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# Clinical investigation of the biopharmaceutical characteristics of nifurtimox tablets – implications for quality control and application

**Short title**

Nifurtimox dose proportionality

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**Highlights** (suggested)

- *In vivo* exposure to nifurtimox was unaffected by tablet dissolution rate *in vitro*
- Robust formulation is desirable in real-world treatment settings
- Exposure to nifurtimox was up to 73% greater in the fed than the fasting state
- Dose-adjusted exposure was similar across the clinically relevant dose range
- Linearity of pharmacokinetics was found for single oral doses up to 240 mg

**ABSTRACT**

Nifurtimox is approved in Chagas disease and has been used in endemic countries since the 1960s. Nifurtimox, available as a 120 mg tablet, is administered with food typically three times daily, and dose is adjusted for age and bodyweight. Accurately or reproducibly fragmenting the 120 mg tablet for dose adjustment in young children and those with low bodyweight is problematic. Based on the existing tablet formulation, new nifurtimox 30 mg and 120 mg tablets have been developed in a format that can be divided accurately into 15 mg and 60 mg fragments. In adults with chronic Chagas disease, we investigated whether nifurtimox bioavailability is affected by tablet dissolution rate, and whether different diets affect nifurtimox bioavailability. In an open-label, three-period cross-over study (n=36; ClinicalTrials.gov, NCT03350295), patients randomly received three 30 mg tablet formulations (slow, medium, or fast dissolution; a 4 × 30 mg dose of one formulation per period). In an open-label, four-period cross-over study (n=24; ClinicalTrials.gov,

NCT03334838) patients randomly fasted or received one of three meal types (high-fat/high-calorie, low-fat, dairy-based) before ingesting nifurtimox (a 4 × 30 mg dose per period). Acceptance criteria for no difference between groups were 90% confidence intervals (CIs) of exposure ratios in the range 0.8–1.25. Nifurtimox bioavailability was unaffected by tablet dissolution kinetics. Ratios of area under the curve at final assessment ($AUC_{(0–tlast)}$ [90% CI]) were: fast/medium dissolution, 1.061 (0.990–1.137); slow/medium dissolution, 0.964 (0.900–1.033); fast/slow dissolution, 1.100 (1.027–1.179). Compared with a fasting state, nifurtimox bioavailability increased by 73% after a high-fat/high-calorie meal ($AUC_{(0–tlast)}$ ratio [90% CI], 1.732 [1.581–1.898]); smaller increases were seen with the other meal types (low-fat: 1.602 [1.462–1.755]; dairy-based: 1.340 [1.222–1.468]). Although type of diet can affect bioavailability, taking nifurtimox with food is most important.

## 1. Introduction

Chagas disease is caused by infection with the protozoan parasite *Trypanosoma cruzi* (Bern, 2015). The World Health Organization (WHO) estimates that 8 million individuals are infected with *T. cruzi* globally, most in Latin America, and that more than 10,000 people die from Chagas disease annually (World Health Organization, 2020). The main route of transmission is contamination of a bite site or of mucous membranes with faeces from carrier insects that contain the parasite, but infection by blood transfusion, organ transplant, or consumption of contaminated food is also possible (Bern, 2015). Untreated, and following an incubation period, the disease has an acute phase of 6–8 weeks, followed by a chronic indeterminate (asymptomatic) or determinate (symptomatic) phase that can last for decades (Álvarez-Hernández et al., 2018; Bern, 2015). Between 70% and 80% of patients have indeterminate disease (Álvarez-Hernández et al., 2018; Bern, 2015), but those who become symptomatic may suffer cardiac, gastroenterological, neurological or combined disorders (Álvarez-Hernández et al., 2018; Bern, 2015; Rassi et al., 2010; World Health Organization, 2002). During the last 30 years, several South American countries have implemented programmes of vector eradication to reduce infection rates (Russomando et al., 2017), such

that in urban areas most new cases of Chagas disease are now attributable to congenital, transplacental infection, which is also the main route of transmission seen in non-endemic countries (Juarez et al., 2018; Pennington et al., 2017). In 2008, a study of over 12,000 young children from areas that had deployed vector-control measures for nearly 10 years determined a seroprevalence rate of 0.24% (Russomando et al., 2017). In contrast, a seroprevalence rate of 22% was estimated in 2019 in a group of 423 school-age children from an area where implementation of vector control measures was inherently problematic (Hopkins et al., 2019).

Chagas disease is treatable if antiparasitic treatment is initiated soon after *T. cruzi* infection (Meymandi et al., 2018). Treatment during the acute phase is 80–90% curative, including in early cases of congenital transmission (Bern, 2015), and treatment in the chronic phase is likely to prevent or curb disease progression (Meymandi et al., 2018). Guidelines recommend treatment with trypanocidal drugs in all patients with acute phase disease or congenital infection, in women of childbearing age (to avoid transplacental transmission), in patients with immunosuppression or at risk of reactivated infection, and in those in the indeterminate phase of the disease or with minimal cardiac involvement (Centers for Disease Control and Prevention, 2019; Edwards et al., 2017; World Health Organization, 2020). Nifurtimox and benznidazole are the only trypanocidal agents indicated in Chagas disease (Álvarez-Hernández et al., 2018), and both are approved by the US Food and Drug Administration (FDA) in paediatric patients (nifurtimox, children aged <18 years weighing >2.5 kg; benznidazole, children aged 2–12 years) (US Food and Drug Administration, 2017; US Food and Drug Administration, 2020). Nifurtimox is on the WHO Model List of Essential Medicines (World Health Organization, 2019), and is licensed for use in Argentina, Chile, El Salvador, Guatemala, Honduras and Uruguay.

Nifurtimox is metabolised in *T. cruzi* by a type I nitroreductase, generating nitrenium ions and saturated open-chain nitriles with cytotoxic activities (Hall et al., 2011). A study in animals found that nifurtimox is rapidly absorbed following ingestion (Duhm et al., 1972), and a small

clinical pharmacokinetic (PK) study in fasting healthy volunteers found that peak drug concentration in serum was reached after approximately 2 h (Paulos et al., 1989). Nifurtimox should be taken with food (US Food and Drug Administration, 2020), which both increases its bioavailability and median $t_{max}$ (approximately 4 h; Stass et al. 2021). Nifurtimox crosses both placental and blood–brain barriers (Duhm et al., 1972), and is also found in breast milk; although breastfeeding while taking nifurtimox is not recommended (Garcia-Bournissen et al., 2010), an infant's exposure to nifurtimox via this route would be lower than that experienced during treatment with nifurtimox (Garcia-Bournissen et al., 2010; Moroni et al., 2019). A major metabolic pathway involves degradation by nitroreductases, including bacterial reductases present in the gut flora (Wilkinson et al., 2008); the drug is rapidly metabolised, with only 0.5% excreted unchanged in urine (Medenwald et al., 1972), and has an elimination half-life of approximately 3 h (Paulos et al., 1989). Investigation of biliary and faecal elimination of nifurtimox and of its metabolites in humans is yet to be reported, nor have any drug–drug interactions been described, although concomitant use of nifurtimox and alcohol is contraindicated (US Food and Drug Administration, 2020). The therapeutic dose of nifurtimox must be adjusted for bodyweight, and the total daily dose is administered orally in three separate doses (morning, noon and evening) with food (US Food and Drug Administration, 2020). The most common adverse reactions in adults are nausea, decreased appetite, headache, amnesia, insomnia, fatigue or abdominal pain (Forsyth et al., 2016; Olivera et al., 2015). Dose adjustment may be necessary during treatment if weight loss occurs, and treatment may need to be discontinued if hypersensitivity reactions occur (US Food and Drug Administration, 2020).

Nifurtimox drug substance is practically insoluble in water (The International Pharmacopoeia – 9th Edition., 2019). Considering its high permeability, nifurtimox is a Biopharmaceutics Classification System class 2 compound, with dose/solubility ratios of >250 mL at pH 1.2–6.8, and is a neutral compound at physiological pH (Fig. 1A). A marketed tablet formulation containing 120 mg active drug, which is mainly distributed by WHO and the Pan American

Health Organization, was developed in the 1960s. The tablets must be divided to administer an approximate weight-adjusted dose, and often they must be pulverised and mixed with a small amount of food for administration to children unable to swallow a whole tablet. These factors present an obstacle to accurate dosing given both the complexity of the dosing regimen and that implementation of the regimen in real-world settings is often the responsibility of individuals with little medical training and few resources. Thus, to facilitate dose adjustment, tablets that can easily be divided have been developed in dose strengths of 30 mg and 120 mg based on the granules of the marketed formulation. For both dose strengths a special format is used which facilitates division of the tablets (van Santen et al. 2002), e.g., for paediatric dosing (Fig.1B). With this format, 30 mg and 120 mg tablets can be divided along score lines to give two equal 15 mg or 60 mg fragments, respectively. This permits administration of smaller and more accurate dose increments than was previously possible. Moreover, the tablets quickly disintegrate in a small quantity of water to form a slurry, which can be administered to patients unable to swallow tablets. The tablets are manufactured from a common blend of granulate, different dose strengths being obtained by adjusting the tablet weight.

