

Study of bioequivalence between two formulations containing dipyrone 300 mg + isometheptene mucate 30 mg + caffeine 30 mg: a randomized, open-label, two period crossover study in healthy adult Brazilian volunteers

Bioequivalência entre duas formulações contendo 300 mg de dipirona + 30 mg de mucato de isometepteno + cafeína 30 mg: um estudo cruzado randomizado, aberto e de dois períodos, em voluntários brasileiros adultos saudáveis

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ABSTRACT

The combination of dipyrone 300 mg, isometheptene mucate 30 mg and caffeine 30 mg in a single tablet is widely used in Brazil for the acute treatment of various forms of primary headaches. This study aimed to evaluate the bioequivalence between two formulations

containing the combination of these active ingredients. An open-label, randomized, single-dose, two-period, two-sequence, two-treatment crossover study was conducted in 80 healthy subjects of both genders. Subjects received a single dose of test coated tablet (Sedamed[®], Cimed Indústria de Medicamentos Ltda.) and reference product (Neosaldina[®], Nycomed Pharma Ltda.) under fasting conditions according to a randomly assigned order with a 7-day washout period. Serial blood samples were collected up to 24h post-dose. Plasma concentrations of active pharmaceutical ingredients were obtained by a validated liquid chromatography-tandem mass spectrometry method. Pharmacokinetic parameters were calculated using non-compartmental methods. There were no serious adverse events during the study and both formulations were safe and well tolerated during the study. Geometric mean ratios (90% confidence intervals) for C_{max} and $AUC_{0,t}$ were 97.04% (94.94 – 99.19) and 98.77% (95.58 – 102.06) for 4-MAA and 100.12% (93.33 – 107.41) and 96.19% (91.24 – 101.42) for isometheptene, respectively. The test formulation of was considered bioequivalent to reference product according to regulatory requirements, and therefore interchangeable.

Keywords:

Bioequivalence, dipyrone, 4-methylaminoantipyrine, isometheptene, LC-MS/MS.

RESUMO

A combinação de 300 mg de dipirona, 30 mg de mucato de isomethepteno e 30 mg de cafeína em um único comprimido é amplamente utilizada no Brasil para o tratamento agudo de várias formas de cefaléia primária. Este estudo teve como objetivo avaliar a bioequivalência entre duas formulações contendo a combinação desses ingredientes ativos. Um estudo cruzado, aberto, randomizado, de dose única, dois períodos, duas sequências e dois tratamentos foi conduzido em 80 indivíduos saudáveis de ambos os sexos. Os volunt receberam uma dose única de comprimido revestido de teste (Sedamed[®], Cimed Indústria de Medicamentos Ltda.) e o produto de referência (Neosaldina[®], Nycomed Pharma Ltda.), em condições de jejum, em uma ordem aleatória e com um período de intervalo entre as administrações de 7 dias. Amostras seriadas de sangue foram coletadas até 24 horas após a dose. As concentrações plasmáticas dos princípios ativos foram obtidas por um método validado por cromatografia líquida acoplada a espectrometria de massa. Os parâmetros farmacocinéticos foram calculados usando métodos não compartimentais. Não houve eventos adversos graves durante o estudo e ambas as formulações foram seguras e bem toleradas durante o estudo. As razões das médias geométricas (intervalos de confiança de 90%) para C_{max} e $ASC_{0,t}$ foram 97,04% (94,94 - 99,19) e 98,77% (95,58 - 102,06) para 4-MAA e 100,12% (93,33 - 107,41) e 96,19% (91,24 - 101,42) para isometepteno, respectivamente. A formulação do teste foi considerada bioequivalente ao produto de referência de acordo com os requisitos regulatórios e, portanto, intercambiáveis.

Palavras-chave:

Bioequivalência, dipirona, 4-metil amina antipirina, isometepteno, CLAE-EM/EM.

