


Bioequivalence and food effect of a fixed-dose combination of macitentan and tadalafil: Adaptive design in the COVID-19 pandemic

Dénes Csonka¹  | Vladislav Fishman² | Jaya Natarajan³ | Hans Stieltjes⁴ | Danielle Armas⁵ | Victor Dishy³ | Juan Jose Perez Ruixo⁴

¹Actelion Pharmaceuticals Ltd, Allschwil, Switzerland

²QVIA RDS, Inc, Durham, North Carolina, USA

³Janssen Research and Development, Raritan, New Jersey, USA

⁴Janssen Research and Development, Beerse, Belgium

⁵Celerion, Inc, Tempe, Arizona, USA

Correspondence

Dénes Csonka, Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, CH-4123 Allschwil, Switzerland.
Email: dcsotka@its.jnj.com

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Actelion Pharmaceuticals

Abstract

The COVID-19 pandemic has forced clinical studies to accommodate imposed limitations. In this study, the bioequivalence part could not be conducted as planned. Thus, the aim was to demonstrate bioequivalence, using an adaptive study design, of tadalafil in fixed-dose combination (FDC) tablets of macitentan/tadalafil with single macitentan and tadalafil (Canadian-sourced) tablets and assess the effect of food on FDC tablets in healthy subjects. This Phase 1, single-center, open-label, single-dose, two-part, two-period, randomized, crossover study enrolled 62 subjects. Tadalafil bioequivalence as part of FDC of macitentan/tadalafil (10/40 mg) with single-component tablets of macitentan (10 mg) and tadalafil (40 mg) was determined by pharmacokinetic (PK) assessment under fasted conditions. The effect of food on FDC was evaluated under fed and fasted conditions. Fasted 90% confidence intervals (CIs) for geometric mean ratios (GMRs) were within bioequivalence limits for tadalafil and macitentan. Fed and fasted 90% CIs for area under the curve (AUC) GMR were within bioequivalence limits. However, 90% CIs for maximum plasma concentration (C_{max}) GMR for macitentan and tadalafil were outside bioequivalence limits. One FDC-treated subject experienced a serious adverse event of transient ischemic attack (bioequivalence part). To address pandemic-imposed limitations, an adaptive study design was implemented to demonstrate that the FDC tablet was bioequivalent to the free combination of macitentan and tadalafil (Canadian-sourced). No clinically significant differences in PK were determined between fed and fasted conditions; the FDC formulation could be taken irrespective of meals. The FDC formulation under fasted and fed conditions was well tolerated with no clinically relevant differences in safety profiles between the treatment groups.

Abbreviations: AEs, adverse events; AUC, area under the curve; BCS, biopharmaceutical classification system; CIs, confidence intervals; C_{max} , maximum plasma concentration; CV, coefficient of variation; EEA, European Economic Area; ET, endothelin; EU, European Union; FDC, fixed-dose combination; GMRs, geometric mean ratios; ICF, informed consent form; PAH, pulmonary arterial hypertension; PDE-5, phosphodiesterase type 5; PK, pharmacokinetic; QC, quality control; SAE, serious adverse event; SD, standard deviation; $t_{1/2}$, terminal half-life; t_{max} , time to reach maximum plasma concentration; US, United States.

Principal Investigator's statement: The authors confirm the Principal Investigator for this paper is Danielle Armas, and she had direct clinical responsibility for subjects.

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KEYWORDS

bioequivalence, fasted, fed, fixed-dose combination, food effect, macitentan, pulmonary arterial hypertension, tadalafil

1 | INTRODUCTION

Pulmonary arterial hypertension (PAH) is a chronic disease characterized by an increase in pulmonary vascular resistance, which leads to right ventricular failure and ultimately death if not treated.^{1,2}

Macitentan, an orally active, nonpeptide, potent dual endothelin (ET) receptor A and ET receptor B antagonist, and biopharmaceutical classification system (BCS) class 2 compound, was granted a marketing authorization for the treatment of patients with PAH in the United States (US), European Economic Area (EEA), and other countries, as Opsumit® 10 mg film-coated tablets of macitentan, administered once daily.

Tadalafil is a selective phosphodiesterase type 5 (PDE-5) inhibitor and BCS class 2 compound, approved in the US, EEA, and other countries under the brand name Adcirca® (20 mg film-coated tablets) for the treatment of PAH at a dosage of 40 mg once daily.

A free combination of macitentan (0.3 mg/kg) and tadalafil (10 mg/kg) was found to have a synergistic effect versus either single agent for the duration of decreased blood pressure in two animal models of systemic hypertension (Dahl salt-sensitive rat and spontaneously hypertensive rat) associated with endothelial dysfunction. This effect was further confirmed in a rat model of pulmonary hypertension. In fact, in rats with hypoxia/sugen-induced pulmonary hypertension, the combination of macitentan (3 mg/kg) and tadalafil (9 mg/kg) had a synergistic effect versus either single agent in decreasing mean pulmonary arterial pressure without increasing the risk of exaggerated systemic vasodilation.

The approval of macitentan for use in monotherapy and combination therapy with PDE-5 inhibitors was based on data generated in the long-term, event-driven SERAPHIN study.³ In SERAPHIN, approximately 61% of patients were receiving a PDE-5 inhibitor, mainly sildenafil, as background therapy at baseline. In patients with background PDE-5 inhibitor-specific therapy at baseline, the hazard ratio for the primary endpoint for macitentan 10 mg versus placebo was 0.62 (95% confidence limits: 0.43, 0.89). Adding long-term treatment with macitentan 10 mg to currently approved and commonly used background PDE-5 inhibitor-specific medicines is thus associated with a clear treatment effect on morbidity/mortality outcome events. In addition, the AMBITION study demonstrated that up-front combination with ambrisentan and tadalafil was associated with a 50% reduction in the primary composite morbidity/mortality endpoint, as compared with ambrisentan and tadalafil alone.⁴

The results of these studies contributed to updated European Society of Cardiology and European Respiratory Society guidelines,⁵ which recommend that combination therapy be given as sequential or up-front combination therapy if treatment targets are not met.