**[Fig.1]**

Using the divisible nifurtimox 30 mg and 120 mg tablets, a phase 3 clinical trial demonstrated the clinical efficacy of nifurtimox over 60 days across all age groups of paediatric patients with Chagas disease (Altcheh et al., 2021). Before embarking on this trial, a PK study in adult patients with Chagas disease demonstrated that four 30 mg tablets were bioequivalent to one 120 mg tablet, and that the bioavailability of nifurtimox was unaffected whether administered in tablet form or as an aqueous slurry (Stass et al., 2021). Following US FDA guidance for industry, a clinical food effect study was also performed to assess the impact of a high calorie, high fat meal on PK (U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research., 1997). The study found that the bioavailability of nifurtimox was substantially increased if the drug was ingested after

a high-fat/high-calorie meal rather than in a fasting state, with both exposure and maximum plasma nifurtimox concentration increasing by approximately 70% (Stass et al., 2021). Such a meal is recommended when undertaking fed–fasting studies of drug exposure, but paediatric patients with Chagas disease would be unlikely to eat such a meal three times daily throughout a course of treatment. We therefore wanted to investigate how different types of meal might affect exposure to nifurtimox, and particularly whether food rich in dairy products (as may be consumed by infants and young children), or with low fat content (likely during a long treatment period), could significantly affect bioavailability, and thus whether diet may impact clinical use of nifurtimox.

Here, we report the findings from an *in vitro* study and from two clinical studies in adult patients with Chagas disease. As part of the process of setting a dissolution specification, the *in vitro* study characterised the relationship between aspects of formulation and tablet dissolution characteristics. The first clinical study then compared the performance of tablet formulations with different dissolution characteristics to investigate whether changes in dissolution rate can affect nifurtimox bioavailability. The second clinical study examined whether different types of meal eaten before tablet ingestion can affect nifurtimox PK characteristics and bioavailability. Finally, each clinical study included a sub-study to compare the dose-dependent bioavailability of nifurtimox at two dose levels. For an adult of normal weight, the maximum dose of nifurtimox typically administered three times daily is 240 mg, so it is important to understand the relationship between dose and the bioavailability of nifurtimox up to this dose level. Safety and tolerability outcomes were also monitored and reported in each of the clinical studies.

## 2. Materials and methods

### *2.1. Formulation development*

Nifurtimox tablets are manufactured as immediate-release formulations; excipients and their distributors are summarized in Table 1. The manufacturing process consists of wet high-shear granulation, wet screening, fluid-bed drying and sieving of the granules, followed by post-blending, tableting and post-drying. A wet granulation process was developed using a high-shear granulator (MGT30 – Loedige, Paderborn/Germany) to provide a free-flowing granulate for tableting. Granulation time was 6–12 minutes and was conducted at room temperature. After high-shear granulation, the granules are dried in a fluid-bed dryer (GPCG2 – Glatt, Binzen/Germany). Tablets were compressed at 12 kN using a rotary tablet press (Kilian T200, Romaco Kilian, Cologne, Germany, or Korsch XL-100, Berlin, Germany). Three side batches of 30 mg tablets, each with a different dissolution profile, were prepared for use in a clinical side-batch study (Study A – see below). The different side batches were obtained by adjusting the manufacturing process parameters for high-shear granulation (variation of granulation time) and for fluid-bed drying (variation of residual moisture in the granules after drying). Shorter granulation time and lower residual moisture resulted in faster tablet dissolution than the clinical formulation (termed ‘medium dissolution’), whereas longer granulation time and higher residual moisture led to slower tablet dissolution.

**Table 1**

Tablet composition

| Excipient | Supplier |
|---|---|
| Calcium hydrogen phosphate dihydrate | Chemische Fabrik Budenheim KG, Budenheim, Germany |
| Maize starch | Cargill Benelux B.V., Sas van Gent, The Netherlands |
| Silica colloidal anhydrous | Evonik Industries AG, Rheinfelden, Germany |
| Sodium lauryl sulfate | BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany |

Dissolution characteristics of the three different batches of 30 mg tablets used in Study A were determined using a US Pharmacopeia 2 paddle apparatus (paddle stirring speed 100 rpm, at 37°C, 12 replicates per tablet formulation, one tablet per dissolution vessel, each vessel containing 900 mL acetate buffer pH 4.5 with 1% sodium dodecyl sulfate). High-performance liquid chromatography using a 125 mm Nucleosil C18 5 µm column (internal diameter 4.0 mm or equivalent) at 40°C determined the amount of nifurtimox dissolved in an injection volume of 10 µL. Nifurtimox was eluted isocratically in acetonitrile/purified water (v/v 50/50) at a flow rate of 1.5 mL/min and detected by absorbance at 275 nM. The method was validated according to current international guidelines.

### *2.2. Clinical study oversight*

Clinical study protocols, amendments and informed consent documentation were approved by an independent ethics committee (Independent Ethics Committee for Clinical Pharmacology Research, Buenos Aires, Argentina). The studies were conducted in accordance with the ethical principles of the Declaration of Helsinki and in accordance with the International Council for Harmonization Good Clinical Practice guidelines. Studies were explained to prospective participants and all gave written informed consent before enrollment. The studies were conducted at FP Clinical Pharma SRL, Buenos Aires, Argentina. Study A (ClinicalTrials.gov Identifier: NCT03350295) was conducted 14 June – 14 December 2018. Study B (ClinicalTrials.gov Identifier: NCT03334838) was conducted 10 June 2019 – 29 January 2020.

### *2.3. Clinical study participants*

Patients were enrolled if they had a diagnosis of chronic Chagas disease based on two positive serological tests for *T. cruzi*, were aged 18–45 years (inclusive), had a body mass index ≥18 and ≤29.9 kg/m$^2$ and were otherwise healthy (no history of heart failure, of gastrointestinal disease that may impair drug absorption, of renal or hepatic conditions that may affect drug metabolism or elimination, of clinically relevant active infections, or any other condition deemed clinically significant by the investigator – see Supplementary material

Table S1 for full eligibility criteria). Women of childbearing age and sexually active men had to use two methods of contraception from enrollment until 12 weeks after completing study participation.

### *2.4. Study designs*

Both clinical studies were Phase 1, single-centre and open-label with a randomised cross-over design. The screening visit was in the 4-week period before first dose of study drug; screening began with the participant's provision of informed consent and concluded with determination of eligibility for pre-dose assessment in the first treatment period. Study A Group 1 (side-batch study), which examined the effect of tablet dissolution rate on nifurtimox bioavailability, had a three-way cross-over design (three treatment periods) and compared three nifurtimox tablet formulations with slow, medium or fast dissolution characteristics (medium dissolution corresponds to the characteristics of the existing clinical formulation), taken after a high-fat/high-calorie meal (Fig. 2A). Study A Group 2, which examined dose-proportionality, had a two-way cross-over design (two treatment periods) and compared the bioavailability of nifurtimox 30 mg or 120 mg taken after a high-fat/high-calorie meal (Fig. 2A). Study B Group 1, which investigated whether different types of food can affect the PK characteristics and bioavailability of nifurtimox, had a four-way cross-over design (four treatment periods) and compared the bioavailability of nifurtimox under fasting conditions and after each of three different meal types (Fig. 2B). Meal types are summarized in Table 2. Study B Group 2, which examined dose proportionality, had a two-way cross-over design (two treatment periods), and compared the bioavailability of nifurtimox 120 mg or 240 mg taken after a high-fat/high-calorie meal (Fig. 2B). Participants arrived at the study site on the morning of each dose after fasting overnight for >10 h, were served a meal (if specified) 30 minutes before study drug was administered in 240 mL of water, then remained at the site for 24 h post-dose. In Study A Groups 1 and 2 and in Study B Group 2, participants received a high-fat/high-calorie meal before they received study drug in each treatment period. In Study B Group 1, participants were either fasting, or received one of the meal options

specified by the intervention sequence to which they were randomised before receiving study drug. Participants had nil by mouth for 2 h post dose, then were allowed up to 240 mL of water between 2 h and 4 h post dose. Standardised meals or snacks were served at 4 h, 8 h and 12 h post dose but only after any study-related actions scheduled for that timepoint had been performed. There was a washout period of ≥5 days between all treatment periods. Participants returned to the study site 7–14 days after the last treatment period for a follow-up visit.

**[Fig. 2]**

**Table 2**

Composition of meal types

| Meal type | Composition |
|---|---|
| Low-fat meal (400–450 kcal)[a] | • 2 slices (40 g) of white bread (toasted)<br>• 20 g butter<br>• 25 g jam<br>• 20 g cheese (45% fat)<br>• 200 mL tea containing 1 cube of sugar |
| Dairy-based meal (250–300 kcal)[a] | • 300 g yoghurt (containing approximately 300 mg calcium)<br>• 150 mL milk. |
| High-fat/high-calorie meal (800–1000 kcal) | • 2 large eggs fried in 10 g butter<br>• 2 slices of fried ham<br>• 2 slices of toast<br>• 20 g butter<br>• 125 g of pan-fried potatoes<br>• 250 mL milk with 3.5% fat<br>• 100–200 mL decaffeinated coffee. |

[a]Calorific values are estimates based on published data tables (Gebhart and Thomas, 2002)

### *2.5. Treatment groups*

There were three interventions in Study A Group 1 (A: fast; B: medium; C: slow dissolution tablets). Each intervention comprised 4 × 30 mg nifurtimox tablets and participants were

randomised 1:1:1:1:1:1 to one of six prespecified intervention sequences (A–B–C; A–C–B; B–A–C; B–C–A; C–A–B; C–B–A). Two interventions were specified in Study A Group 2 (D: 1 × 30 mg nifurtimox tablet; E: 1 × 120 mg nifurtimox tablet; tablets at both doses were formulated with a medium dissolution rate) and participants were randomised 1:1 to one of two specified intervention sequences (D–E; E–D) (Fig. 2A); summaries of participant characteristics at baseline are given in Section 3 “Results”. There were four interventions in Study B Group 1 (F: fasting; G: low-fat meal; H: dairy-based meal; I: high-fat/high-calorie meal). Each intervention comprised 4 × 30 mg nifurtimox tablets and participants were randomised 1:1:1:1 to one of four prespecified intervention sequences (F–H–I–G; G–I–H–F; H–G–F–I; I–F–G–H). Two interventions were specified in Study B Group 2 (I: 4 × 30 mg nifurtimox tablets; J: 8 × 30 mg nifurtimox tablets; both were formulated with a medium dissolution rate) and participants were randomised 1:1 to one of two specified intervention sequences (I–J; J–I) (Fig. 2B).

