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Bioequivalence of two oral formulations of nitazoxanide in healthy volunteers

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Abstract

Objective: This study evaluated the bioequivalence (BE) between two Nitazoxanide 20mg/ml oral suspension formulations. **Methods:** We carried out a single-center, open-label, randomized, single-dose, two-sequence, two-period. Subjects received single oral doses of 500 mg Nitazoxanide oral suspension. Whole blood samples were collected pre-dose and at specified intervals up to 12 h post-dose to assess pharmacokinetic parameters. **Results:** Thirty healthy adult subjects completed the study. We found the 90% confidence intervals for the geometric mean ratios for the Tixozanide area under the curve AUC_{0-t} and $AUC_{0-\infty}$ and maximum plasma concentration (C_{max}) were within the established limits of 80% to 125 % of BE. **Conclusion**: The criteria for BE were met for the nitazoxanide formulations from Eurofarma Laboratórios S.A.

Keywords: Nitazoxanide, antiparasitic, bioequivalence, pharmacokinetics

Bioequivalência de duas formulações de nitazoxanida em voluntários sadios

Resumo

Objetivo: Este estudo avaliou a bioequivalência (BE) entre duas formulações de suspensão oral de nitazoxanida 20mg/ml. **Metodologia**: Foi realizado um estudo monocêntrico, aberto, randomizado, de dose única, de duas sequências e de dois períodos. Os indivíduos receberam doses orais únicas de 500 mg de suspensão oral de nitazoxanida. Foram colhidas amostras de sangue total antes da dose e em intervalos especificados até 12 horas após a dose para avaliar os parâmetros farmacocinéticos. **Resultados:** Trinta indivíduos adultos saudáveis completaram o estudo. Os intervalos de confiança de 90% para as relações das médias geométricas para a área sob a curva da tixozanida AUC_{0-t} e $AUC_{0-\infty}$ e concentração plasmática máxima (C_{max}) estavam dentro dos limites estabelecidos de 80% a 125% de BE. **Conclusão:** Os critérios para BE foram cumpridos para as formulações de teste de nitazoxanida 20mg/ml da Eurofarma Laboratórios S.A.

Palavras-chave: Nitazoxanida, antiparasitário, bioequivalência, farmacocinética

Introduction

Nitazoxanide (NTZ) is a synthetic, broad-spectrum antiparasitic, derived from nitrothiazolyl-salicylamide, for oral administration. It is a bright yellow crystalline powder, slightly soluble in ethanol and practically insoluble in water. Chemically it is 2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide. The molecular formula is C12H9N3O5S and its molecular weight is 307.3[1]. NTZ is a prodrug, rapidly hydrolysed non-enzymatically or by plasma esterases with a $t_{½}$ of about 6 min to tizoxanide (TZX) or desacetyl nitazoxanide [(2-hydroxy-N-(5-nitro-2-thiazolyl) benzamide)]. TZX is further glucuronidated to form tizoxanide glucuronide (TG). TZX is further degradeted to more hydrophilic compounds and be eliminate predominantly by urine (see Figure 1).

In Brazil, nitazoxanide is currently approved for treating intestinal infections caused by protozoa and helminths, as well as for gastroenteritis caused by rotavirus and norovirus. It is available commercially in two pharmaceutical forms: a 20mg/ml oral solution and a 500mg tablet[2].

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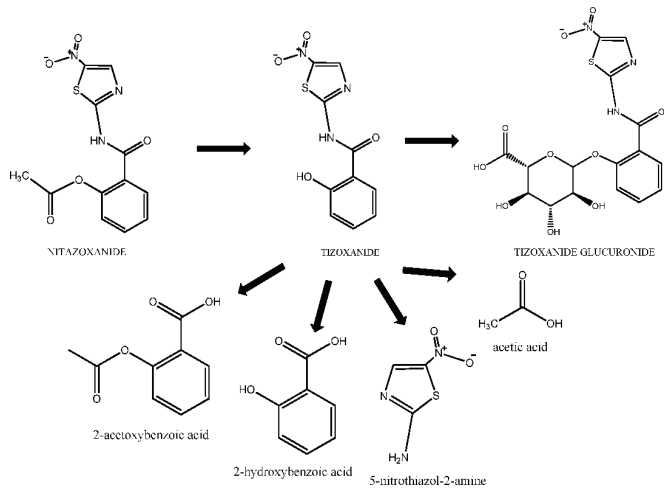
Figure 1. Metabolites pathway after administration of nitazoxanide