Statement 1

What is already known about this subject

- FDC tablet containing 10/40 mg of macitentan/tadalafil was bioequivalent to a free combination of 10 mg macitentan and 40 mg tadalafil (US- and EU-sourced).
- Patients not requiring dose adjustments could use a FDC tablet instead of a free combination of macitentan and tadalafil.

Statement 2

What this study adds

- FDC tablet containing 10/40 mg of macitentan/tadalafil was bioequivalent to free combination 10 mg macitentan and 40 mg tadalafil (Canadian-sourced).
- No clinically significant food effect was observed with the FDC tablet.
- Example of how adaptive study design can be used for a bioequivalence study in special circumstances.

The Canadian Cardiovascular Society/Canadian Thoracic Society Position Statement on Pulmonary Hypertension (2020)⁶ notes that combination PAH-targeted medical therapy is standard of care for most PAH patients and recommends initial dual oral combination therapy in intermediate-risk treatment-naïve PAH patients.

A fixed-dose combination (FDC) of macitentan and tadalafil would offer PAH patients the advantages provided by the concomitant use of macitentan and a PDE-5 inhibitor in a single, once daily dose tablet. This should facilitate compliance and reduce the risk of medication errors.

Bioequivalence of macitentan, its active metabolite apocitentan (ACT-132577), and tadalafil was established for FDC in two Phase 1 studies of a total of 160 healthy subjects, in which tadalafil as a single-component was sourced from the US or European Union (EU) to fulfil regional regulatory requirements.⁷

Health Canada generally requires that the reference products used in bioequivalence studies are the Canadian marketed product⁸; hence, a Phase 1 clinical bioequivalence study was conducted using Canadian-sourced tadalafil. Although both tadalafil and macitentan can be administered as individual components with or without food, the food effect on the FDC tablets was evaluated in line with regulatory guidelines.⁹⁻¹²

Thus, the study objective was to ensure bioequivalence of tadalafil in the FDC formulation with that of Canadian-sourced tadalafil.¹³ Furthermore, the effect of food on the pharmacokinetics (PK) of a 10/40 mg FDC of macitentan and tadalafil was assessed.

2 | MATERIALS AND METHODS

2.1 | Study subjects

In this study, eligible subjects were healthy adults between the ages of 18 and 55 years, and had systolic blood pressure of 100–145 mm Hg, diastolic blood pressure of 50–90 mm Hg, pulse rate of 45–99 beats per minute, body mass index between 18.5 and 30.0 kg/m², and body weight not less than 50 kg. Key exclusion criteria included known allergy, hypersensitivity, or intolerance to any active substance or drugs of the same class, or any excipient of the drug formulations; hepatic aminotransferases (alanine and/or aspartate) >1.5 × upper limit of normal at screening; history or clinical evidence of any disease and/or existence of any surgical or medical condition which could have interfered with the absorption, distribution, metabolism, or excretion of the study drug(s).

The female subjects were not pregnant, and both the male and female subjects agreed to remain on an acceptable birth control method throughout the study from signing of the informed consent form (ICF) onward. The subject was included in the study if they met all inclusion criteria and did not meet any of the exclusion criteria.

Written informed consent was obtained from each subject in the study prior to any study procedure and after adequate explanation of the aims, methods, objectives, and potential hazards of the study.

2.2 | Study design

The present study was a single-center, open-label, single-dose, two-period, randomized, crossover Phase 1 study in healthy adult male and female subjects. The study comprised a bioequivalence part and a food effect part (originally planned as two parallel groups). The FDC formulation containing 10/40 mg of macitentan/tadalafil was investigated. Subjects were first allocated to Group 1 (FDC vs. free-combination, fasted), and once Group 1 completed then to Group 2 (FDC, fed vs. fasted) and within each group subjects were randomized to 1 of the 2 possible treatment sequences per group. In Group 1, Treatment A was a single oral dose of an FDC of macitentan/tadalafil (10/40 mg) in fasted conditions (test). Treatment B was a single oral dose of a free combination of 10 mg macitentan and 40 mg Canadian-sourced tadalafil in fasted conditions (reference). In Group 2, Treatment C was a single oral dose of an FDC of macitentan/tadalafil (10/40 mg) in fed conditions (test). Treatment D was single oral dose of an FDC of macitentan/tadalafil (10/40 mg) in fasted conditions (reference).

The FDC was a film-coated tablet that contained 10 mg of macitentan and 40 mg of tadalafil and was administered orally.

Macitentan was provided as a 10-mg film-coated tablet, and tadalafil was provided as a 20-mg film-coated tablet, each for oral administration.

All intake of study drug took place at the study site on Day 1. On Day 1 of all treatments, subjects fasted overnight for at least 10 h. Intake of water was not allowed from approximately 1 h before until approximately 1 h after study drug intake (except for the water used for study drug intake and for breakfast, if applicable). In the case of intake in fed conditions during Treatment C (Group 2), a high-fat, high-calorie breakfast was served per Food and Drug Administration guidance.¹⁴ The high-fat breakfast was ingested entirely in 30 min or less. Within 10 min after completion of the breakfast, but no more than 30 min after the start of breakfast, study drug was administered. Study drug intake in subsequent treatment periods in an individual subject was to be separated by a washout period of at least 10 days. The maximum duration on study for each subject including follow-up was 6 weeks (excluding screening).

Full PK profiles were to be determined up to 216 h after study drug administration in each treatment period.

In both studies, the FDC tablets and the reference treatments macitentan and Canadian-sourced tadalafil were provided by Actelion Pharmaceuticals Limited.

The study was approved by an institutional review board (Advarra, Columbia, MD, US) and was performed in accordance with the Declaration of Helsinki and with the laws and regulations of the US.