1 INTRODUÇÃO

Headache, an extremely common clinical symptom, is often encountered in clinical practice. Due to the subjective nature of headaches and the wide variation in frequency,

severity and duration of episodes, the presence of associated symptoms and the lack of biological markers, medical treatment of headaches is often empirical and difficult [1]. The combined use of analgesic (dipyrone) and adjuvants such as caffeine and isometepten which also have their own antinociceptive activities has been increasingly considered in pain therapy, mainly because some types of pain are not easy to relieve with conventional analgesic [2]. The combination of dipyrone 300 mg, isometheptene 30 mg and caffeine 30 mg in a single tablet is widely used in Brazil for the acute treatment of various forms of primary headaches. This combination seems to produce consistent efficacy and good tolerability; However, as seen for most over-the-counter (OTC) drugs, evidence to support its use is scarce [1].

Dipyrone is an effective non-opioid analgesic, antipyretic and anti-inflammatory drug, a prodrug that has extensively investigated. It is widely used in the clinic, either alone or in combination with other medicines. Dipyrone is rapidly absorbed after oral administration and is hydrolyzed in the gastrointestinal tract to the more potent active metabolite 4-methylaminoantipyrine (4-MAA) than dipyrone, which is reaching peak levels within 1-2 h and the elimination half-life of is 2.0 - 3.5 hours [3, 4, 5]. Due to the analgesic effect of dipyrone correlates with the time course of 4-MAA concentrations in plasma, pharmacokinetic parameters pertaining to this active metabolite will be appropriate for assessing the bioequivalence of dipyrone formulations.

Isometheptene, 6-methylamino-2-methylheptene, is an antispasmodic drug belonging to the aliphatic amine series of adrenergic agents. It was introduced into clinical practice for the treatment of spastic conditions of biliary and urinary tracts. It is an indirect-acting agent given for vasoconstrictor effect in the treatment of migraine administered in combination with caffeine and dipyrone. It is well absorbed after oral administration and is completely metabolized preferably by oxidation of the dimethylalyl group into two isomers which are fully excreted within 3.5 hours [6, 7].

Caffeine is a xanthine derivative that has discrete action on the central nervous system and has a vasoconstrictor effect on the cranial arteries, reducing blood flow and oxygen tension in the brain and may contribute directly to relieve various types of headaches, especially migraines, and increase the potency of painkillers. Its oral absorption is complete and fast, needing 1 h to reach the maximum plasma concentration and has a linear elimination, with an elimination half-life of approximately 5 h [8].

In Brazil, the registration of similar products follows the same criteria established by ANVISA for generic products. Similar products need to prove that they are therapeutic

equivalents and therefore interchangeable with their respective reference product [9,10]. Some drugs are candidates for biowaiver studies based on the biopharmaceutical classification system (BCS) such as dipyron and caffeine. The BCS aims to provide a regulatory tool that can replace certain bioequivalence studies with *in vitro* dissolution tests [11]. Studies of relative bioavailability and pharmaceutical equivalence are used to determine the drug concentration of the test formulation compared to the reference medication. Based on these studies, therapeutic equivalence is confirmed for the generic and similar products, which represents a less costly alternative for the general population.

The aim of this study was to evaluate the bioavailability of two medications containing 300 mg of dipyron, 30 mg of isometheptene mucate and 30 mg of caffeine in tablets to determine their bioequivalence. The test product, Sedamed[®] was obtained from Cimed Indústria de Medicamentos Ltda, while the reference was Neosaldina[®] from Nycomed Pharma Ltda.