The antiprotozoal activity of NTZ is believed to be due to interference with the pyruvate ferredoxin oxidoreductase (PFOR) enzyme dependent electron transfer reaction which is essential to anaerobic energy metabolism. Studies have shown that the PFOR enzyme from Giardia lamblia directly reduces nitazoxanide by transfer of electrons in the absence of ferredoxin. The DNA derived PFOR protein sequence of *Cryptosporidium parvum* appears to be similar to that of *Giardia lamblia*. Interference with the PFOR enzyme dependent electron transfer reaction may not be the only pathway by which nitazoxanide exhibits antiprotozoal activity[3-5].

After oral administration of nitazoxanide oral suspension, peak plasma concentrations of its active metabolites tizoxanide and tizoxanide glucuronide are observed within one to four hours[6]. Therefore, nitazoxanide is not detected in plasma. The relative bioavailability of the oral suspension compared to the tablet is 70%. When nitazoxanide tablets are administered under fed conditions, the AUC of tizoxanide and tizoxanide glucuronide in plasma increases by almost two-fold and C_{max} by almost 50%. When nitazoxanide oral suspension is administered with food, the AUC of tizoxanide and tizoxanide glucuronide increases by approximately 45-50% and C_{max} by ≥10% [7].

Bioequivalence studies are essential for health services as they ensure that generic drugs are as safe and effective as their brand-name counterparts, providing the same therapeutic benefits. By confirming the similarity in bioavailability between generic nitazoxanide formulation and original Anitta®, the present study facilitates the approval of cost-effective alternative, enhancing patient access to affordable medication.

Methods

The study protocol and the informed-consent form were approved by an independent ethics and research committee of *INVESTIGA - INSTITUTO DE PESQUISA, Campinas, SP,* Brazil, prior to the study initiation, under CAAE registry number 19535113.6.0000.5480 and approval number 338.685. The study was conducted in accordance with the principles of Helsinki and its amendments and the International Conference on Harmonisation Guideline for Good Clinical Practice. The subjects were lectured about the study details and all their questions were clarified to facilitate an informed decision on whether to join the study or not. Those who accepted to participate signed the Informed Consent Form.

Participants were recruited from the CAEP database. Thirty-six healthy male and female volunteers between 18 and 49 years, were enrolled, meeting the criteria of being between 18 and 50 years of age, non-smokers or former smokers who have abstained for over a year, weighing 50 kg or more, with a body mass index (BMI) ranging between 18.70 kg/m² and 29.99 kg/m² and testing negatively for HIV-1 and HIV-2 in serum tests. Additionally, the absence of evidence of infections with hepatitis B or hepatitis C viruses. A physical examination was conducted in each participant. Subject's health was based on unremarkable findings on a clinical health evaluation, which consisted on the following: a personal interview; complete physical examination (blood pressure [BP], heart rate, weight, height, temperature and respiratory rate), diagnostic testing that include 12-lead ECG, and laboratory testing (complete blood cell count, metabolic and liver function tests [alanine and aspartate amino transferase], biochemistry [glucose, blood urea, nitrogen and creatinine, and serological tests for hepatitis B and C and HIV antibodies], urinalysis, and, in women, a pregnancy test. Candidates were excluded if laboratory values were significantly out of the reference range and/or if all tests had not been completed. The primary exclusion criteria included also a previous allergic reaction to nitazoxanide or related medications, any indication of organ dysfunction, a history of gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological, psychiatric, or hematological illness, diabetes or glaucoma, and a history of using psychotropic drugs or consuming over two units of alcohol daily. In the last 48 hours before confinement, subjects were excluded whether have consumed alcohol, tobacco or foods rich in xanthines.