2.2.1 | Bioequivalence part (group 1)

For the bioequivalence part, the primary objective was to demonstrate bioequivalence of the primary PK parameters (maximum plasma concentration [C_{max}], area under the plasma analyte concentration-time curve (AUC) from time 0 to time of the last quantifiable concentration [$AUC_{(0-last)}$], and AUC from time 0 to infinity [$AUC_{(0-\infty)}$]) of tadalafil administered as FDC (test) tablets of macitentan/tadalafil (10/40 mg) and co-administered as a free combination (reference) of 10 mg macitentan and 40 mg Canadian-sourced tadalafil tablets in fasted conditions in healthy adult subjects.

The secondary objectives included investigation of secondary PK parameters (time to reach maximum plasma concentration [t_{max}] and terminal half-life [$t_{1/2}$]) of tadalafil and the primary (as stated previously) and secondary (as stated previously) PK parameters of macitentan (and apocitentan), administered as FDC tablets or co-administered as free combination tablets in fasted conditions in healthy adult subjects. Also, to evaluate the safety and tolerability of macitentan and tadalafil administered as FDC tablets or co-administered as free combination tablets in fasted conditions in healthy adult subjects.

In the two sequential treatment periods, each subject was planned to receive two treatments (Treatment A and Treatment B) in a random order, that is, the sequence A-B or B-A.

Because of the coronavirus disease (COVID-19) pandemic, the study was placed on hold since the study site closed for approximately 2 months in accordance with local laws and to adjust to the Centers for Disease Control recommendations for operating during the pandemic. Because of this hold, the study design was amended into an adaptive design per Health Canada bioequivalence guidance,⁸ and an interim analysis of the bioequivalence part of the study (Group 1) was planned. The interim analysis allowed for a decision on either continuing with the bioequivalence part of the study and adjusting the sample size, if sufficient power was not achieved with the initial sample size, or stop the bioequivalence part of the study and conduct the bioequivalence assessment if sufficient power was achieved with the sample size available at the interim analysis. Detailed changes are explained in the Pharmacokinetic Analysis section.

2.2.2 | Food effect part (group 2)

For the food effect part, the primary objective was to evaluate the effect of food on the primary PK parameters (C_{max} , $AUC_{(0-last)}$, and $AUC_{(0-\infty)}$) of tadalafil and macitentan (and apocritentan) administered as FDC tablets (10/40 mg, respectively) in healthy adult subjects.

The secondary objectives included investigation of secondary PK parameters (t_{max} , $t_{1/2}$) of tadalafil and macitentan, administered as FDC tablets or co-administered as free combination tablets in fasted conditions in healthy adult subjects. Also, to evaluate the safety and tolerability of macitentan and tadalafil administered as FDC tablets or co-administered as free combination tablets in fasted conditions in healthy adult subjects.

In the two sequential treatment periods, each subject received two treatments (fed [Treatment C] and fasted [Treatment D]) in a random order, that is, the sequence C-D or D-C.

2.3 | Sample size calculation

2.3.1 | Bioequivalence part (group 1)

A formal sample size calculation was performed for the primary PK endpoints.

Using a conservative estimate of the intrasubject coefficient of variation (CV) of 20% for AUCs and C_{max} of tadalafil and a 5% level of significance, a sample size of 37 subjects who completed the study was sufficient to conclude bioequivalence with 80% power, when the true ratio of treatment means equaled 90% or 110%. To account for potential dropouts and have at least 37 subjects who completed all assessments, 46 subjects were to be enrolled in this part of the study.

Because the study was placed on hold due to the COVID-19 pandemic, a study amendment to conduct an interim analysis of the bioequivalence part was performed. At that time, 33 subjects had completed both Treatment Periods (for C_{max} determinations, it was 34 subjects), and were available for the analysis.

As specified in Health Canada's guidance on the conduct and analysis of comparative bioavailability studies,⁸ Method C of Potvin et al,¹⁵ was utilized for planning the interim analysis of results from the bioequivalence part of this study. This method uses an adaptive sample size sequential approach for studies with a crossover design. Here, the second stage sample size is based on the estimated intrasubject variance from the first stage, if there was not sufficient power to conduct the assessment with the data of the first stage.

Adaptive study design

Bioequivalence part (Group 1): Adaptive study design changes had significant impact on the PK analysis. The analysis was changed to a 2-stage analysis, which included an interim analysis after Stage 1, with an option to terminate the study if pre-set stopping criteria were met, per the following procedure: the power to conclude bioequivalence was to be determined using the variance estimate from Stage 1 data and an α -level of .05. If the power was estimated to be $\geq 80\%$, bioequivalence testing was to be performed with Stage 1 data using an α -level of .05. The bioequivalence part was to be terminated regardless of whether bioequivalence criterion was met or not. If the power was estimated to be $< 80\%$, bioequivalence testing was to be performed with Stage 1 data using an α -level of .0294. If the bioequivalence criterion was met, the bioequivalence part of the study was to be terminated. If the bioequivalence criterion was not met, the sample size for the second stage of the bioequivalence part was to be calculated based on the variance estimated at the first stage and an α -level of .0294. The study would then continue to the second stage. At the second stage, bioequivalence was to be evaluated using data from both groups and an α -level of .0294. The study was to be terminated regardless whether bioequivalence was met or not. Bioequivalence limits of 80.00% to 125.00% were to be used for bioequivalence testing in both stages.

2.3.2 | Food effect part (group 2)

Using an estimated intrasubject CV of 20%⁷ for AUCs and C_{max} of tadalafil, a sample size of 12 subjects was considered sufficient for the point estimate of the relative bioavailability of tadalafil in fed conditions versus fasted conditions to fall within 86.4% and 115.8% of the true value with 90% confidence. Using an estimated intrasubject CV of 23% for AUCs and C_{max} of macitentan, a sample size of 12 subjects who completed was sufficient for the point estimate of the relative bioavailability of macitentan in fed conditions versus fasted conditions to fall within 84.5% and 118.3% of the true value with 90% confidence. To account for potential dropouts and have at least 12 subjects who completed all assessments, 16 subjects were enrolled in this part of the study.