2 SUBJECTS AND METHODS

2.1 SUBJECTS AND ETHICS

Eighty (80) adult healthy subjects of both genders, aged 18 to 50 years and body mass index (BMI) between 18 and 30 kg / m² were enrolled in the study. Volunteers were equally distributed among groups and the same number of men and women were used. The good health conditions of the volunteers were confirmed through an evaluation including medical case history, physical examination, vital signs measurements, anthropometric data, 12-lead electrocardiogram (ECG) and laboratory tests (hematology, biochemistry, urinalysis, hepatitis B and C and HIV), as well as pregnancy testing for women. Some of the exclusion criteria were reactions of hypersensitivity to drugs, clinically significant case history or presence of renal, pulmonary, neurological, psychiatric, hematological, cardiologic, endocrine, immunological and diseases neoplasms. The use of any medicine, including those sold without a prescription, could not be taken regularly for at least 14 days and even irregularly, within 7 days before the beginning of the first study period. The participation in any clinical study within six months prior to the study initiation, pregnancy or lactation, significant loss or blood donation in quantities higher than 450 mL, and alcohol and / or drug abuse also prevented the individuals participation in the study.

It was conducted in accordance with national and international standards and research guidelines involving human beings: Good Clinical Practices Guidelines [12] Declaration of

Healsink [13] and Resolutions n. 466/2012 [14]. The protocol was approved by the Ethical Committee of the Faculdade de Ciências Médicas Dr. José Antônio Garcia Coutinho da Universidade do Vale do Sapucaí, Pouso Alegre, Brazil (approval number: 534.968). After explaining the nature and purpose of the research, the volunteers provided written informed consent before starting the study. All study stage were carried out by ICMP&D - Instituto Cláudia Marques de Pesquisa e Desenvolvimento (Pouso Alegre, Brazil), a company certified by ANVISA as center of bioequivalence.

2.2 STUDY DESIGN

The study design was randomized, open-label, single dose, fasting, two-period, two-sequence crossover with a 7-day washout period. The administration of one tablet of each product (test and reference) was initiated, regarding the list of randomization, at 07:00 am, after overnight fasting about 10 hours. No food was allowed for four hours after the dose intake. Subjects received standardized meals at 4 hours (lunch), 8 hours (snack) and 11 hours (dinner) after the drug intake in each treatment. The volunteers did not have alcoholic beverage, coffee, drinks containing xanthines, or foods outside the prescribed diet during the study. The study consist of prestudy screening visit, 2 treatment periods with each confinement period lasted 36 hours on average and poststudy follow-up. During the study period, the volunteers were under medical surveillance by registered physicians to report any adverse events all times.

A sample size of 80 volunteers was estimate based on coefficient of variation (% CV) for isometheptene C_{max} of 33.42% taken from previous similar studies bioequivalence assessments under fasted conditions with 24 volunteers.

2.3 INVESTIGATIONAL MEDICINAL PRODUCTS

Test product: Sedamed[®] combination of dipyron 300 mg, isometheptene mucate 30 mg and caffeine 30 mg in coated tablet (batch n. A029/13). The test product was manufactured by Cimed Indústria de Medicamentos Ltda. (Pouso Alegre, Brazil). Reference product: Neosaldina[®] combination of dipyron 300 mg, isometheptene mucate 30 mg and caffeine 30 mg in dragea (batch n. L 220708). The reference product was manufactured by Nycomed Pharma Ltda. (Jaguariúna, Brazil). Both test and reference investigational medicinal product were swallowed whole with 200 mL water with the fasting period.

2.4 BLOOD SAMPLE COLLECTION

A total of 22 blood sample (7.5 mL) from each volunteer was collected for pharmacokinetic analysis at 0 hours (before dosing) and after dosing at 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2, 2.33, 2.66, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14 and 24 hours for determination of 4-MAA (dipyron metabolite) and isometheptene plasma concentrations. The sample schedule was based on the pharmacokinetic parameters of previous studies (isometheptene), known data in the literature for dipyron (4-MAA) and exceeded 3-5 times the mean half-life of approximately 3.5 hours for both substances. All blood samples were drawn by using an intravenous catheter insert into a forearm vein, except samples collected at 24 hours, which were drawn by direct venipuncture. Samples were collected into heparinized tubes. Plasma was separated after centrifugation at 3,500 rpm for 10 minutes at 4 °C immediately after a collection. The plasma was transferred into polypropylene tubes and kept frozen at ≤ -20 °C until analysis.