### *2.6. Reporting and sampling schedule*

Eligibility, patient characteristics, demographic data, medical and surgical histories, and physical examinations were undertaken at the screening visit; a physical examination was also undertaken at the follow-up visit. Participants were questioned about adverse events (AEs) and previous or concomitant medications at the screening visit, during each treatment period and at follow-up. Blood samples were taken at the screening visit for laboratory tests and virology, and for laboratory tests in each treatment period before administration of study drug, at 1 day post dose, and at the follow-up visit. Blood samples were also taken for PK analyses in each treatment period up to 30 min before study-drug administration, and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 15 and 24 h post dose. At the screening visit, and in each treatment period before study drug administration, urine samples were taken for safety analyses and drug testing, and an alcohol breath test was performed; urine samples were also taken for safety analyses at the follow-up visit.

### *2.7. Nifurtimox quantitation*

Nifurtimox was assayed using liquid chromatography with tandem mass spectrometry detection (inVentiv Health Clinical, Quebec, Canada) as previously reported (Stass et al., 2021). Only values above the lower limit of quantitation were used to determine PK parameters. For all studies, the concentration–time courses of nifurtimox were prepared separately by intervention.

### *2.8. Pharmacokinetic calculations*

All PK parameters were calculated using non-compartmental methods according to the sponsor's current guidelines using WinNonlin (Version 5.3 or higher). The main parameters were $AUC_{(0–tlast)}$; AUC from zero to infinity (AUC); $C_{max}$; $AUC_{(0–tlast)}$/dose; AUC/dose (Study A); and $C_{max}$/dose. Additional parameters were $t_{max}$; AUC adjusted for dose and body weight ($AUC_{norm}$); $C_{max}$ adjusted for dose and body weight ($C_{max,norm}$); the percentage AUC from the last data point greater than the lower limit of quantitation to infinity ($\%AUC_{(tlast–\infty)}$); and $t_{½}$. The logarithms of $AUC_{(0–tlast)}$, $AUC_{(0–tlast)}$/dose, AUC, AUC/dose, $C_{max}$ and $C_{max}$/dose were analysed assuming log-normally distributed data using analysis of variance (ANOVA) adjusting for sequence, subject (sequence), period and treatment effect. The bioavailabilities of interventions A and C in Study A Group 1 (formulation-effect set [$FES_1$]) were defined as comparable to that of reference intervention B if they met the criteria stipulated in the EMA guidance on bioequivalence (EMA, 2010): the 90% confidence interval (CI) of the ratios (A/B; C/B) of their point estimates (least-squares [LS] geometric means) for $AUC_{(0–tlast)}$, AUC and $C_{max}$ had to lie within the acceptance interval 0.80–1.25. Similarly, in Study B Group 1 (food-effect set [$FES_2$]) interventions G, H and I were defined as having bioavailability comparable to that of reference intervention F if the 90% CIs of the ratios (G/F; H/F; I/F) of their LS geometric means for $AUC_{(0–tlast)}$ and $C_{max}$ lay within the acceptance interval 0.80–1.25. In Study A Group 2 and Study B Group 2 in the relative-bioavailability sets (RAS), the bioavailability of interventions E and D or of interventions I and J were comparable if the 90% CIs of the ratio of their LS means for $AUC_{(0–tlast)}$/dose, AUC/dose (Study A only), and $C_{max}$/dose lay within the acceptance interval 0.80–1.25. Intervention ratios were calculated

by re-transformation of the logarithmic data using the intra-individual standard deviation of the ANOVA. Analysis sets used in the two studies are defined in Table 3.

**Table 3**

Analysis sets and definitions.

| Study | Analysis set | Definition |
|---|---|---|
| A & B | Safety set (SAF) | All participants who received at least one dose of study drug |
| A & B | Pharmacokinetic set (PKS) | All participants who completed at least one treatment with a valid set of PK samples |
| A | Formulation-effect set ($FES_1$) | All participants in Study A Group 1 who completed the reference intervention (B) and at least one other intervention (A, C) with a valid set of PK samples |
| B | Food-effect set ($FES_2$) | All participants in Study B Group 1 who completed the reference intervention (F) and at least one other intervention (G, H, I) with a valid set of PK samples |
| A & B | Relative-bioavailability set (RAS) | All participants in Study Groups 2 who completed both interventions (D, E; or I, J) with valid sets of PK samples |

*2.9. Statistical analyses*

The F2 test was used to determine whether dissolution profiles for the three test formulations were similar; the criterion for similarity was F ≥ 50 (Moore & Flanner, 1996; ICH, 2021). For comparison of dissolution in Study A Group 1, it was estimated that 32 participants would have 80% power ($\alpha = 0.05$) to rule out an effect on bioavailability if the 90% CIs of the intervention ratios for $AUC_{(0–tlast)}$ and $C_{max}$ met the acceptance criteria (see Section 2.8), and the within-individual coefficients of variation (CVs) for the two parameters were <13.75% and <26.36%, respectively; 36 participants were recruited to allow for potential dropouts. No sample size calculation was performed for the evaluation of food effect in Study B Group 1. As well as the specified acceptance criteria (Section 2.8) for the 90% CIs of the intervention ratios for $AUC_{(0–last)}$/dose and $C_{max}$/dose, an intra-individual CV of 28% for each parameter was assumed based on previous studies. No sample size calculation was undertaken for evaluation of dose-dependent bioavailability (Groups 2) in either study. In addition to meeting the acceptance criteria, a threshold intra-individual CV of 14% for $AUC_{(0–tlast)}$/dose

and for $C_{max}$/dose was assumed based on previous studies; 12 participants were enrolled in each study. Statistics calculated at each sample point for nifurtimox concentration in plasma included: geometric mean, geometric standard deviation and coefficient of variation; median and range. Means were only calculated if at least two-thirds of the sample data were measured and were above the lower limit of quantitation. Demographic characteristics, medical and surgical history, and prior and concomitant medications were summarised descriptively in the safety set (SAF). All analyses were conducted with SAS release 9.2 (SAS Institute Inc., Cary, NC, USA).

### *2.10. Safety and tolerability*

All AEs were summarised descriptively for each study period from randomisation until the end of follow-up; treatment-emergent AEs (TEAEs) were events starting or worsening after first dose of study drug until 30 days after the last dose. The incidence and severity of AEs were reported based on Medical Dictionary for Regulatory Activities terms (version 22.1). Laboratory parameters and vital signs were also summarised descriptively.

## 3. Results

### *3.1. Formulation development*

During process development it was shown that variations in moisture and in particle-size distribution of the granules had the most impact on *in vitro* dissolution rate. The higher the residual moisture of the granules, the slower, and the greater the variability in, the *in vitro* dissolution rate. Larger granule particle-size distribution before tableting also led to slower tablet dissolution. Loss on drying and particle-size distribution (based on sieve residue values) for the fast, medium, and slow dissolution formulations used in Study A are summarised in Table 4; dissolution profiles of the three formulations are in Fig. 3. Statistical comparison of the three formulations using the F2 test determined that the slow-dissolution formulation was dissimilar to both the medium- and fast-dissolution formulation ($F2 < 50$), but the medium- and fast-dissolution formulations were similar ($F2 = 53$).

**Table 4**

Moisture level and particle size characteristics by tablet dissolution rate

| Characteristic | Fast dissolution | Medium dissolution | Slow dissolution |
|---|---|---|---|
| Loss on drying (%) | 1.2 | 2.4 | 4.4 |
| Sieve residue[a] (%) | | | |
| >63 µm | 66 | 93 | 83 |
| >125 µm | 43 | 77 | 78 |
| >250 µm | 27 | 51 | 71 |

[a] After fluid-bed drying.

**[Fig. 3]**

*3.2. Study A – Patient disposition and baseline characteristics*

Overall, 54 individuals were screened and 48 were randomised. All 48 participants were included in the SAF and the PKS, 36 in Group 1 were included in the $FES_1$ and 12 in Group 2 were included in the RAS. In Group 1, 29 participants (80.6%) were women, mean (range) age was 31.4 (19–43) years and mean (range) body mass index (BMI) was 25.5 (19.2–29.9) kg/$m^2$. The only prior medication taken by individuals in Group 1 was hormonal contraceptives (n=4); concomitant medication was administered to eight participants on study for symptomatic relief of headache (n=4), nausea (n=2), vomiting (n=1) and abdominal discomfort (n=1). In Group 2, eight of 12 participants (66.7%) were women, mean (range) age was 34.5 (28–45) years and mean (range) BMI was 26.9 (22.0–29.8) kg/$m^2$. No prior medication had been taken by individuals in Group 2; two participants received concomitant treatment for symptomatic relief of headache (n=1) and nausea (n=1). Demographic data by treatment sequence in Groups 1 and 2 are in Table 5.