Food Effect part (Group 2): The start of enrolment of this part of the study was delayed and commenced only after the study site reopened with strict infection control measures in place. The food effect part of the study was then completed as originally planned. Thus, the originally planned 2-way crossover design was used for food effect evaluation. Adaptive design was not used for food effect evaluation.

2.4 | Sample collection and analytical methods

Venous blood samples for determination of plasma concentrations of macitentan and its active metabolite apocitentan, and tadalafil were collected at planned timepoints.

Blood samples (to an overall 500 ml maximum per subject) were collected at predose, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 7.5, 8, 8.5, 9, 10, 12, 24, 48, 72, 120, 168, and 216 h postdose.

Plasma concentrations of macitentan, its active metabolite apocitentan, and tadalafil were analyzed using validated bioanalytical methods, and liquid chromatography for separation and tandem mass spectrometry for analysis. For macitentan and metabolite and for tadalafil, the methods consisted of a protein precipitation with acetonitrile, followed by quantification using liquid chromatography tandem mass spectrometry. A 5500 (macitentan and metabolite) or 5000 (tadalafil) triple-quad mass spectrometer (SCIEX) using a Turbo Ionspray in the positive mode was used for ionization of the molecules. For chromatography, an Acquity BEH C18 column (Waters) with 0.1% formic acid and acetonitrile as mobile phase constituents was used. Stable isotope-labeled analogues were used as internal standards. The range of quantitation was 1.00 to 2000 ng/ml for both macitentan and apocitentan, and 0.500 to 1000 ng/ml for tadalafil. All assay acceptance criteria were met.

For macitentan and apocitentan, the method was successfully validated with respect to linearity, selectivity, response, precision and accuracy, recovery, matrix effect, selectivity, and effect of carry-over. Stability in plasma samples, in the presence of tadalafil, was demonstrated for at least 26 h at room temperature, and up to 150 days storage at -20 and -70°C , and for 5 freeze/thaw cycles.

For tadalafil, the method was successfully validated with respect to the same parameters. Stability in plasma samples, in the presence of macitentan and metabolite, was demonstrated for at least 24 h at room temperature, 127 days at -20 and -70°C , and for 5 freeze/thaw cycles.

These results demonstrated that the validated method was suitable for the determination of macitentan, apocitentan, and tadalafil in human plasma samples. The tested long-term stability period was covering the age of the samples at the time of analysis.

The in-study descriptive statistics of the quality control (QC) samples of macitentan showed that the interbatch precision (%CV) was $\leq 7.0\%$, whereas the interbatch accuracy (% bias) was in the range of 1.3% to 6.5.

For both assays, the incurred-sample reanalysis results confirmed that the measured concentrations were reliable.

2.4.1 | Pharmacokinetic analysis

PK parameters ($AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, C_{max} , t_{max} , and $t_{1/2}$) were derived by noncompartmental analysis using Phoenix[®]WinNonlin[®] version 8.1 (Certara). PK parameters were summarized by study treatment with arithmetic mean, geometric mean, standard deviation (SD), intrasubject CV (in %), and 95% Confidence Interval (CI) of the arithmetic and geometric means. Median and range were calculated for

t_{max} and mean SD are presented for all other PK parameters (Table 2). Geometric mean ratio (GMR) with 95% CI and intrasubject CV% are presented for PK parameters in Table 3.

Determination of bioequivalence was based on 90% CI for the ratios of the geometric means (test/reference) for tadalafil $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, and C_{max} . For acceptance of bioequivalence, the 90% CIs had to be within the range of 80.00 and 125.00, when rounded to two decimal places.¹³

For bioequivalence testing, the treatments (test and reference) were compared with a linear mixed-effects model using log-transformed values of the primary PK parameters as dependent variables, treatment, treatment sequence, and period as fixed effects, and subject as a random effect.¹⁶ GMRs (test/reference) and 90% CIs were calculated from the corresponding re-transformed contrasts for treatment of the mixed-effects models. In addition, the intrasubject variability was estimated from the mixed-effects model. Determination of the 90% CIs for the GMRs for $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, and C_{max} for tadalafil, macitentan, and apocitentan provided a means to test if the GMRs fell within the bioequivalence limits (80.00% to 125.00%).

The PK analysis was performed on data from all subjects who received at least one dose of study drug and who had at least 1 PK parameter value.

2.5 | Safety evaluation

The safety evaluation included change from baseline to each scheduled timepoint of measurement and included treatment-emergent adverse events (AEs), clinical laboratory tests, electrocardiogram, vital signs, and physical examination. Data were listed by study group, subject number, sex, and summarized descriptively by treatment. Safety and tolerability were evaluated throughout the study from signing of the ICF until the subject's last study-related activity or follow-up.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the International Union of Basic and Clinical Pharmacology/British Pharmacological Society Guide to PHARMACOLOGY,¹⁷ and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.¹⁸

3 | RESULTS

3.1 | Study subjects

A total of 62 subjects were planned, enrolled, and randomized; 46 subjects were enrolled in Group 1 and 16 subjects were enrolled in Group 2.

TABLE 1 Arithmetic mean (SD) and range of demographic characteristics in the randomized subjects

Variable	Group 1: Bioequivalence FDC vs. free combination (fasted) (N = 46)			Group 2: Food effect FDC (fed vs. fasted) (N = 16)		
	AB	BA	Total	CD	DC	Total
Females, n (%)	18.0 (78.3)	17.0 (73.9)	35.0 (76.1)	4.0 (50.0)	1.0 (12.5)	5.0 (31.3)
Males, n (%)	5.0 (21.7)	6.0 (26.1)	11.0 (23.9)	4.0 (50.0)	7.0 (87.5)	11.0 (68.8)
Age, y	38.2 (9.0) 21–52	39.1 (8.2) 23–51	38.7 (8.5) 21–52	40.8 (6.8) 32–48	39.3 (7.7) 29–53	40.0 (7.1) 29–53
Weight, kg	70.2 (8.6) 53.1–83.6	70.8 (14.5) 50.7–106.9	70.5 (11.8) 50.7–106.9	74.9 (10.5) 59.7–88.9	81.0 (4.9) 75.5–91.4	77.9 (8.6) 59.7–91.4
Height, cm	163 (7) 147–175	165 (11) 151–192	164 (9) 147–192	171 (13) 151–186	174 (7) 166–183	172 (10) 151–186
BMI, kg/m ²	26.5 (2.8) 20.2–30.5	26.0 (3.4) 19.7–29.5	26.2 (3.1) 19.7–30.5	25.7 (2.0) 22.5–28.9	26.8 (2.0) 23.3–29.4	26.3 (2.0) 22.5–29.4