Before the enrolment of the subjects, the laboratory data were reviewed by investigators at the clinical unit, and general health condition and drugs' tests (cocaine, tetrahydrocannabinol, amphetamine, methamphetamine, benzodiazepines, morphine. Selected candidates were compensated for their participation after the study.

The test product was 20 mg/mL nitazoxanide oral suspension developed by Eurofarma Laboratórios S.A. and the reference product was Annita®, 20 mg/mL nitazoxanide oral suspension, manufactured and distributed by Farmoquímica S.A. The dose administered to the subjects was 500 mg.

This study was single-center, open-label, randomized, crossover 2-periods, with 2 treatments, Test (T) and Reference (R), being balanced by two treatment sequences (RT and TR – test/reference and reference/test) and by gender. After screening, the formulations were administered as a single orally dose followed by blood sampling for up to 12 hours from dosing and by a 7-days washout period. Randomization of the order of treatments was assigned by the quality assurance personnel at the clinical unit, in a 1:1 ratio using a computer-generated table.

The sample size was estimated by statistical method considering a power of 90% to constructed of a Confidence Interval of 0.9 for the range of the acceptance criteria of bioequivalence (80-125%) with an intra-subject coefficient of variability of 20%. The estimated size was 36 subjects including 20% of dropout. The statistical analysis was based on the ANOVA considering the calculation of pharmacokinetic parameters C_{max}, ASC_{0-t}, $ASC_{0-\infty}$, which interval of confidence values (90%) must be within the acceptable limit for the ratio between the geometric means of the test and reference products (80-125%), according to the Brazilian legislation.

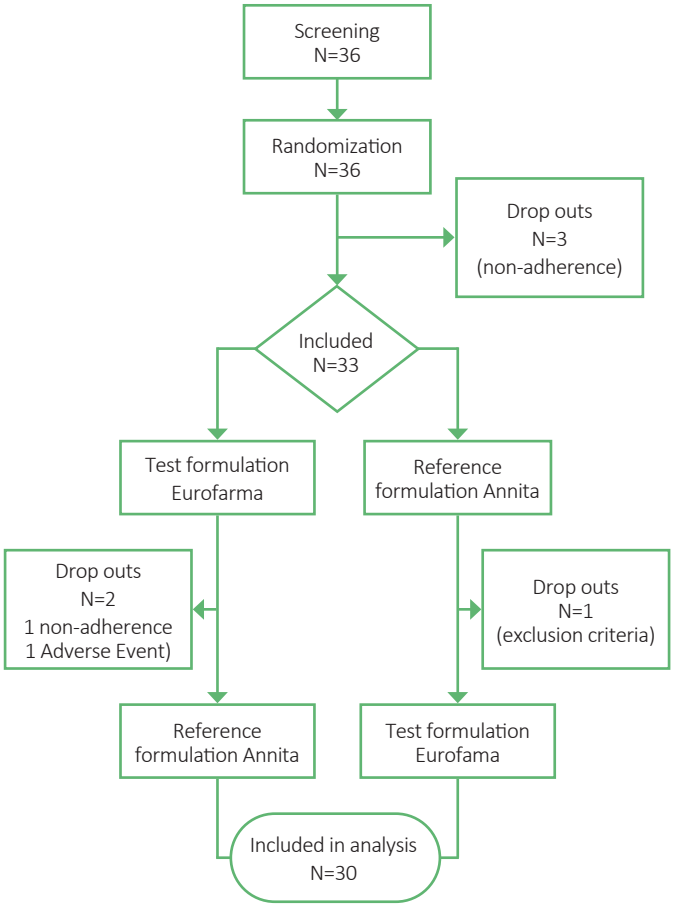
Blood sampling was performed through a peripheral venous catheter of adequate diameter and kept in a vein in one of the arms for multiple blood collections during hospitalization. The cannula was kept unobstructed by injecting 1 mL of a 100 UI/mL heparin solution in saline.