2.5 BIOANALYTICAL METHOD

The bioanalytical methods were validated according to Resolution ANVISA n.27/2012 [15]. The validation parameters evaluated were as follows: selectivity, linearity, intra and interassay for both precision and accuracy, matrix effect, carryover, and stability under different conditions.

Samples containing 4-methylaminoantipyrine and isometheptene were quantified by ultra performance liquid chromatography (Acquity UHPLC, Waters) coupled tandem mass spectrometry (MS-MS TQ detector, Waters) method using a Waters® Acquity UPLC BEH C8 (2,1 x 50,0, 1,7 μ m) column at 40°C \pm 5°C. The mobile phase composed of 0.1% formic acid + methanol and ammonium acetate solution + 0.1% formic acid (55:45, v/v) at a flow rate of 0.200 mL/min.

Plasma samples (200 μ L) were extract by protein-precipitation with methanol (1,000 μ L) using 25 μ L solution of ondansetron (1.0 μ g/mL) as the internal standard (IS). An aliquot of 7.0 μ L was injected into the UHPLC system and the total run time set as 2 min.

Mass spectrometry detection was conducted using electrospray ionization source in positive mode, the multiple reaction monitoring (MRM) method was used and the transitions monitored were 4-MAA 218.28 > 97.15, m/z isometheptene 142.44 > 69.16 m/z and m/z ondansetrona 294.12 > 170.09.

2.6 TOLERABILITY ASSESSMENTS

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 adverse events (AEs). Subjects were asked to spontaneously report any AE to the investigators at any time during the study, including the washout period. AEs that were life-threatening, led to death, hospitalization, disability, and/or medical intervention to prevent permanent impairment or damage were considered serious.

2.7 PHARMACOKINETICS AND STATISTICAL ANALYSES

Sample size calculation was based on the within-subject variability of isometheptene C_{max} from a pilot study ($n = 24$) that had a % CV of 33.42% (data on file, Instituto Claudia Marques, Pouso Alegre, Brazil, study: ICM BEQ 027 12, completed January 2014). This calculation was performed considering the following values: $1 - \beta = 0.8$, $\alpha = 0.05$, % CV = 33.42, and an equivalence range of 80% to 125%; which yielded a sample size of 72 subjects. In this research a sample size of 80 subjects was used, which included 8 additional subjects (with respect to the required sample size) considered in case of dropouts.

Individual plasma concentration–time curves were constructed; C_{max} and T_{max} were directly obtained from these curves, the area under the plasma concentration-time curve from time baseline to the last measurable concentration (AUC_{0-t}) was calculated according the noncompartmental method using the trapezoidal rule. From the terminal log-decay phase, the elimination rate constant (K_{el}) was estimated using linear regression, and $T_{1/2}$ was estimated using the following equation: $T_{1/2} = \ln 2 / K_{el}$, where \ln was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-t} + (C_t / K_{el})$, where C_t was the last measurable plasma concentration.

To assess the bioequivalence between the test and reference formulations, C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were considered as the primary variables. ANOVA for a 2 x 2 crossover design using log-transformed data for these parameters was carried out at the 5% significance level ($\alpha = 0.05$). The 90% CIs of the geometric means ratios (test/reference) of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were calculated using log-transformed data. The test and the reference formulations were to be considered bioequivalent if the 90% CIs of these parameters fell within a

predetermined range of 80% to 125% and if the probability of exceeding these limits was <0.05 . All pharmacokinetic and parametrical-statistical analyses were performed using software Phoenix WinNonlin version 6.3 (Certara Corporation, Princeton, NJ, USA).