Abbreviations: %, percentages are calculated with corresponding randomized count in the respective treatment as denominator; BMI, body mass index; FDC, fixed-dose combination; N, maximum number of subjects with data; n, number; SD, standard deviation; vs., versus; y, year.

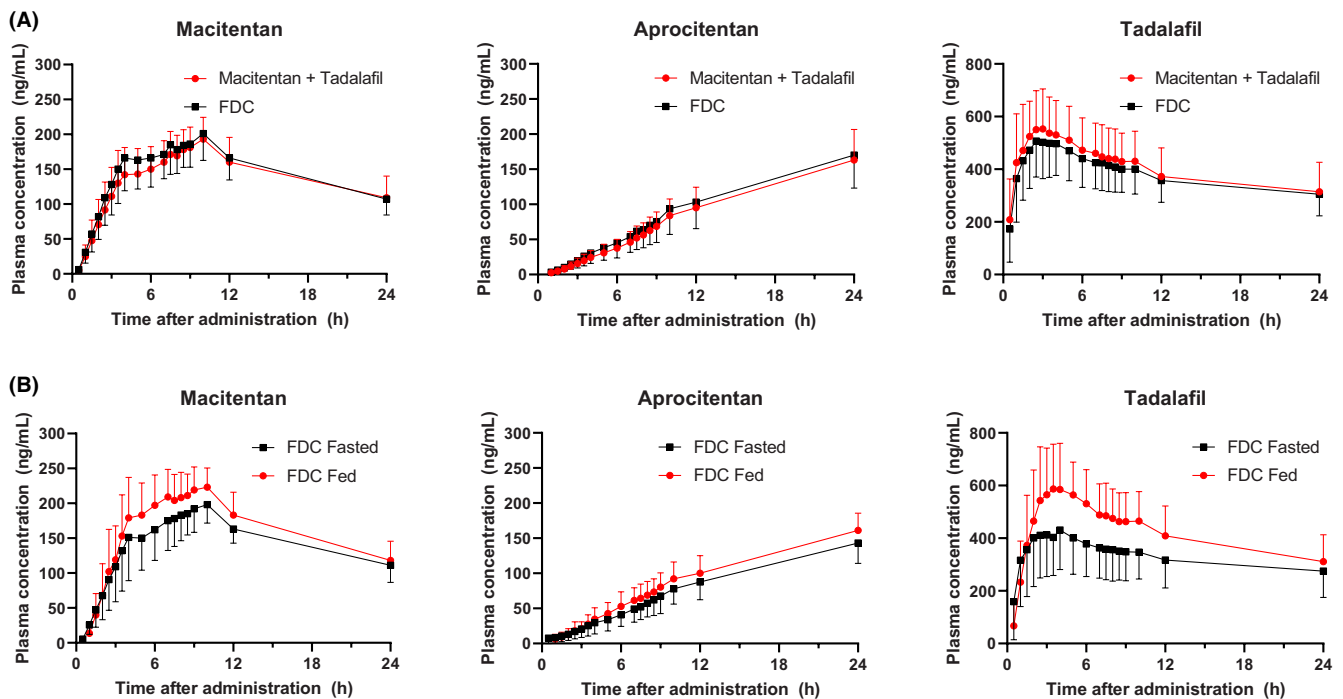


FIGURE 1 Arithmetic mean (SD) plasma concentration versus time profiles for macitentan, apocritentan and tadalafil during the first 24 h after administration of (A) FDC tablets or free combination of macitentan and Canadian-sourced tadalafil (bioequivalence), and (B) after administration of FDC tablets to subjects under fed or fasted conditions (food effect), and who were included in pharmacokinetics analysis of data. FDC, fixed-dose combination; SD, standard deviation. Data are plotted on a linear scale

In Group 1, all subjects received study drug, and 23 subjects were assigned to each of Treatment Sequence AB and BA. Of the 46 subjects, 33 subjects completed the study. In Group 1, (FDC vs. free combination, fasted), 13 subjects discontinued the study (10 subjects for “other” reasons [the study was put on hold due to COVID-19 pandemic, and this group was later discontinued], 1 subject due to an AE of transient ischemic attack, 1 subject because of noncompliance with study drug, and 1 subject withdrew from the study).

In Group 2, all subjects received study drug, and eight subjects were assigned to each of Treatment Sequence CD and DC. A total of 14 of 16

subjects in Group 2 were evaluable for PK analysis. In Group 2 (FDC [fed vs. fasted]), 2 subjects discontinued the study by withdrawal of consent after completing Period 1. Of the 16 subjects, 14 completed the study.

3.2 | Demographics and baseline characteristics

The demographics and baseline characteristics were generally similar for all treated subjects in both groups with the exception of sex as a higher proportion of females were included in the study (Table 1).