To ensure reliable baseline plasma measurements, subjects underwent a 12-hour overnight fast with a 1-week washout period which exceeds the 7 half-lives of tizoxanide as required by ANVISA. During hospitalization, the subjects were under medical surveillance, and, During the washout period, the subjects remained in contact with the investigators to report any adverse events (AEs).

Out of the initial 36 volunteers enrolled, only 30 remained due to dropout or exclusions as showed in Figure 2. The reasons to exclusions are described in Results section. Samples from each of the 30 remaining subjects were sent to the analytical facility, and the resulting data were evaluated by the statistician.

The volunteers were confined the day before administration and remained fasted at least eight hours before and four hours after the drug administration. All volunteers received a single dose of nitazoxanide, administered orally in a single dose. Drug administrations were performed around 7:00 am according to the randomization list.

Figure 2. Distribution of subjects across the study



Tolerability was determined using clinical assessment, monitoring of vital signs (BP, heart rate, and armpit body temperature) at baseline, after the drug administration during hospitalization, and at the end of the clinical stage of the study.

Laboratory results were also considered. The subjects were interviewed (using open-ended questions) by the investigators during hospitalization and at the end of the clinical stage of the study concerning the occurrence AEs. Subjects were asked to spontaneously report any AE to the investigators at any time during the study, including the washout period. Data for all AEs were recorded on a case-report form designed by the principal investigator.

Blood samples were obtained prior to dosing (baseline) and 0:15, 0:30, 1:00, 1:15, 1:30, 1:45, 2:00, 2:15, 2:30, 2:45, 3:00, 3:20, 3:40, 4:00, 4:30, 5:00, 6:00, 8:00 and 12:00 h post-dose. After each sampling, the samples were immediately centrifuged at 3500rpm for 10 minutes at 4°C for plasma separation from serum. The plasma was then transferred to pre-labeled cryogenic tubes in two aliquots, one for analysis and other for backup, and stored at -20ºC until shipedt to the laboratory for the bioanalysis.

The analyses were conducted with the objective of quantifying the major metabolite tizoxanide, as determined by the Brazilian health authority ANVISA in the context of the "List 2: Analyte for Establishing Relative Bioavailability/Bioequivalence" [11] The methodology employed was comparable to the approach employed in the quantification of samples[3, 11-13], with a bioanalytical approach utilizing heparinized plasma samples for the extraction of tizoxanide, followed by the determination of tizoxanide levels via liquid chromatography coupled to a tandem mass spectrometer.

The bioanalytical method was validated using high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). The main metabolite, tizoxanide, was quantified in human heparinized plasma. The method met all criteria for acceptability, including specificity/selectivity, linearity, precision, accuracy, carryover, ion suppression, recovery, robustness, and stability testing. Tizoxanide is quantified because the parent drug, nitazoxanide, undergoes rapid hydrolysis to the deacetylated species and is no longer detectable during the bioanalysis[14-17].