**Table 5**

Patient characteristics in Study A (SAF).

| Characteristic | Group 1 intervention sequences | | | | | | Group 2 intervention sequences | | Total (N=48) |
|---|---|---|---|---|---|---|---|---|---|
| | A–B–C (n=6) | A–C–B (n=6) | B–A–C (n=6) | B–C–A (n=6) | C–A–B (n=6) | C–B–A (n=6) | D–E (n=6) | E–D (n=6) | |
| Women, n (%) | 4 (67) | 5 (83) | 6 (100) | 4 (67) | 4 (67) | 6 (100) | 4 (67) | 4 (67) | 37 (77) |
| Age, years[a] | 28.7 (19–40) | 30.7 (19–39) | 33.5 (25–43) | 27.8 (21–38) | 31.8 (22–41) | 35.8 (24–42) | 36.8 (32–42) | 32.2 (28–45) | 32.2 (19–45) |
| Weight, kg[b] | 68 (14.7) | 61 (7.0) | 69 (12.7) | 58 (6.8) | 72 (10.4) | 63 (8.7) | 70 (8.9) | 73 (14.0) | 67 (11.3) |
| BMI, kg/m$^2$[,b] | 26 (4.5) | 25 (1.7) | 28 (1.7) | 22 (2.3) | 26 (2.5) | 27 (3.1) | 27 (2.2) | 27 (2.9) | 26 (3.1) |

BMI, body mass index; SAF, safety set.

[a] Mean (range)

[b] Mean (standard deviation).

All participants received study drug under fed conditions: treatment A, 4 × 30 mg tablets, fast in vitro dissolution rate; treatment B, 4 × 30 mg tablets, medium in vitro dissolution rate; treatment C, 4 × 30 mg tablets, slow in vitro dissolution rate; treatment D, 1 × 30 mg tablet, medium in vitro dissolution rate; treatment E, 1 × 120 mg standard tablet

### *3.3. Study A Group 1 – Effect of tablet dissolution rate on bioavailability*

In Group 1, the onset of increase in nifurtimox concentration in plasma with each formulation was consistent with the respective rates of dissolution, i.e. plasma concentrations of nifurtimox increased earlier with the fast-dissolution formulation than with the slow-dissolution formulation, but there were no significant differences in absorption rate between the three formulations (Fig. 4A). From the time of ingestion, quantifiable plasma concentrations of nifurtimox appeared after 0.25–0.75 h with the fast-dissolution tablets, after 0.25–1.0 h with the medium-dissolution tablets, and after 0.25–2.0 h with the slow-dissolution tablets. Maximum plasma nifurtimox concentration ($C_{max}$) was slightly greater with the fast-dissolution tablets than with the medium- and slow-dissolution formulations (Table 6) and was reached after 3 h ($t_{max}$) compared with 4 h for the slower-dissolving formulations. Exposure to nifurtimox (AUC) was slightly greater with fast-dissolution tablets than with the other formulations, but when the different tablet formulations were compared based on the intervention ratios for their respective AUC, $AUC_{(0–tlast)}$ and $C_{max}$ values, the 90% CI for each parameter lay within the 0.80–1.25 acceptance interval, indicating that the fast- and slow-dissolution formulations met the specified criteria for bioequivalence with the reference medium-dissolution formulation. Intervention ratios (90% CIs) for AUC were: fast/medium, 1.060 (0.993–1.130); slow/medium, 0.985 (0.924–1.051); and fast/slow, 1.075 (1.008–1.147). Intervention ratios (90% CIs) for $AUC_{(0–tlast)}$ and for $C_{max}$ are in Fig.4B.

**[Fig. 4]**

### *3.4. Study B – Patient disposition and baseline characteristics*

In total, 42 adults with Chagas disease were screened; 36 were randomised and completed

**Table 6**

Pharmacokinetic parameters of nifurtimox in Study A (PKS).

| | Group 1<br>Dissolution and bioavailability | | | Group 2<br>Dose-dependent bioavailability | |
|---|---|---|---|---|---|
| Parameter[a] | Intervention A<br>4 × 30 mg tablets,<br>fast dissolution<br>(N=36) | Intervention B<br>4 × 30 mg tablets,<br>medium dissolution<br>(N=36) | Intervention C<br>4 × 30 mg tablets,<br>slow dissolution<br>(N=36) | Intervention D<br>1 × 30 mg tablet,<br>medium dissolution<br>(N=12) | Intervention E<br>1 × 120 mg<br>standard tablet<br>(N=12) |
| AUC, µg.h/L | 1932<br>(1122–2784) | 1823<br>(1014–2807) | 1796<br>(669–2485) | 450<br>(319–617)[b] | 1937<br>(1001–2514) |
| $AUC_{(0–tlast)}$, µg.h/L | 1844<br>(1080–2694) | 1738<br>(939–2751) | 1676<br>(485–2415) | 351<br>(131–501) | 1842<br>(969–2469) |
| $C_{max}$, µg/L | 372<br>(176–705) | 359<br>(195–885) | 355<br>(89–750) | 93<br>(30–206) | 425<br>(204–768) |
| $t_{max}$, h[c] | 3 (2–6) | 4 (1–6) | 4 (2–8) | 4 (2-6) | 3 (2–6) |
| $t_{½}$, h | 2.7<br>(1.3–4.9) | 2.6<br>(1.5–3.8) | 2.8<br>(1.3–7.2) | 2.4<br>(1.3–3.1)[c] | 2.4<br>(1.4–3.9) |

[a] Geometric mean (range) unless stated otherwise.

[b] N=11.

[c] Median (range)

AUC, area under the concentration curve; $AUC_{(0–tlast)}$, area under the concentration curve from baseline to last measurable concentration; $C_{max}$, maximum observed concentration; PKS, pharmacokinetic set; $t_{max}$, time to reach $C_{max}$; $t_{½}$, half-life.

the study. All 36 participants were included in the SAF and the PKS; the 24 patients in Group 1 were included in the $FES_2$ and the 12 patients in Group 2 in the RAS. In Group 1, 23 participants (95.8%) were women, all participants were white and 21 (87.5%) were Hispanic or Latino. Average (range) age was 31.3 (19–42) years, average (range) BMI was 25.3 (18.3–29.6) $kg/m^2$. The only prior medication taken by individuals in Group 1 was hormonal contraceptives (n=5); 13 participants received concomitant medication for symptomatic relief of headache or nausea, or for hormonal contraception. In Group 2, 10 (83.3%) participants were women, and all participants were white-Hispanic or white-Latino. Average (range) age was 35.6 (30–44) years, average (range) BMI was 27.8 (20.9–29.7) $kg/m^2$. The only prior medication taken by individuals in Group 2 was hormonal contraceptives (n=4); four participants in Group 2 continued to receive hormonal contraception concomitantly with study drug. Demographic data for Groups 1 and 2 are summarised in Table 7.

### *3.5. Study B Group 1 – Effect of food type on bioavailability*

The effect of different diets on exposure to nifurtimox is illustrated by the plasma concentration curves for each intervention (Fig. 5A). Exposure assessed by $AUC_{(0–tlast)}$ was greater in the fed than in the fasting state, and a high-fat/high-calorie meal was associated with greater exposure than low-fat or dairy-based meals. The same pattern among the different interventions was seen for $C_{max}$ (Table 8). The 90% CIs for the intervention ratios associated with each meal type were not bounded by the acceptance interval of 0.80–1.25, indicating a food effect on exposure with each type of meal. Intervention ratios relative to the fasting group for $C_{max}$ showed a similar pattern to those for $AUC_{(0–tlast)}$ (Fig. 5B). $t_{max}$ was longer in the fed than in the fasting state, but $t_{½}$ was similar across all four interventions.

**Table 7**

Patient characteristics in Study B (SAF).

| Characteristic | Group 1 intervention sequences | | | | | Group 2 intervention sequences | | |
|---|---|---|---|---|---|---|---|---|
| | F–H–I–G (n=6) | G–I–H–F (n=6) | H–G–F–I (n=6) | I–F–G–H (n=6) | Total (N=24) | I–J (n=6) | J–I (n=6) | Total (N=12) |
| Women, n (%) | 5 (83) | 6 (100) | 6 (100) | 6 (100) | 23 (96) | 5 (83) | 5 (83) | 10 (83) |
| Age, years[a] | 24.3 (19–30) | 34.3 (20–42) | 34.0 (28–42) | 32.7 (25–42) | 31.3 (19–42) | 35.0 (32–41) | 36.2 (30–44) | 35.6 (30–44) |
| Weight, kg[b] | 63 (11.4) | 61 (8.1) | 68 (12.3) | 55 (8.0) | 62 (10.6) | 65 (9.9) | 71 (6.7) | 68 (8.8) |
| BMI, kg/m$^2$[,b] | 25 (4.6) | 26 (2.9) | 27 (3.1) | 23 (3.6) | 25 (3.6) | 27 (3.1) | 29 (1.1) | 29 (2.5) |

BMI, body mass index; SAF, safety set.