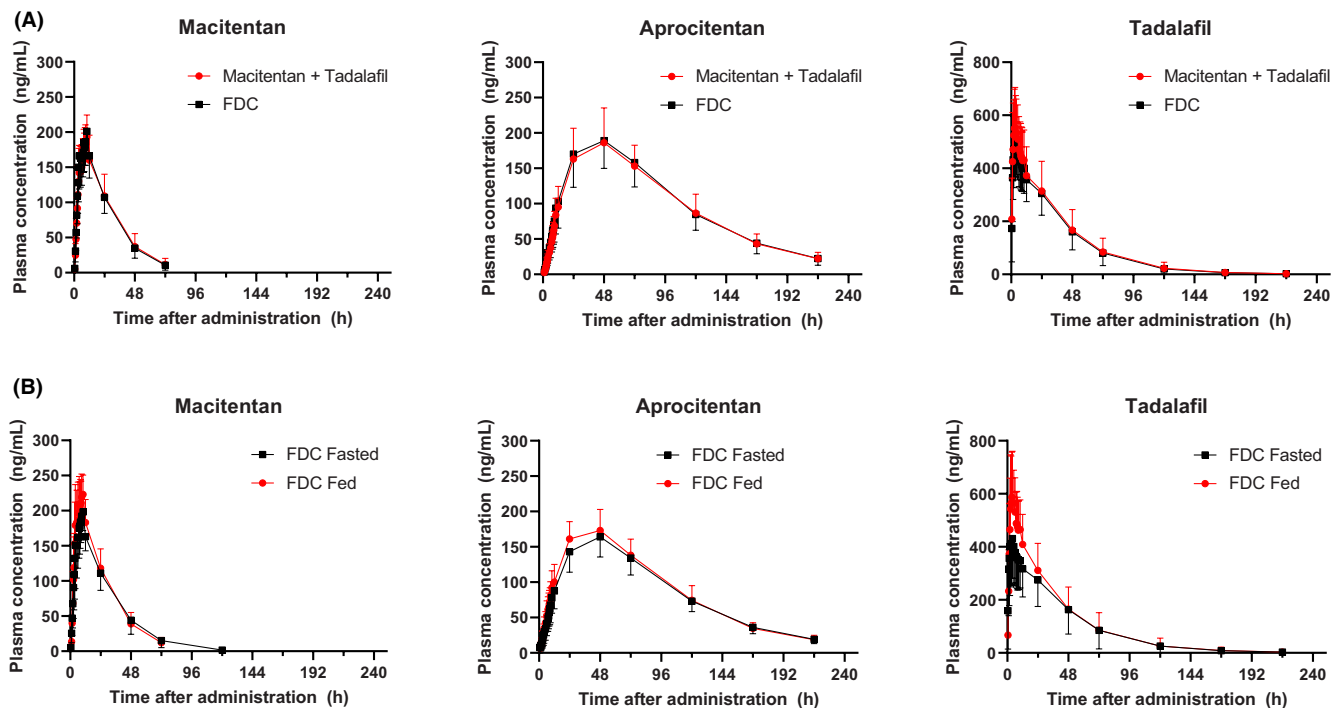


FIGURE 2 Arithmetic mean (SD) plasma concentration versus time profiles for macitentan, aprocitantan and tadalafil over 216 h in subjects included after administration of (A) FDC tablets or free combination of macitentan and Canadian-sourced tadalafil (bioequivalence), and (B) after administration of FDC tablets to subjects under fed or fasted conditions (food effect), and who were included in pharmacokinetics analysis of data. FDC, fixed-dose combination; SD, standard deviation. Data are plotted on a linear scale

3.3 | Pharmacokinetics

3.3.1 | Bioequivalence part (group 1)

The power to conclude bioequivalence at Stage 1 using the variance estimated from Stage 1 data and an α -level of .05 was 80.9% to >99.9%. The bioequivalence criterion was met using 90% CIs, and the study did not proceed to Stage 2 (Table 3).

The mean plasma concentrations and exposure PK parameters $AUC_{(0-last)}$, $AUC_{(0-\infty)}$, C_{max} , t_{max} , and $t_{1/2}$ for tadalafil, macitentan, and aprocitantan were similar following administration of the FDC tablet or tadalafil in the free combination tablet (Table 2, Figures 1 and 2). The 90% CIs for the GMRs (FDC vs. free combination) of $AUC_{(0-last)}$, $AUC_{(0-\infty)}$ and C_{max} for tadalafil and macitentan were within the bioequivalence limits (80.00% to 125.00%) for (Table 3).

3.3.2 | Food effect part (group 2)

The mean AUC , t_{max} , and $t_{1/2}$ values of tadalafil were comparable under fed versus fasted conditions (Table 2, Figures 1 and 2). However, the mean C_{max} value for tadalafil was approximately 42.5% higher under fed (test) conditions compared with fasted (reference) conditions.

The 90% CI for the GMRs of AUC_{0-last} and $AUC_{0-\infty}$ were both within the equivalence limits for tadalafil (Table 3). For the C_{max} of tadalafil, the geometric means ratios for the test was 44.97% higher compared to reference.

The mean AUC , t_{max} , and $t_{1/2}$ values of macitentan were comparable between fed and fasted conditions (Table 2). However, the mean C_{max} value for macitentan was approximately 15.9% higher under fed (test) conditions compared with fasted (reference) conditions.

The 90% CI of the GMRs of macitentan fell within the 80.00% to 125.00% equivalence limits for $AUC_{(0-last)}$ and $AUC_{(0-\infty)}$; however, they were outside the equivalence limits for C_{max} , albeit minimally (16.10% higher for test compared with reference) (Table 3). The 90% CI of GMRs of aprocitantan for all 3 PK parameters were within the equivalence limits.

3.3.3 | Safety

In the present study, the AE profile for the FDC and free combination were consistent with the known safety profiles of macitentan and tadalafil.

No subject died and one subject in Group 1 had a serious adverse event (SAE) of transient ischemic attack 12 days after single dose administration of the FDC; the SAE was considered to be related to the study drug and led to the subject's discontinuation of the study but the SAE resolved without sequela.