3 RESULTS AND DISCUSSION

3.1 SUBJECTS

Eighty healthy subjects were randomized in this study and seventy-eight completed the study (38 men and 40 women). Two volunteers dropped out of the study after the second period due to personal reasons. Data from these participants (78) were included in the pharmacokinetic and bioequivalence analyses. The mean age of subjects was 34.2 years (range = 18 - 50 years) and mean body mass index was 24.08 Kg/m^2 (range = $18.55 - 29.60 \text{ Kg/m}^2$).

3.2 BIOANALYSIS

Bioanalytical methods using liquid chromatography for determining isomethepten in human plasma were not found in the researched literature, isometheptene in human plasma. In searching PubMed using the terms 'isometheptene' and 'chromatography' and 'human' has only one method described by Lyris E, et al., (2005) [16] in human urine and analysis by gas chromatography-mass spectrometry. As describe in the literature bioanalytical methods to quantify dipyrone metabolite (4-MAA), only one of them used LC / MS - MS, the same was performed by Ojha A, Rathod R, Padh H., (2009) [17].

Criterion of accepting biowaiver for immediate release caffeine solid oral drug products is considered scientifically justified, if the test product contains only those excipients reported in this paper in their usual amounts and the test product is rapidly dissolving, as well as the test product fulfils the criterion of similarity of dissolution profiles to the reference product. In the case of this study caffeine was bioaiwer and the other two drugs were evaluated for bioequivalence criteria: dipyrone (4-MAA) and isometheptene. The bioanalytical method was developed internally in our laboratory based on the characteristics of the two analytes: 4-MAA (metabolite dipyrone) and isometheptene.

Seletivity analyzed blank plasma samples, free of analyte and internal standard, with the addition of caffeine at $2.0 \mu\text{g} / \text{mL}$ to assess interference of this substance in the determination of the analytes under study, 4-methylaminoantipyrine, isometheptene, and the internal standard, ondansetrone. The method was selectivity, its proved to be adequated by

showing that substances in the blank plasma did not interfere at the retention times of 4-methylaminoantipyrine (0.81 min), isometheptene (1.01 min) and ondansetron (0.85 min), even in the presence of caffeine.

The intrassay precision, expressed as the % CV, was in the range of 0.2% to 6.9% (4-MAA) and 1.0% to 9.5% (isometheptene) and interassay precision was 2.0% to 3.5% (4-MAA) and 3.5% to 5.2% (isometheptene). The accuracy was in the range 98.6% to 108.0% (4-MAA) and 94.1% to 104.1% (isometheptene). The samples remained stable during the four cycles of freezing and thawing in the short-term of 7 hours, post-processing of 18 hours, and long-term of 58 days afterward, indicating adequate stability of the plasma samples of volunteers from time of collection to the last analysis. All validation parameters were in accordance with what was defined in protocol and resolution.

Plasma concentrations of the samples were derived from the linear regression equation of the line using the $1/x^2$ weighting, obtained with the standard curve (isometheptene and 4-methylaminoantipyrine concentration as a function of the internal standard area ratios), as it presents the lower value for sum of the relative errors of the nominal values of the calibration standards versus their values obtained by the curve equation. We chose to use this mathematical model due to the wide range of concentrations employed in the quantification of the samples. This model is the simplest and best accommodates the variations inherent in the experimental method.

3.3 PHARMACOKINETICS AND STATISTICAL ANALYSES

The curve of mean plasma concentration versus time of 4-MAA and isometheptene after administration of test and reference products to 78 volunteers are shown in Figures 1 and 2, demonstrating similar absorption, distribution and elimination for both medications.