[a] Mean (range)

[b] Mean (standard deviation).

All participants in groups F–I received a nifurtimox 120 mg dose based on 4 × 30 mg tablets with a medium dissolution rate: intervention F, under fasting conditions; intervention G, after a low-fat meal; intervention H, after a dairy-based meal; intervention I, after a high-fat/high-calorie meal. Participants randomised to intervention J, received a nifurtimox 240 mg dose, based on 8 × 30 mg tablets with a medium dissolution rate, after a high-fat/high-calorie meal.

**[Fig. 5]**

### *3.6. Study A and B Groups 2 – Dose proportionality*

In Study A Group 2, exposure to nifurtimox was compared in individuals receiving 30 mg or 120 mg drug in the fed state. PK parameters are summarised in Table 6. Nifurtimox concentration in plasma was estimated at fewer time points in the 30 mg dose group than in the 120 mg dose group because drug concentration was below the lower limit of quantitation more frequently (e.g. at earlier time points in the terminal phase, Fig. 6A). This difference and wide variation in nifurtimox plasma concentration between individuals affected interpretation of dose proportionality. The intervention ratios (90% CIs) of the 120 mg to the 30 mg dose for the dose-adjusted PK parameter values were: AUC/dose, 1.107 (0.985–1.244); $AUC_{(0–tlast)}$/dose, 1.314 (1.118–1.544); and $C_{max}$/dose, 1.139 (0.924–1.405). The 90% CI for the AUC/dose intervention ratio was bounded by the acceptance interval, indicating a linear increase in exposure with increasing dose. Based on the $AUC_{(0–tlast)}$/dose intervention ratio, dose-adjusted exposure was apparently 31% greater with the 120 mg than the 30 mg dose, and the upper limit of the 90% CI for the $C_{max}$/dose ratio was outside the acceptance interval.

In Study B Group 2, exposure to nifurtimox ($AUC_{(0–tlast)}$) increased dose-dependently (Fig. 6B) but was slightly greater with nifurtimox 240 mg than with nifurtimox 120 mg after dose adjustment (Table 8). Comparing both dose-adjusted values with that determined for the high-fat/high-calorie intervention in Group 1, dose-adjusted exposure was similar overall, in the range 0.018–0.020 h/L.

**Table 8**

Pharmacokinetic parameters of nifurtimox in Study B (PKS).

| | Group 1 Food effect | | | | Group 2 Dose-dependent bioavailability | |
|---|---|---|---|---|---|---|
| Parameter[a] | Intervention F 4 × 30 mg tablets, fasting (N=24) | Intervention G 4 × 30 mg tablets, low-fat meal (N=24) | Intervention H 4 × 30 mg tablets, dairy-based meal (N=24) | Intervention I 4 × 30 mg tablets, high-fat/ high-calorie meal (N=24) | Intervention I 4 × 30 mg tablet, high-fat/ high-calorie meal (N=12) | Intervention J 8 × 30 mg tablets high-fat/ high-calorie meal (N=12) |
| AUC (0–$t_{last}$), µg.h/L | 1290 (577–2030) | 2070 (1070–3370) | 1730 (1070–3140) | 2230 (1360–2920) | 2100 (1460–3020) | 4830 (3140–6310) |
| AUC (0–$t_{last}$)/dose, h/L | 0.0107 (0.0048–0.0170) | 0.0172 (0.0090–0.0280) | 0.0144 (0.0089–0.0262) | 0.0186 (0.0113–0.0243) | 0.0175 (0.0122–0.0252) | 0.0201 (0.0131–0.0263) |
| $C_{max}$, µg/L | 238 (104–543) | 427 (149–697) | 356 (169–882) | 455 (180–932) | 391 (204–951) | 889 (603–1450) |
| $C_{max}$/dose, L | 0.0020 (0.0009–0.0045) | 0.0036 (0.0012–0.0058) | 0.0030 (0.0014–0.0074) | 0.0038 (0.0015–0.0078) | 0.0033 (0.0017–0.0079) | 0.0037 (0.0025–0.0060) |
| $t_{max}$, h[b] | 2.75 (0.75–6.00) | 4.00 (1.50–6.00) | 4.00 (1.50–6.03) | 4.00 (2.00–8.00) | 5.00 (2.5–8.00) | 4.00 (2.50–8.00) |
| $t_{½}$, h | 2.92 (1.49–6.90) | 3.02 (1.90–15.0) | 2.90 (1.67–15.0) | 3.10 (1.81–4.99) | 3.38 (2.04–6.64) | 3.22 (2.20–4.18) |

[a] Geometric mean (range) unless stated otherwise.

[b] Median (range).

AUC, area under the concentration curve; AUC (0–tlast), area under the concentration curve from baseline to last measurable concentration; Cmax, maximum observed concentration; PKS, pharmacokinetic set; tmax, time to reach Cmax; t½, half-life.

Median $t_{max}$ was slightly longer in the 120 mg dose group than in the 240 mg dose group, but the respective ranges for $t_{max}$ were similar to that seen in Group 1 (2.5–8.0 vs 2.0–8.0); $t_{½}$ was also slightly longer in Group 2 than in Group 1 (3.2–3.4 h vs 2.9–3.1 h). Intervention ratios (90%CI) for dose-adjusted PK parameter values in the 120 mg dose versus the 240 mg dose group were: $AUC_{(0–tlast)}$/dose, 0.868 (0.792–0.951) and $C_{max}$/dose, 0.879 (0.706–1.09). The intervention ratios suggested comparability of PK, although the lower limits of the 90% CIs for both parameters were outside the acceptance interval.

**[Fig. 6]**

### *3.7. Safety*

All TEAEs occurring in Study A and Study B were of mild or moderate intensity. There were no serious TEAEs or deaths; TEAEs occurring in at least of 5% of participants overall in either group in each study are summarised in Table S2. Overall, nifurtimox had a favourable safety profile and was well tolerated in both the fed and fasting states. Further description of the safety findings is provided in the supplementary text.

## 4. Discussion

Divisible nifurtimox 30 mg and 120 mg tablets have been developed as immediate-release formulations. Characterizing the relationship between changes in drug dissolution rate and bioavailability is a key part of the process of defining a specification. Dissolution specification was set based on FDA guidance (U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research., 1997) and on ICH Q6A guidance (European Medicines Agency., 2000). As recommended therein, the dissolution specification proposal was set based on human PK data for fast- and slow-dissolving batches, exposure to which was shown here to be equivalent (Study A, Group 1), as well as on dissolution profiles of clinical batches with proven efficacy in a pivotal Phase 3 study

(Altcheh et al., 2021). Dissolution curves of the Phase 3 batches (Supplementary material, Figure S1) lay between the curves of the fastest and slowest dissolution batches tested in Study A. Overall, it can be concluded that the dissolution method over discriminated, as it could detect very sensitively the impact of variations in granule moisture and particle size, which were identified as the drivers of altered *in vitro* dissolution kinetics, even though these variations had no impact on the *in vivo* performance of the drug product.

A food effect was demonstrated in Study B Group 1 that supports ingestion of low-fat or high-fat/high-calorie meals before taking nifurtimox so as to optimise systemic drug exposure; exposure was lower following a dairy-based meal. However, this observation does not impact posology. A Phase 3 study demonstrated the clinical effectiveness of nifurtimox in South American patients ranging in age from infants to adolescents (Altcheh et al., 2021). Patients' diets on study were not prespecified and therefore meals with different dietary composition representative of a real-world setting were warranted in the study. Thus, although diet affects PK, dosing regimens do not have to be adjusted according to diet, and accordingly, taking nifurtimox with food is mandated by the drug label but the type of food is not stipulated (US Food and Drug Administration, 2020). This is important because individuals typically take nifurtimox three times daily for at least 60 days, and compliance with such a dosing regimen is likely to be facilitated by dietary flexibility.

It is not possible to state definitively why drug bioavailability associated with ingestion of nifurtimox in the fed state should be greater than in the fasting state, nor what aspects of the different meal types investigated here are relevant to the changes in bioavailability observed. As a poorly soluble molecule, one could speculate that the effect in the fed state might be attributable to nifurtimox having a longer residence time in the stomach and small intestine because of decreased gastric motility, delayed gastric emptying, and increased transit time, all of which would provide more time for drug dissolution and possibly reduce pre-systemic metabolism by bacterial reductases. Bile acid secretion may also play a role in solubility, and increased splanchnic blood flow in the fed-state could also increase drug absorption. Such

effects may explain the difference in bioavailability observed with the different meal types (e.g., a high-fat diet having the greatest effect on gastric motility and therefore the greatest impact on bioavailability) (O'Shea et al. 2019; Pentafragka et al. 2019).