The majority of the subjects in the study had at least one AE. The proportion of subjects who had at least one AE was similar for Group 1 and Group 2 and varied between 97.8% and 93.8%. Headache was the most frequently reported AE for Group 1 and Group 2, with an incidence of 84.8% and 75.0%, respectively. Other

TABLE 2 Summary of pharmacokinetic parameters of macitentan, tadalafil, and apocritentan, and tadalafil by treatment in Group 1 (FDC vs. free combination, fasted) and Group 2 (FDC fed vs. fasted), pharmacokinetic analysis set

Parameter: Group 1 Fasted	Macitentan ^a		Tadalafil ^a		Aprocritentan ^a	
	FDC	Free combination	FDC	Free combination	FDC	Free combination
N	34	34 ^b	34	34	34	34
C _{max} , ng/ml	210 (39.7)	206 (32.6)	545 (143)	610 (120)	187 (36.4)	191 (51.2)
AUC _{0–last} , ng·h/ml	5846 (1378)	5804 (1504)	20,593 (7376)	20,769 (7062)	20,930 (4982)	20,832 (4525)
AUC _{0–∞} , ng·h/ml	5934 (1384)	5903 (1490)	20,733 (7511)	20,913 (7174)	22,638 (5688)	22,500 (5222)
t _{max} , h	10.0 (2.48–10.2)	10.0 (3.55–12.0)	3.00 (1.00–24.0)	2.50 (1.00–7.00)	48.0 (24.0–72.0)	48.0 (24.0–72.0)
t _{1/2} , h	13.9 (2.87)	14.6 (2.67)	22.8 (7.76)	22.7 (7.26)	50.7 (9.99)	49.4 (6.92)
Parameter: Group 2 FDC	Fed	Fasted	Fed	Fasted	Fed	Fasted
N	15	15	15	15	15	15
C _{max} , ng/ml	241 (22.8)	208 (31.4)	677 (127)	475 (162)	176 (27.8)	165 (29.3)
AUC _{0–last} , ng·h/ml	6431 (1377)	6224 (1550)	22,330 (9697)	19,731 (10,439)	18,934 (3148)	18,095 (3061)
AUC _{0–∞} , ng·h/ml	6539 (1380)	6338 (1532)	22,503 (9969)	19,875 (10,667)	20,329 (3566)	19,499 (3546)
t _{max} , h	9.00 (4.00–10.0)	10.0 (4.00–10.0)	4.00 (1.50–10.0)	2.50 (1.50–12.0)	48.0 (24.0–48.1)	48.0 (24.0–48.0)
t _{1/2} , h	14.1 (2.50)	15.5 (2.68)	24.5 (7.29)	23.4 (6.64)	49.4 (6.02)	49.7 (7.63)

Abbreviations: AUC, area under the plasma concentration–time curve; AUC_{0–∞}, AUC from time 0 to infinity; AUC_{0–last}, AUC from time 0 to time *t* of the last measured concentration above the lower limit of quantification; C_{max}, maximum plasma concentration; FDC, fixed-dose combination; h, hours; N, maximum number of subjects with data; PK, pharmacokinetic; SD, standard deviation; t_{1/2}, terminal half-life; t_{max}, time to reach maximum plasma concentration.

Treatment A = FDC of macitentan and tadalafil in fasted conditions (test).

Treatment B = free combination of macitentan and Canadian-sourced tadalafil in fasted conditions (reference).

Treatment C = FDC of macitentan and tadalafil in fed conditions (test).

Treatment D = FDC of macitentan and tadalafil in fasted conditions (reference).

^aData are mean (SD) except t_{max}, which is median (range).

^bOverall, 33 subjects who completed both treatment periods with evaluable PK (C_{max}, AUC_{0–last} and AUC_{0–∞}) parameters (additionally for C_{max}, 1 subject prematurely discontinued the study, yet reliable C_{max} was calculated, therefore *n* = 34) for determination of bioequivalence.

frequently reported AEs included myalgia, back pain, nausea, and constipation. Most of the recorded AEs were mild in severity. The AE nature, severity and frequency were similar between treatment sequences (FDC vs. co-administration of tadalafil and macitentan), supporting the safety of the FDC formulation. This was also the case with respect to AE severity and rate in the fasted and fed conditions.

4 | DISCUSSION

This study evaluated the bioequivalence of the FDC of macitentan/tadalafil (10/40 mg) with a free combination of 10 mg macitentan and 40 mg (Canadian-sourced) tadalafil under fasted conditions in healthy subjects, as well as the effect of a high-fat high-calorie meal on the PK of the FDC of macitentan/tadalafil (10/40 mg) in healthy subjects.

Demographics and baseline characteristics were generally similar in both the groups with the exception of sex with a higher proportion of females in the study. However, with a crossover study design, the influence of sex on the results was lowered due to the intra-subject comparison and the relatively low within-subject variability.

Bioequivalence of the FDC was demonstrated to the free combination of macitentan and tadalafil (Canadian-sourced) under fasted conditions. When administered in the free combination, the PK profiles of

macitentan and tadalafil, respectively, were similar to those observed in previous studies.^{7,10,12,19} Also, mean plasma concentration–time profiles in fasted conditions for the FDC were similar to the free combination for tadalafil and macitentan/aprocritentan. The 90% CIs for the GMRs for AUC_{0–last}, AUC_{0–∞}, and C_{max} for tadalafil and macitentan/aprocritentan were within the bioequivalence limits (80.00% to 125.00%).

Mean plasma concentration–time profiles in fasted conditions for the FDC were similar to the fed combination for tadalafil and macitentan/aprocritentan. The 90% CIs for the GMRs for AUC_{0–last} and AUC_{0–∞}, for tadalafil and macitentan/aprocritentan, were within the equivalence limits (80.00% to 125.00%). However, the 90% CIs for the C_{max} GMRs for macitentan was slightly higher (just outside the 90% CI equivalence limits) and for tadalafil was markedly higher, and outside equivalence limits, following administration of FDC tablets. Despite the mean (SD) tadalafil C_{max} being higher under fed conditions, it was similar to the C_{max} observed under fasted conditions.^{7,20} The mean C_{max} for tadalafil for the FDC under fasted conditions in Group 2 was lower than for the free combination (fasted) of tadalafil in Group 1, which had a more similar C_{max} to the FDC administered under fed conditions in Group 2.