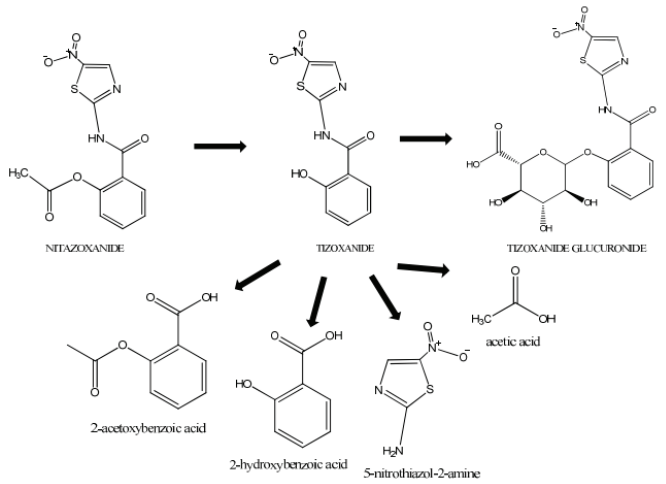
Linearity was assessed to determine the method's ability to accurately relate the analyte concentration to the instrumental response. The linear equation (y = a + bx [weighted 1/x]) was obtained and applied to the peak response (plasma analyte versus internal standard area ratios), where "y" represents response and "x" represents analyte concentration. The method demonstrated linearity for concentrations ranging from 0.0500 µg/mL to 20.0000 µg/mL.

The summarized results of the bioanalytical method's validation are shown in table 1.

Table 1. Validated parameters of the bioanalytical method.

Analytical technique	LC-MS/MS
LC MS/MS system	API5000 (Sciex/Applied Biosystems, Canada)
Method used during quantification	MPH118_02
Analyte	Tixozanide
Internal standard	Tixozanide-d4
Biological matrix	Human plasma
Anticoagulant	Heparin
Linearity	0.0500 µg/mL to 20.0000 µg/mL
Equation of the curve	y = a + bx (1/x)
Lower limit of quantification (LQL)	0.0500 µg/mL
Low quality control (LQC)	0.1500 µg/mL
Medium quality control (MQC)	8.0000 µg/mL
High quality control (HQC)	16.0000 µg/mL
Post-processing stability time	166 hours
Freeze and thaw cycles	3 cycles
Short-term stability time	16 hours

Figure 2. Structure and metabolic pathway of Nitazoxanide into its metabolites. Adapted from Gupta et. al. [14]



Method validation

The bioanalytical method was validated according to the requirements of the Brazilian legislation RDC-027/12[12]. Primary solutions were built in methanol/water (80/20; v/v) + 0.1 % triethylamine, and, plasma was contaminated in the proportion of 1 part of primary solution for 9 parts of plasma. The method presented 166 days of post processing stability, 3 cycles of freezing and thawing and16 hours of bench top stability in addition to 58 days of long term and 78 days of primary solutions kept on refrigerator. The validated concentration range was 0.05 µg/mL to 20 µg/mL.

Results

Out of 36 subjects, six were dropped from the trial. A total of 30 subjects completed the clinical stage, and their data was used for pharmacokinetic calculations. Four participants showed non-adherence (desistence, 3 before the first period, 1 before the second period), one experienced an adverse event (diarrhea), and one was excluded due to the use of forbidden medication before second hospitalization (tramadol and ketoprofen).

Pharmacokinetics Parameters

The validated method was used to measure 1260 collected samples. Statistical evaluation was conducted using Phoenix WinNonlin software, version 5.3 (Pharsight, USA). The main pharmacokinetic parameters are shown in Table 2.

To demonstrate the bioequivalence of both formulations and their interchangeability, we performed an analysis of variance (ANOVA) on the ln-transformed data after normality testing. The ANOVA results were used to construct a 90% confidence interval (CI) for the µT/µR (ratio of geometric means for the test and reference products) of the analyzed pharmacokinetic parameters. The statistical significance of effects was determined based on the calculated p-values, with significance values greater than 0.05 indicating no statistical significance. Bioequivalence was assumed when the 90% CI of the point for AUC_{0-t}, $AUC_{0-\infty}$ and for C_{max} were within the required range.