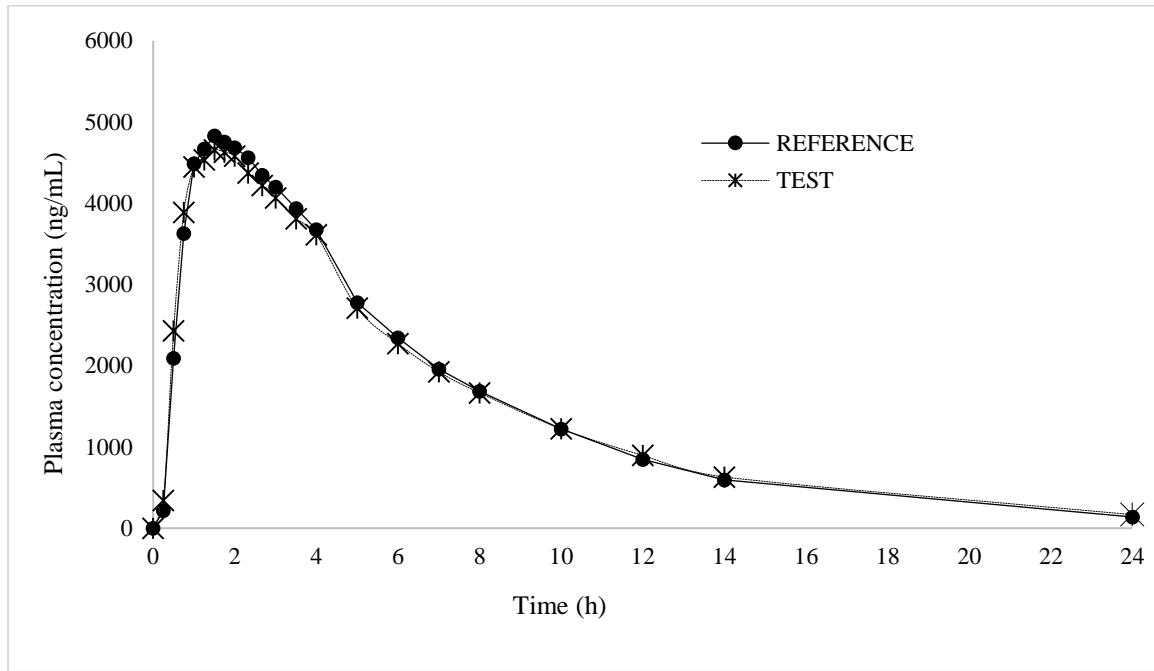


Figure 1. Mean plasma concentrations of 4-MAA after oral administration of reference and test products to 78 healthy volunteers.