A study hold during the COVID-19 pandemic meant that the bioequivalence part of the study could not be conducted as planned. However, changing the study design to an adaptive design provided the flexibility to assess bioequivalence based on the data collected up to study hold or adapt the sample size until sufficient power was

TABLE 3 Results of bioequivalence assessment in Group 1 (FDC vs. free combination, fasted) and Group 2 (FDC, fed vs. fasted)

Parameter	Group 1 FDC vs. free combination Treatment A/B		Group 2 FDC (fed vs. fasted) Treatment C/D		Aprocitentan (N = 15)
	Macitentan (N = 34)	Tadalafil (N = 34)	Macitentan (N = 15)	Tadalafil (N = 15)	
C_{max}, ng/ml					
Geometric means ratio	101.49	88.30	116.10	144.97	107.25
90% CI	(96.95–106.25)	(82.68–94.31)	(107.12–125.83)	(128.96–162.97)	(102.08–112.69)
CV _w %	11.2	16.1	12.4	17.7	7.4
Power (%)	>99.9	80.9	NA	NA	NA
AUC_{0-last} ng·h/ml					
Geometric means ratio	100.96	98.87	102.32	111.07	104.50
90% CI	(96.99–105.09)	(94.33–103.64)	(97.90–106.94)	(103.93–118.71)	(99.60–109.63)
CV _w %	9.6	11.3	6.6	9.9	7.2
Power (%)	>99.9	>99.9	NA	NA	NA
$AUC_{0-∞}$ ng·h/ml					
Geometric means ratio	100.66	98.82	102.13	111.04	103.98
90% CI	(96.77–104.70)	(94.27–103.60)	(97.85–106.61)	(103.92–118.65)	(99.21–108.99)
CV _w %	9.5	11.3	6.4	9.9	7.0
Power (%)	>99.9	>99.9	NA	NA	NA

Abbreviations: AUC, area under the plasma concentration-time curve; $AUC_{0-∞}$, AUC from time 0 to infinity; AUC_{0-last} , AUC from time 0 to time of the last quantifiable concentration; CI, confidence interval; C_{max} , maximum plasma concentration; CV_w, intrasubject (within-subject) coefficient of variation; FDC, fixed-dose combination; N, maximum number of subjects with data; NA, not applicable; vs, versus.

Treatment A = FDC of macitentan and tadalafil in fasted conditions (test).

Treatment B = free combination of macitentan and tadalafil in fasted conditions (reference).

Treatment C = FDC of macitentan and tadalafil in fed conditions (test).

Treatment D = FDC of macitentan and tadalafil in fasted conditions (reference).

achieved to conduct the bioequivalence assessment. Despite the lower than planned sample size at the interim analysis of Stage 1 (an expected consequence of having been conservative in selecting 20% as intrasubject CV for the sample size calculation), the study had adequate power to assess bioequivalence with α -level of 0.05, and met its primary endpoint. The parallel group study design of the bioequivalence and food effect parts allowed postponement of the conduct and analysis of the food effect part until the study site re-opened with strict infection control measures in place. Ultimately, study objectives were met despite the limitations imposed by the COVID-19 pandemic.

Although there was no difference observed in terms of the extent of absorption (i.e., amount of absorption) with comparable AUCs, the increased C_{max} suggests that a high-fat breakfast transiently enhanced the rate of absorption for macitentan and tadalafil. However, the increase in C_{max} was not considered clinically significant. This can be explained as the increase in C_{max} with food was generally smaller in subjects with higher fasting C_{max} values, and the geometric mean of tadalafil C_{max} under fed conditions was similar between the FDC formulation and tadalafil (666 ng/ml vs. 586 ng/ml, ratio: 1.14),²¹ and in another study C_{max} was 614 ng/ml for tadalafil in a fasted state.²¹ Another consideration is the magnitude of the intersubject variability, which was 34.0% in C_{max} of tadalafil under fasting conditions, and was comparable to the increase in C_{max} (45%) under fed conditions.

5 | CONCLUSION

To address pandemic-imposed limitations, an adaptive study design was implemented to demonstrate bioequivalence between the FDC tablet (10/40 mg of macitentan/tadalafil) and free combination of 10 mg macitentan and 40 mg tadalafil (Canadian-sourced). No clinically significant differences in PK were determined between fed and fasted conditions; thus, similar to recommendations for the individual drug components, the FDC formulation could be taken without regard to meals. Both the FDC formulation and free combination under fasted and fed conditions were well tolerated, with no clinically relevant differences in safety profiles between the treatment groups.

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AUTHOR CONTRIBUTION

DA was responsible for the study implementation and had direct responsibility for the subjects. DC were responsible for the PK analysis and for the interpretation of the data. HS was responsible for the bioanalytical activities. JN and VD were responsible for the statistical analysis. JJPR

and VD were responsible for the interpretation of the data. All authors reviewed, contributed to, and approved the manuscript.

ETHICS STATEMENT

The study protocol and amendment were reviewed by an Institutional Review Board. This study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and applicable regulatory requirements.

DISCLOSURE

DC is an employee of Actelion Pharmaceuticals Ltd, a Janssen Pharmaceutical Company of Johnson & Johnson. DA is an employee of Celerion, Inc. Celerion, Inc. received funds from the Sponsor to perform work on the reported study. JN and VD are employees of Janssen Research and Development, USA, and own shares in Johnson & Johnson. JJPR is an employee of Janssen Research and Development, Belgium and owns shares in Johnson & Johnson. HS is an employee of Janssen Research and Development, Belgium. VF is an employee of IQVIA, Inc.; IQVIA, Inc. received funds from the Sponsor to perform work on the reported study.

DATA AVAILABILITY STATEMENT

The data sharing policy of the Sponsor is available at <https://www.janssen.com/clinical-trials/transparency>. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access (YODA) Project site at <http://yoda.yale.edu>.

ORCID

Dénes Csonka  <https://orcid.org/0000-0001-7797-2615>

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