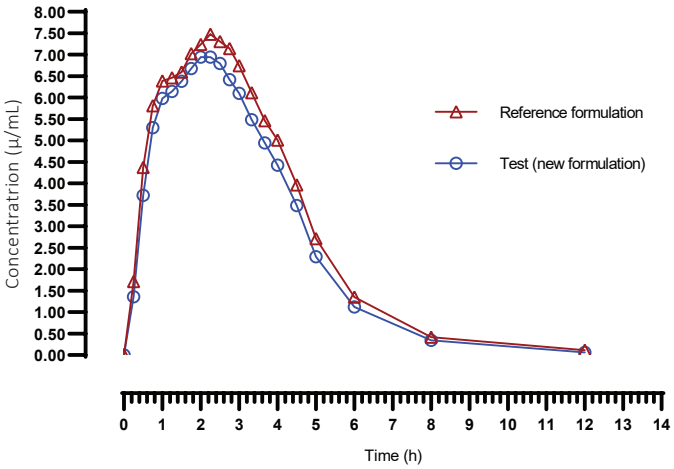
Table 2. Pharmacokinetic parameters for Tizoxanide generic (Eurofarma) and reference formulation (Anitta)

Pharmacokinetics Parameters		Tizoxanide	
		ANITTA®	EUROFARMA
Kel (h^{-1})	Arithmetic average	0.5277	0.5679
	Geometric Mean	0.5084	0.5549
	Minimum	0.1649	0.3413
	Maximum	0.8864	0.8221
	DP	0.1350	0.1223
	CV	25.5799	21.5415
$t_{1/2}$ (h)	Arithmetic average	1.4354	1.2799
	Geometric Mean	1.3635	1.2492
	Minimum	0.7819	0.8432
	Maximum	4.2039	2.0311
	DP	0.5933	0.2944
	CV	41.3330	23.0061
Tmax (h)	Arithmetic average	1.8557	1.9610
	Geometric Mean	1.6783	1.8029
	Minimum	0.5000	0.7500
	Maximum	3.6700	3.6700
	DP	0.7637	0.7858
	CV	41.1551	40.0704
C_{max} ($\mu g*ml^{-1}$)	Arithmetic average	8.7252	8.4050
	Geometric Mean	8.0675	7.8693
	Minimum	4.0040	4.3000
	Maximum	18.7250	16.7980
	DP	3.6689	3.2149
	CV	42.0497	28.2494
AUC_{0-t} ($\mu g*h*ml^{-1}$)	Arithmetic average	32.2841	28.9533
	Geometric Mean	29.1943	26.6860
	Minimum	12.8430	12.2725
	Maximum	81.8465	56.8275
	DP	15.8149	12.0280
	CV	48.9867	41.5428
$AuC_{0-\infty}$ ($\mu g*h*ml^{-1}$)	Arithmetic average	32.7470	29.1632
	Geometric Mean	29.6695	26.8996
	Minimum	12.9634	12.3503
	Maximum	82.0222	57.0057
	DP	15.7698	12.0424
	CV	48.1564	41.2932
AUC_{0-t} / $AuC_{0-\infty}$	Arithmetic average	0.9851	0.9921
	Geometric Mean	0.9840	0.9921
	Minimum	0.7530	0.9803
	Maximum	0.9979	0.9973
	DP	0.0442	0.0043
	CV	4.4849	0.4325

It was observed that the arithmetic means of the ratios between AUC_{0-t} and $AUC_{0-\infty}$ (reference 0.9851 and test 0.9921) are greater than 80% for the reference and test drug, in compliance with Brazilian legislation.

Figures 3 show the mean curves of plasmatic concentrations of tizoxanide (reference and test drugs) versus time, of the 30 research participants who completed the trial and were submitted to analysis.

Figure 3. Mean curves of tizoxanide plasma concentrations of reference (ANITTA®) and test (Nitazoxanida Eurofarma Laboratórios S.A.) after a 500 mg single dose of oral suspension each.



Safety and Tolerability

A total of 20 adverse events were recorded in the trial: 11 corresponding to the reference formulation and 9 to the test product. Ten out of 36 subjects reported the 20 adverse events.

None of the AEs were considered serious in severity. Nineteen adverse events were classified as mild (11 in the reference product and 8 in the test one) and one as moderate. In addition, all of the AEs spontaneously resolved under medical surveillance during the clinical stage of the study, and have included values of laboratory tests out of normality range, with no clinical significance.