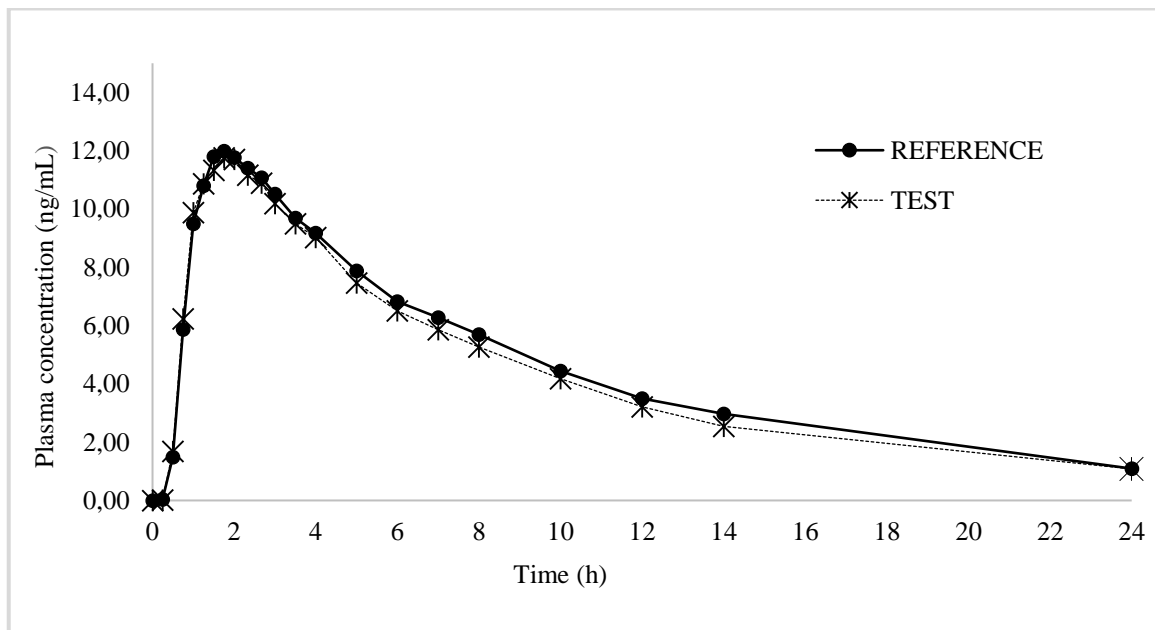


Figure 2. Mean plasma concentrations of isometheptene after oral administration of reference and test products to 78 healthy volunteers.

Average values for C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, T_{max} , $T_{1/2}$ and K_{el} of the two products did not differ significantly, suggesting that plasma profiles generated by the test product are comparable to those produce by the reference product and values are listed in Table 1.

Table 1. Pharmacokinetics parameters of 4-MAA and isometheptene after the administration of single dose of a test and a reference tablet formulation in healthy volunteers (mean \pm standard error, n = 78).

Parameter	4-MAA		Isometheptene	
	Reference	Test	Reference	Test
C_{max} (ng/mL)	5168.25 \pm 1009.27	5021.56 \pm 1007.56	14.38 \pm 15.95	14.43 \pm 14.67
AUC_{0-t} (ng/mL*h)	33989.84 \pm 13893.12	34197.45 \pm 15449.94	107.53 \pm 210.74	101.49 \pm 183.49
AUC_{0-inf} (ng/mL*h)	36276.36 \pm 16300.67	36669.24 \pm 18295.49	122.61 \pm 255.64	117.86 \pm 225.318
T_{max} (h)	1.486 \pm 0.467	1.421 \pm 0.538	1.449 \pm 0.561	1.514 \pm 0.715
$T_{1/2}$ (h)	3.883 \pm 1.674	4.061 \pm 1.815	3.503 \pm 1.856	3.502 \pm 1.937
K_{el} (1/h)	0.206 \pm 0.073	0.202 \pm 0.081	0.230 \pm 0.070	0.233 \pm 0.072

C_{max} , maximum plasma concentration; AUC_{0-t} , area under the concentration-time curve from zero to last measurable concentration; AUC_{0-inf} , area under the concentration-time curve extrapolated to infinity; T_{max} , time to reach C_{max} ; $T_{1/2}$, elimination half-life; K_{el} , elimination constant.

The plasma half-life of dipyron metabolite (4-MAA) was similar in both products, with a mean of 3.9 hours. Suarez-Kurtz, et al., 2001 [3] found a plasma half-life of 3.9 hours, the average value was the same as that found by us. The time taken to reach maximum concentration (T_{max}) in our study was 1.4 hours (4-MAA). Suarez-Kurtz, et al., (2001) [3] found a mean T_{max} of 1.55 whereas Ojha, Rathoh, Padh (2009) [4] found T_{max} of 1.25 in male volunteers. Isometheptene was absorbed rapidly, achieving mean peak plasma level around 1.5 hours for both test and reference products and $T_{1/2}$ of 3.5 hours was found. In a similar pilot study conducted for us at this research center, we found similar values for 4-MAA, $T_{1/2}$ 3.5 hours and T_{max} 1.5 hours, and isometheptene we also found similar values $T_{1/2}$ 3.1 hours and T_{max} 1.6 hours. The T_{max} and $T_{1/2}$ values obtained for both analytes made it possible to use a single collection schedule design.