Dose-proportionality studies provided some preliminary information about the dose–bioavailability relationship for nifurtimox across the clinically most relevant dose range (30–240 mg/dose). In Study A Group 2, dose-adjusted exposure was greater with the 120 mg than with the 30 mg dose, but this discrepancy can be attributed to the greater number of time points at which the plasma concentration of nifurtimox could be determined following the 120 mg than the 30 mg dose, and to the small sample size, which renders this part of the study exploratory in nature. Also, the upper limit of the 90% CI for the $C_{max}$/dose ratio for these two doses was outside the acceptance interval. Again, this can be attributed to the smaller number of evaluable time points with the 30 mg dose as well as the small sample size, but would also be affected by the variability in $t_{max}$ observed in the two dose groups. In Study B Group 2, the lower limits of the 90% CIs for $AUC_{(0–tlast)}$/dose and for $C_{max}$/dose were also outside the acceptance interval, implying a trend toward greater exposure at the 240 mg dose than at the 120 mg dose. However, a separate population PK (popPK) analysis of a much larger dataset that pooled data from multiple clinical studies, including Study A here, determined that the dose–bioavailability relationship is linear across the 30–240 mg dose range (Ince I, personal communication). That exposure differences were not seen when a much larger dataset was analysed suggests that the discrepancy seen here may relate to the small sample size and possibly to the exploratory nature of the study.

Determining that the dose–bioavailability relationship is linear is key to assessment of the risk–benefit profile across the dose range used, in that the efficacy and safety profiles are not disproportionately affected by dose variation. The importance of this relationship is underlined by the fact that the regimen must be tailored to a range of age groups and bodyweights, from newborn infants to adults.

Taken together, the observations that different dissolution profiles do not affect PK and that the dose–bioavailability relationship is linear have important ramifications for the exchangeability of different dosage forms: drug exposure, and efficacy and safety outcomes, are the same following ingestion of four 30 mg tablets or one 120 mg tablet. A separate equivalence study has confirmed the equivalence of drug exposure following ingestion of one 120 mg or of four 30 mg tablets, and following ingestion of four whole 30 mg tablets or of four 30 mg tablets dispersed as a slurry in water (Stass et al., 2021). Moreover, all tablet fragments met US Pharmacopeia requirements on weight uniformity as part of an investigation of uniformity of dosage (USP Convention, 2011). This finding taken together with the performance of the tablet slurry indicates that tablet fragmentation should not alter nifurtimox bioavailability. The safety and tolerability profile of nifurtimox was consistent with that seen in other studies. No new safety signals were identified, no serious events occurred and there were no deaths. Study-drug related instances of QTc prolongation were clinically asymptomatic and normalised within 24 hours without sequelae.

We have demonstrated that diet affects the PK profile of nifurtimox but based on findings of a recent clinical study (Altcheh et al., 2021), this is not expected to impact the drug's clinical effectiveness in Chagas disease. We have also shown that intentional changes in tablet dissolution characteristics have essentially no effect on the PK profile of nifurtimox, ensuring that the tablets will perform predictably irrespective of the batch variation that falls within the product specification. Based on the findings reported here regarding the stability of the formulation, and its dissolution and absorption characteristics, patients receiving a 60-day supply of nifurtimox can be confident that the drug will perform comparably until the end of the treatment period.

The linearity of pharmacokinetics across the range of clinically relevant therapeutic doses means that minor deviations from recommended dosing, which may arise during dose adjustment for age and bodyweight, should not be associated with disproportionally large variations in drug-related effects. Moreover, despite the complexity of the dosing regimen,

the flexibility of the dosage form (divisible and water-dispersible tablets) and the information accumulated about the biopharmaceutical properties of nifurtimox provide reassurance about the predictability of the risk–benefit profile in paediatric patients who are following the newly approved dosing recommendations (US Food and Drug Administration, 2020).

## 5. Conclusions

The findings presented here show how both biopharmaceutical and dosing conclusions were reached that underpin the clinical development of nifurtimox, as well as providing proof of concept for the dosing recommendations approved in children. Treatment of Chagas disease requires a vulnerable population of patients to follow a complex dosing regimen for an extended period. The availability of nifurtimox 30 mg and 120 mg tablets that are divisible and water-dispersible will facilitate the effective delivery of this therapy in these individuals.

## Conflicts of interest

Heino Stass, Sarah Just, Ibrahim Ince, Stefan Willman, Cecilia Freitas and Uwe Münster are employees of Bayer AG. Boris Weimann, Ethel Feleder and Gustavo Yerino are employees of organisations that received funding from Bayer AG for undertaking parts of the work reported here.

## Data statement

Study designs and synopses of the study data can be viewed and downloaded from https://clinicaltrials.bayer.com/ (studies 16006 and 16007).

**CRediT authorship contribution statement**

**Heino Stass:** Conceptualization; Methodology; Validation; Formal analysis; Investigation; Resources; Data Curation; Writing - Original Draft; Writing - Review & Editing; Visualization; Supervision; Project administration.

**Sarah Just:** Methodology; Validation; Formal analysis; Investigation; Resources; Writing - Original Draft; Writing - Review & Editing; Visualization.

**Boris Weimann:** Methodology; Validation; Formal analysis; Investigation; Resources; Writing - Original Draft; Writing - Review & Editing.

**Ibrahim Ince:** Methodology; Validation; Formal analysis; Investigation; Resources; Writing - Original Draft; Writing - Review & Editing.

**Stefan Willman:** Methodology; Validation; Formal analysis; Investigation; Resources; Writing - Original Draft; Writing - Review & Editing.

**Ethel Feleder:** Methodology; Validation; Formal analysis; Investigation; Resources; Data Curation; Writing - Review & Editing.

**Cecilia Freitas:** Methodology; Validation; Formal analysis; Investigation; Resources; Writing - Original Draft; Writing - Review & Editing.

**Gustavo Yerino:** Methodology; Validation; Formal analysis; Investigation; Resources; Data Curation; Writing - Review & Editing.

**Uwe Münster:** Methodology; Validation; Formal analysis; Investigation; Resources; Writing - Original Draft; Writing - Review & Editing; Visualization.

## Acknowledgements

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**Fig. 1**. Nifurtimox – A. Chemical structure (reproduced from Wikimedia Commons https://commons.wikimedia.org/wiki/File:Nifurtimox_Structure.svg). B. Special tablet format (upper panels) that can be snapped reproducibly into two equal fragments (lower panels; nifurtimox 30 mg, images from Bayer AG).

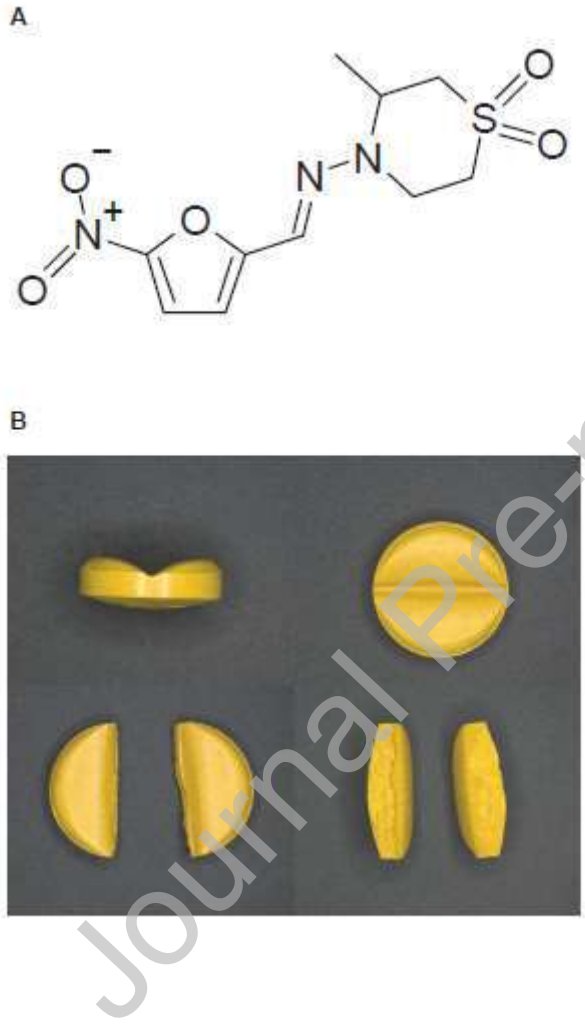
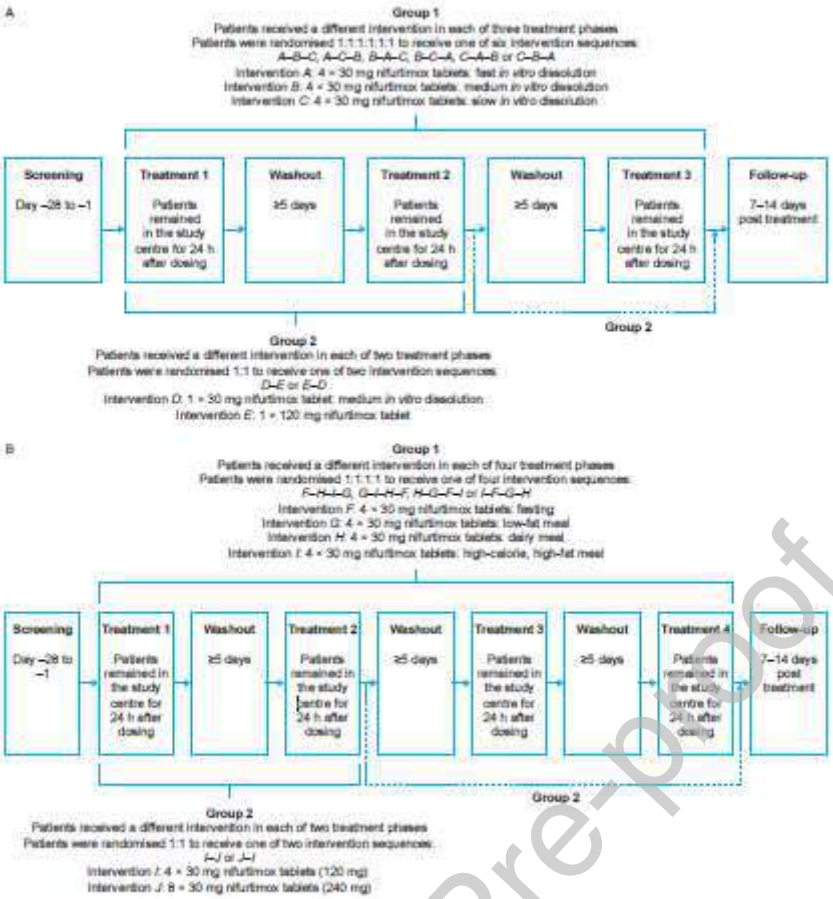





