samples (samples above upper limit of quantitation) were diluted appropriately with blank plasma prior to the analysis. Subsequently, 20 µl of the internal standard solution (1.25 µg/ml miconazole) and 500 µl of a 0.02 M phosphate buffer (pH 8.5) was added, followed by, after a short vortex mixing stage, 4 ml methyl-tert-butyl ether (HPLC grade, Chromasolv, Sigma – Aldrich, Bornem Belgium) extraction solvent. After a 10 min extraction on a rotatory mixer and a 5 min phase separation centrifugation (1500 x g), 3 ml of the upper layer was transferred and dried down under N_2 at 40 °C. The resulting dry residue was dissolved in 100 µl of the LC mobile phase, centrifuged and injected into the LC-MS system.

Method validation was conducted with reference to the FDA's guidance for bioanalytical method validation. The calibration curve was constructed by least squares linear regression of the peak area ratio of ITRA/internal standard against nominal concentration with a weighting of concentration. The measurement range of the analytical method was 15.3 – 510 ng/ml for ITRA in dog plasma. Imprecision and trueness were calculated on results of repeated analysis (n = 4 to n = 6) of quality controls (60.8, 152.2, and 303.4 ng/ml). For all levels of the QC samples, imprecision and trueness measurements comply with the FDA guidance specifications on maximum tolerable bias and imprecision.

2.7 Statistical analysis

Peak plasma concentration (C_{max}), extent of absorption ($AUC_{0\text{-}24h}$), and time to reach the highest plasma concentration (T_{max}) were calculated. Data were statistically analyzed using SPSS 19 (SPSS, Chicago, USA). Analysis of variance (ANOVA) was performed on the log-transformed C_{max} and $AUC_{0\text{-}24h}$ with a significance level of 0.05. Bonferroni post-hoc analysis was performed for pairwise comparisons between treatment groups. T_{max} was compared using the Friedman's nonparametric test ($p< 0.05$).

3. RESULTS AND DISCUSSION

3.1 Cocrystal formation

ITRA, a BCS class II drug, was processed via wet milling to assess its ability to form a nanosuspension when co-milled with different dicarboxylic acids, adding Tween® 80 to the suspension as stabilizing and wetting agent for ITRA. Photon correlation spectroscopy data (Table 1) indicated that it was not possible to obtain a nanocrystalline ITRA suspension when combined with different concentrations of succinic, maleic and glutaric acid. However, the size of the suspended ITRA particles was correlated with the adipic acid concentration used during wet milling, yielding nanosuspensions with a narrow particle size distribution (i.e. polydispersity index (PDI) below 0.3) when at least 4% adipic acid was added. No reduction in particle size (>5 µm) was observed when milling was performed without adipic acid also milling without Tween®80 did not resulted in particle size reduction (>5 µm). Sedimentation and agglomeration of the suspension was evaluated for 24 h. Suspensions containing succinic, maleic and glutaric acid already showed precipitation and agglomeration 1 h post-milling. While the suspension containing addipic acid was stable for at least 12 h.

Previous researchers described the formation of cocrystals between different dicarboxylic acids and ITRA [9]. In order to evaluate the possible interactions and the formation of cocrystals between ITRA and adipic acid, the nanosuspension containing 5% ITRA, 8% adipic acid and 1.25% Tween®80 was spray-dried. To formulate the ITRA nanoparticles into a solid dosage form the nanosuspension was spray-dried without additives (ITRA-SD1) and in combination with mannitol (ITRA-SD2) which was dissolved in the nanosuspension as a carrier for the nanoparticles. Spray-drying without mannitol resulted in a low yield of the spray-drying process (<15 %), while the material recovered after spray-drying increased to 70% when mannitol was added. Importantly spray-dried particles of ITRA-SD1 were difficult to redisperse in water and showed a significantly higher particle size (>1.5 µm) compared to the ITRA nanosuspension. In contrast the addition of mannitol yielded a spray-dried powder (ITRA-SD2) which could easily be redispersed into nanoparticles with a particle size (802.3 ±

21.0 nm) similar to their size prior to spray-drying, indicating that embedding of ITRA in a mannitol matrix via spray-drying was an efficient method to prevent nanoparticle agglomeration during the dehydration step. Previous research showed already that adding a matrix former, such as mannitol, prior to the drying process was necessary to preserve the dissolution potential of the nanosuspension by preventing agglomeration of the nanococrystals [23].

3.2 Characterization of itraconazole cocrystals

Changes in crystalline state can occur both during milling and drying processes. To ensure that the changes are due to the formation of cocrystals and that no amorphous ITRA is formed, (M)DSC, XRD, Raman and ATR FT-IR were performed on the spray-dried samples.

Figure 1 shows the MDSC thermograms for ITRA, adipic acid, mannitol and the spray-dried formulation. The onset melting temperature of ITRA, adipic acid and mannitol were detected at 167.0, 151.7 and 168.4 °C, respectively. After milling and spray-drying a reduction of the onset melting temperature was observed for adipic acid and ITRA, the onset melting temperature of mannitol remained unchanged. In addition a recrystallization peak appeared in the reversed heat cycle at 157.3 °C (data not shown). ITRA showed distinct crystalline peaks for 2θ at 20.4° and 23.6° (Figure 2), while the crystalline state of adipic acid was identified by sharp diffraction peaks at 21.6°, 25.7° and 31.2°. Mannitol is not shown in the XRD profile as the highly crystalline signal of mannitol masked the peaks of ITRA and adipic acid. The peaks of ITRA and adipic acid have shifted in the spray-dried formulations. Based on the MDSC and XRD data, it is evident wet milling and spray-drying affected the crystal structure of both compounds. To further elucidate the interactions between ITRA and adipic acid, Raman and ATR FT-IR spectroscopy were conducted on the samples. Figure 3 shows the results of the Raman analysis. The ITRA peaks in the Raman spectra at 812 and 822 cm^{-1}, representing the triazole ring of ITRA [24], had merged into a single peak at 818 cm^{-1} after milling and spray-drying of the nanosuspension. The peak at 1010 cm^{-1}, corresponding to the C-N stretch in the triazole ring, was broadened and had a much lower intensity, demonstrating the weakening of this