Results of Bioequivalence

Table 3 presents the summarized results of the bioequivalence assessment between the ANITTA® (reference) and Eurofarma Laboratórios S.A. (test) formulations:

The results of this investigation indicated that there were no statistically significant differences between the two products in either the mean concentration-time profiles or in the obtained pharmacokinetic parameters. 90 % confidence limits for the log-transformed data of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were within the acceptable range of 0.80-1.25.

Both the preparations were well tolerated with no adverse reactions seen throughout the study.

Discussion

General results

This study successfully demonstrated the bioequivalence (BE) between two formulations of nitazoxanide 20 mg/ml oral suspension. The pharmacokinetic (PK) parameters of the test formulation were found to be within the established bioequivalence (BE) limits of 80% to 125% for the geometric mean ratios of tizoxanide area under the curve (AUC_{0-t} and $AUC_{0-\infty}$) and maximum plasma concentration (C_{max}) in accordance with the stipulations of Anvisa's Resolution 1,170/2006[18].

These results were subjected to a comprehensive evaluation in comparison to existing literature, with particular attention paid to aspects such as design, conduction, outcomes, and safety, as discussed in the subsequent paragraphs. Additionally, the concept of bioequivalence was explored in the context of the global landscape, with a special focus on Brazil. Furthermore, the strengths and weakness of bioequivalence studies were also considered.

Outcomes

In comparison to other bioequivalence studies, our findings are in accordance with the overall outcomes observed in similar research, particularly with regard to the design of the trial. The pharmacokinetic parameters found were also in accordance with the other studies, which showed similar results, considering the potential influence of ethnic differences on the raw values[13, 19, 20].

Safety

Adverse events (AEs) represent an important aspect of bioequivalence studies, as they provide insights into the safety and tolerability of the formulations under investigation. In our study, a total of 20 AEs were reported, with 11 were be associated with the reference formulation arm and 9 with the test product arm. Notably, none of these AEs were classified as serious. Nineteen AEs were mild, and one was moderate. This safety profile aligns with that of other bioequivalence studies, where mild to moderate adverse events (AEs) are frequently observed and managed without significant medical intervention[19, 21].

In comparison, the incidence and severity of AEs in this study are consistent with those reported in other bioequivalence trials. For instance, studies on generic formulations of commonly used medications have documented similar AE profiles, predominantly characterized by mild events that resolve spontaneously or with minimal medical supervision[19, 21-23]. The recorded AEs in our study, including episodes of diarrhea and abnormal laboratory values, were managed effectively under medical supervision and did not present significant health risks to the participants.

Table 3: Bioequivalence pharmacokinetic calculated parameters after a 500 mg single dose of oral suspension.

	Ratio (Test/Reference)	Geometric Mean	IC (90%)	CV (%) Intra
Tizoxanide	Cmax ($\mu g.mL^{-1}$)	98.0147	(89.0454 - 107.8874)	22.0577
	AUC_{0-t} ($\mu g.h.mL^{-1}$)	92.1134	(85.1332 - 99.6659)	18.0416
	$AUC_{0-\infty}$ ($\mu g.h.mL^{-1}$)	91.2994	(84.2529 - 98.9353)	18.3949

However, it should be noted that, in comparison to efficacy trials, the relatively small sample size of 36 subjects, with 30 completing the clinical stage, represents a limitation of this study, although this is sufficient to demonstrate bioequivalence. Furthermore, the presence of homogeneity in the population characteristics and their healthy state may have influenced the results.

While this sample size is typical for bioequivalence studies and sufficient for describing PK parameters, larger studies could provide more comprehensive safety data and enhance the robustness of the findings. Additionally, the exclusion of six subjects due to non-adherence, AEs, and protocol violations serves to illustrate the difficulties inherent in maintaining participant compliance in clinical trials.