**Fig. 2.** Study design.

A. Study A – Group 1: formulation equivalence; Group 2: dose proportionality.

B. Study B – Group 1: food effects; Group 2: dose proportionality.

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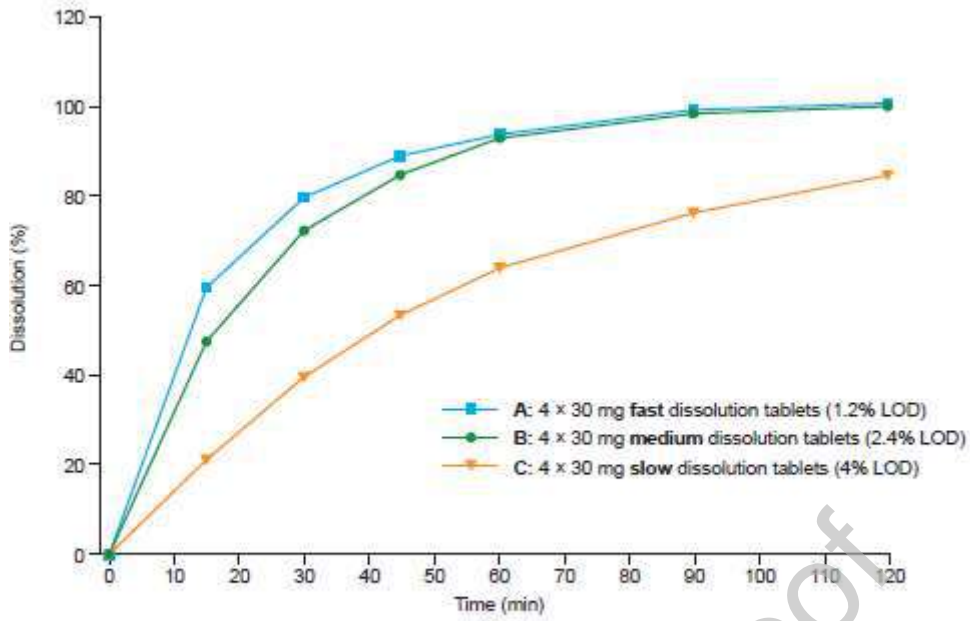
265 **Fig. 3**. *In vitro* dissolution characteristics of the 30 mg tablets used in Study A interventions
266 A (fast), B (medium) and C (slow). LOD, loss on drying. Comparison using the F2 test
267 determined the dissolution profiles of A and B were similar and that those of A and C and of
268 B and C were dissimilar.




269

270 **Fig. 4.** Formulation equivalence – A. Nifurtimox concentration in plasma (Study A, Group 1,
271 interventions A, B and C; $FES_1$). Data are geometric mean and standard deviation; semi-
272 logarithmic plot. $FES_1$, formulation-effect analysis set; LLOQ, lower limit of quantitation. B.
273 Intervention ratios (90% confidence interval) for $AUC_{(0-tlast)}$ and $C_{max}$. The two horizontal lines
274 represent the 0.8–1.25 acceptance interval for equivalence.

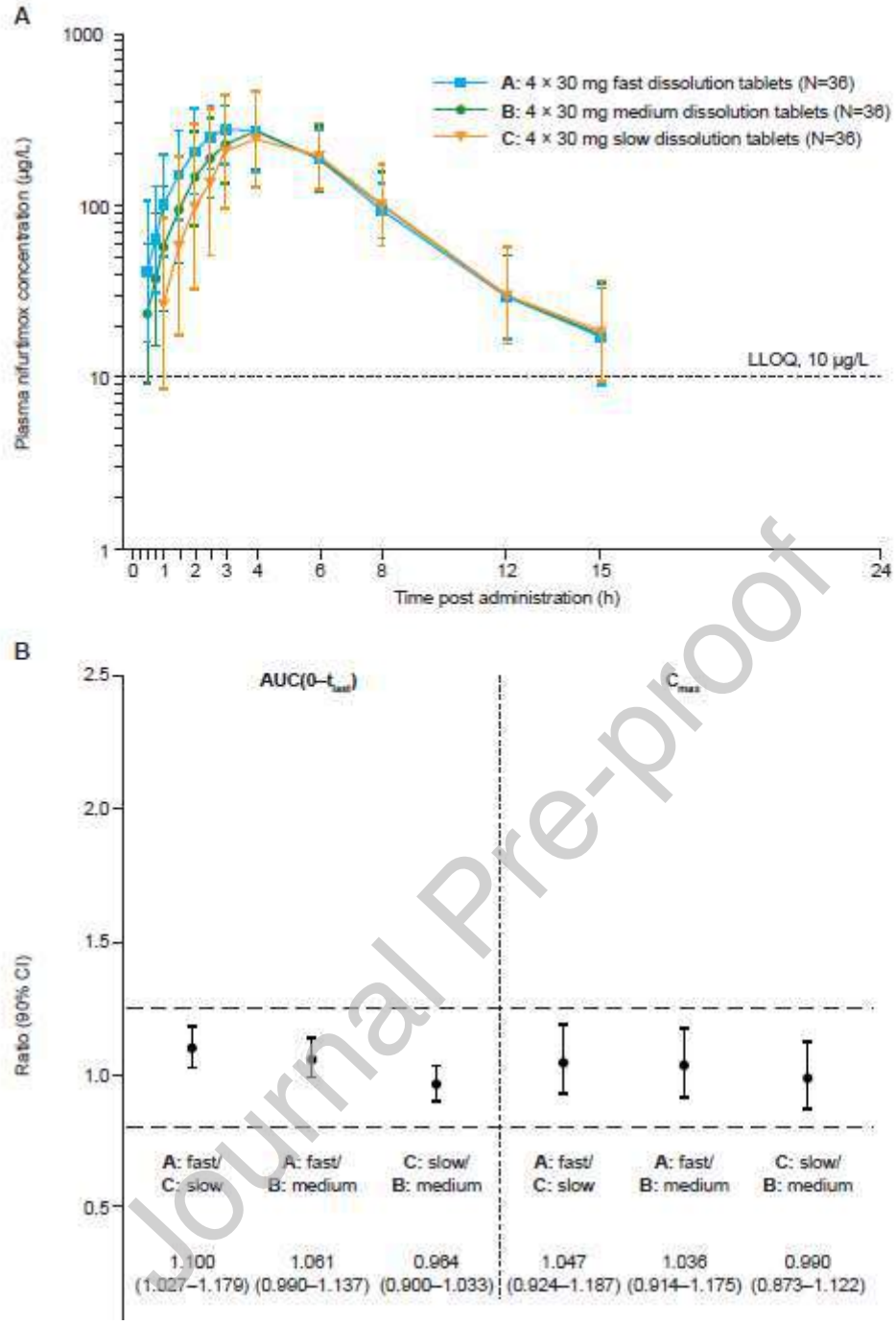





**Fig. 5.** Food-effect – A. Nifurtimox concentration in plasma (Study B, Group 1, Interventions F, G, H and I; $FES_2$). Data are geometric mean and standard deviation; semi-logarithmic plot. $FES_2$, food-effect analysis set; LLOQ, lower limit of quantitation. B. Intervention ratios (90% confidence interval) for $AUC_{(0–tlast)}$ and $C_{max}$. The two horizontal lines represent the 0.8–1.25 acceptance interval for equivalence.