bond due to interactions between the triazole group of ITRA and the carboxylic group of adipic acid during milling and spray-drying. Other typical peaks for ITRA remained unaffected. ATR FT-IR spectroscopy (Fig. 4) indicated that hydrogen bounds and van der Waals interactions were formed between ITRA and adipic acid in the spray-dried formulation. Mannitol did not influence the physical interactions between ITRA and adipic acid. While the IR spectrum of the physical mixture (Fig. 4A) can be considered as the sum of the spectra of the pure substances, the FT-IR spectrum of the spray-dried nanosuspension (Fig. 4B) differed significantly from the physical mixture: e.g. ITRA vibrations at 1551 and 1509 cm^{-1} ($\nu_{C=C}$ and $\nu_{C=N}$ stretches) shifted to higher wavenumbers at 1555 and 1515 cm^{-1}; the 1379, 1140 and 1043 cm^{-1} absorptions disappeared and the intensity of the 943 and 735 cm^{-1} absorptions was lower. Moreover, the 823 cm^{-1} ITRA vibration disappeared and a new band appeared at 820 cm^{-1}, indicating van der Waals interactions between the CH_2-chain of adipic acid and the triazole ring of ITRA in the spray-dried formulation which confirmed the observations made, based on the Raman spectra. Another difference was observed in the $\nu_{C=O}$ stretch vibration region around 1704 cm^{-1}: whereas a shoulder was present at lower wavenumber (around 1684 cm^{-1}) for the physical mixture, the spray-dried formulation showed a clear shoulder at higher wavenumber (around 1704 cm^{-1}). This indicated a hydrogen bond formation between the carboxyl group of adipic acid and the carbonyl group of ITRA. Based on these data it is evident that cocrystals between adipic acid and ITRA are formed. Similar cocrystals between ITRA and dicarboxylic acids (in a molecular ratio of 2/1) have already been described by Remenar et al. [9]. In our study only the combination of ITRA and adipic acid resulted in nanosized particles after manufacturing a nanococrystalline suspension.

In a second step the cocrystalline nanosuspension was layered on MCC beads in order to overcome the problems with the flowability, bulk density and hygroscopicity of the spray-dried powder. Beads which are the result of a layering process using a fluidized bed can be easily filled into capsules.

3.3 Dissolution of cocrystals

To evaluate if the cocrystals enhanced the dissolution rate of ITRA, dissolution tests at pH 1 and pH 3 were performed on the different nanococrystalline formulations (i.e. nanosuspension, spray-dried powder ITRA-SD2 and layered beads ITRA-BL1, both filled into hard gelatin capsules). At pH 1 the in-vitro ITRA release from the reference formulation (Sporanox®) was limited to 15% and 35% after 20 and 60 min, respectively. In contrast, complete dissolution of the nanococrystalline suspension, the spray-dried powder ITRA-SD2 and the layered beads was observed after 4, 8 and 20 min, respectively (Fig. 5A). The slight delay in dissolution time from the processed nanococrystalline formulations can be related to the hydration of the powder, the disintegration of drug aggregates and/or the wetting and opening of the hard gelatin capsule [25]. The small increase in dissolution time of the layered beads (compared to the spray-dried formulation) can be due to agglomeration of the nanococrystals during layering and/or release from the coat. A small increase in particle size (912.1 ± 43.1 nm) was obtained after the dissolution of ITRA particles from the layered beads. While there was a complete ITRA release for all formulations at pH 1, a slower and incomplete release of ITRA was observed at pH 3 for both nanosuspension-based formulations and Sporanox® (Fig 5B), indicating a pH dependent release of ITRA.

3.4 Bioavailability study

Finally, the bioavailability of ITRA after oral administration of the different formulations (containing 200 mg ITRA) to dogs was assessed (Fig.6). The main pharmacokinetic parameters are reported in Table 2. Although clear differences were observed in-vitro at pH 1, no significant differences in bioavailability were observed between the different formulations in-vivo. This can be due to the gastric pH of the dogs. It is known that the pH of the dogs can vary between 1 – 8 with a mean value significantly higher than the gastric pH in humans [26]. Whereby the pharmacokinetic results are more related to the in-vitro results at pH 3, indicating an incomplete release of the nanococrystals but still faster compared to the release of Sporanox®. These results are comparable to the results of the in-vivo study as the T_{max} of the formulations was obtained 3 h after administration while the T_{max}

of Sporanox® was observed only 6 h after administration. Although this trend in relation with the T_{max} was clearly observed no significant differences were noticed by statistical analysis of the pharmacokinetic parameters ($p>0.05$).

4. CONCLUSION

A fast drug release of itraconazole was obtained by a dual concept: the manufacturing of nanosized cocrystals of itraconazole and adipic acid via wet milling. The processing of the liquid cocrystalline nanosuspension and bead layering (using mannitol as matrix former) yielded immediate release solid dosage forms compared to a commercially available reference formulation The formation of stable nanosized cocrystals via this approach seems a good alternative for amorphous systems to increase the solubility and obtain a fast drug release of BSC class II drugs.

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