Brazilian Scenario

In countries with centralized health systems, such as Brazil's *SUS* (Unified National Health System), the continuous supply of government-subsidized medications is of paramount importance. This is contingent upon the conclusion of long-term negotiations with pharmaceutical companies, which result in the acquisition of medications at prices below those of branded products. Additionally, competition from multiple market players, including industry and distributors, is essential for maintaining affordable access to medications[24].

Strengths and Weakness

Bioequivalence studies are crucial in the pharmaceutical industry for ensuring that generic drugs are therapeutically equivalent to their brand-name counterparts. These studies evaluate whether a generic drug releases its active ingredient into the bloodstream at a similar rate and extent as the original drug. Bioequivalence studies (BES) are commonly used to confirm the therapeutic equivalence of generic drugs, making them a vital part of the drug approval process[25, 26].

Bioequivalence studies offer several advantages for Public Health. They are generally less costly and time-consuming compared to full clinical trials, making them a more efficient pathway for bringing generic drugs to market. Regulatory agencies have established guidelines for these studies, ensuring consistent and reliable evaluation processes. This helps facilitate the development of generic formulations by focusing solely on achieving pharmacokinetic equivalence. Additionally, the introduction of generic drugs promotes market competition, often leading to lower prices for consumers, and for government too[22, 24, 27].

Despite their strengths, bioequivalence studies also have limitations. They primarily focus on pharmacokinetic parameters, often using a single-dose scheme, which may not fully capture a drug's efficacy and safety profile. The study populations are usually small and homogeneous, typically consisting of healthy volunteers, which may not accurately represent the broader, more diverse patient population. This can result in overlooked variations in drug metabolism and response. Furthermore, differences in regulatory requirements across regions can complicate study design and approval processes, leading to delays and increased production costs[22, 27, 28]. These limitations highlight the need for a broader approach to ensure comprehensive drug evaluation and safety[26].

These considerations align with the broader experience and observations within the field, reflecting both the current study and extensive long-term experience of this group. They accurately represent the predominant strengths and limitations of bioequivalence studies.

Conclusion

The proposed bioanalytical method was found to be sensitive, robust, and reproducible. It was successfully applied to a bioequivalence study of tizoxanide in 30 human volunteers after oral administration and under fasting conditions. This demonstrated the bioequivalence between the test formulation (Eurofarma Laboratórios S.A.) and the reference formulation ANITTA® (Farmoquímica S.A.), The pharmacokinetic (PK) parameters of the test formulation from Eurofarma Laboratórios S.A. once they were found to be within the established bioequivalence (BE) limits of 80% to 125% for the geometric mean ratios of tizoxanide area under the curve (AUC_{0-t} and $AUC_{0-\infty}$) and maximum plasma concentration (C_{max}). This finding corroborates the pharmacokinetic equivalence of the test and reference formulations in accordance with the stipulations of Anvisa's Resolution 1,170/2006[18].

Based on statistical results, both the oral suspension of nitazoxanide from test. and the reference product comply with the regulatory requirements to be considered bioequivalent. Therefore, based on their biopharmaceutical performance, the test product is interchangeable with the reference product. Thus, these findings indicate that the two products are bioequivalent in terms of rate and extent of drug absorption metabolism and excretion.

Funding

This study was sponsored by *Eurofarma Laboratórios*. Itapevi, São Paulo, Brazil with the purpose of register a new generic formulation. The study was conduct in partnership with UNIFAG.

Conflict of interest

The authors declare that, with the exception of CKA, who act as project manager and is an associate of Eurofarma Laboratórios S/A, there are no other potential conflicts of interest in this study or in the publishing work.

Collaboration

VMR and CS curated the data, wrote the first draft, and perform revisions. CLG and SGS validated the method and analyzed the samples. CKA supported the clinical phase of the study. KIX managed and organized the data of clinical phase. FBCP provided scientific support and manage the paper elaboration. All authors reviewed and approved the final version.

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