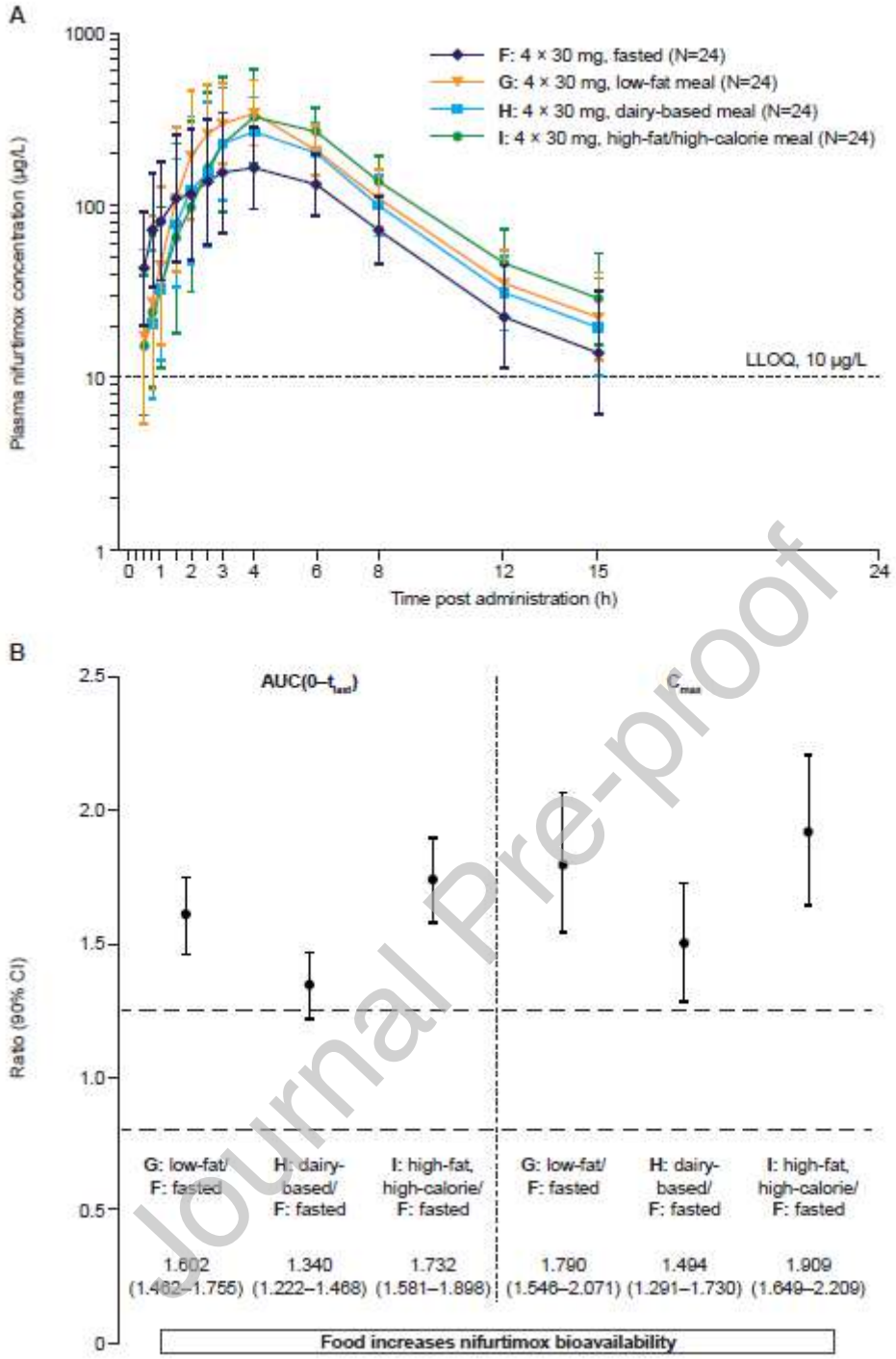
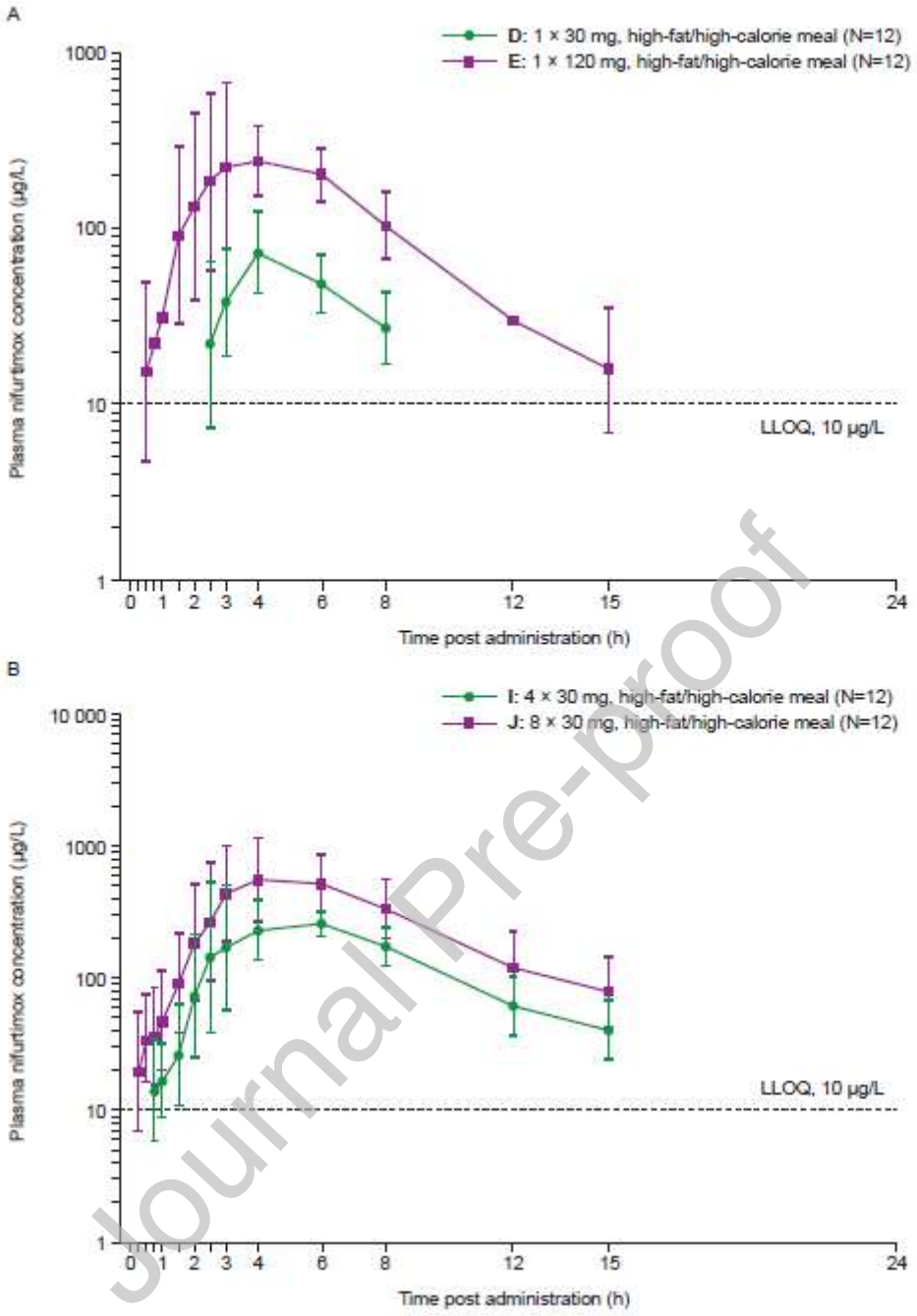



**Fig. 6.** Dose proportionality – A. Nifurtimox concentration in plasma, Study A, Group 2, Interventions D and E (RAS). B. Intervention ratios (90% confidence interval) for $AUC_{(0–tlast)}$ and $C_{max}$, Studies A and B, Groups 2. C. Nifurtimox concentration in plasma, Study B, Group 2, Interventions I and J (RAS). Data are geometric mean and standard deviation; semi-logarithmic plot. LLOQ, lower limit of quantitation; RAS, relative bioavailability analysis set.

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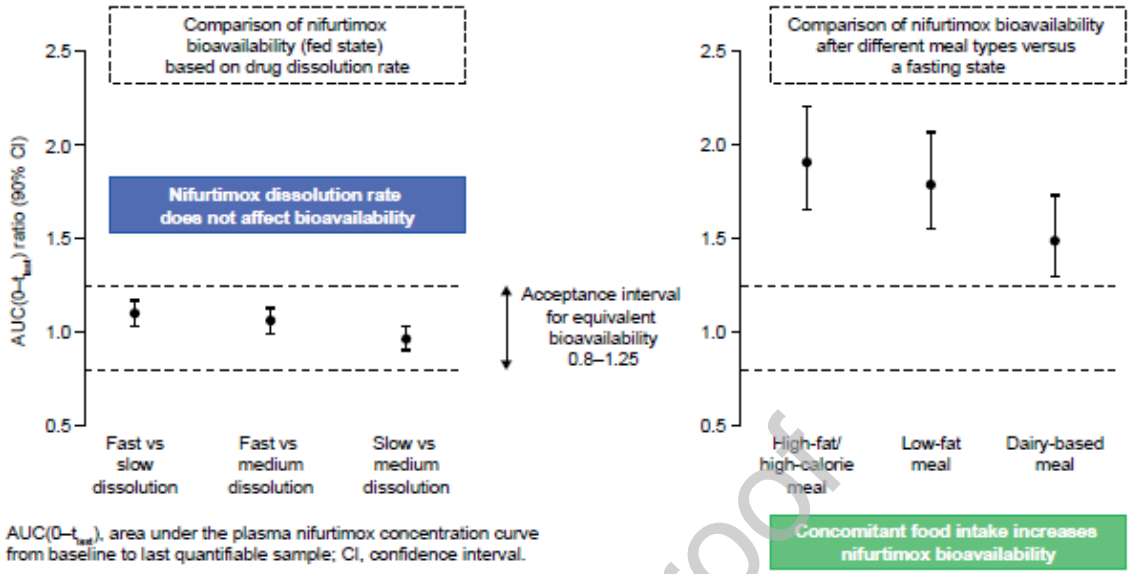


AUC(0–$t_{last}$), area under the plasma nifurtimox concentration curve from baseline to last quantifiable sample; CI, confidence interval.

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