In the present study, predose samples did not exhibit any detectable 4-MAA and isometheptene levels in all subjects, so the occurrence of residual or carry-over effects can be excluded. Also, procedures regarding volunteers' diet and rest, single dose, drug administration and sample workup and storage were the same in the two periods of the study. On the basis of these considerations, the present study is acceptable, despite the sequence effect.

Power of the test used in this study was 100%, indicating that number of volunteers in the study was sufficient to ensure reliability of the results. As can be seen from Table 2 geometric mean ratios teste and reference using the log-transformed data of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ (90% CI); the limits of acceptance for bioequivalence, the power values of the test and the within-subject % CV.

Table 2. Geometric mean ratio, confidence intervals (90%), power and CV_{ws} .

Parameter	Ratio (%)	90% CI (%)	Power (%)	CV_{ws} (%)
4-MAA				
C_{max}	97.04	94.94 – 99.19	100.00	8.23
AUC_{0-t}	98.77	95.58 – 102.06	100.00	12.36
AUC_{0-inf}	98.89	95.41 – 102.50	100.00	13.49
Isometheptene				
C_{max}	100.12	93.33 – 107.41	99.97	26.80
AUC_{0-t}	96.19	91.24 – 101.42	100.00	20.02
AUC_{0-inf}	98.09	93.38 – 103.04	100.00	18.49

C_{max} , maximum plasma concentration; AUC_{0-t} , area under the concentration-time curve from zero to last measurable concentration; AUC_{0-inf} , area under the concentration-time curve extrapolated to infinity; CI, confidence interval; CV_{ws} , coefficient of variation within subject.

The 90% CIs for 4-MAA and isometheptene C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were 94.94 – 99.19%, 95.58 – 102.06%, 95.41 – 102.50% and 93.33 – 107.41%, 91.24 – 101.42% and 93.38 – 103.04%, respectively, which were within the acceptable range of 80.00 – 125.00% as recommended by ANVISA.

3.4 TOLERABILITY

Eight of the 80 subjects reported a total of 8 AEs. The most commonly AEs reported were headache (5 events reported by 5 subjects [62.5%]), four after administration of reference formulation and one after the administration of test formulation. Other AEs were backache, facial rash and excessive sweating (3 events reported by 3 subjects [37.5%]) after the administration of the reference formulation for the first two. All of the AEs were considered as possibly related to the study drug. None of the AEs were considered serious in severity. They were regarded as mild. In addition, all of the AEs spontaneously resolved under medical surveillance during the clinical stage of the study, without the administration of any medication.

4 CONCLUSION

The results of this study that the two formulations teste and reference are bioequivalent and therefore interchangeable, as the 90% CI of the pharmacokinetic parameters evaluated were within the range established by regulatory agency-ANVISA.

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REFERENCES

de Souza Carvalho D, Barea LM, Kowacs PA, Fragoso YD. Expert Rev Neurother. 2012 Feb;12(2):159-67. Efficacy and tolerability of combined dipyrone, isometheptene and caffeine in the treatment of mild-to-moderate primary headache episodes.

Díaz-Reval MI(1), Galván-Orozco R, López-Muñoz FJ, Carrillo-Munguía N. [Synergism of caffeine on antinociceptive effects of metamizole]. [Article in Spanish]. Cir Cir. 2008 May-Jun;76(3):241-6.

Suarez-Kurtz G, Ribeiro FM, Estrela RC, Vicente FL, Struchiner CJ. Limited-sampling strategy models for estimating the pharmacokinetic parameters of 4-methylaminoantipyrine, an active metabolite of dipyrone. Braz J Med Biol Res. 2001 Nov;34(11):1475-85.

Ojha A, Rathod R, Padh H. Quantification of 4-methylaminoantipyrine, the active metabolite of dipyrone, in human plasma. Bioanalysis. 2009 May;1(2):293-8.

Levy M, Muszkat M, Rich B, Rosenkranz B, Schlattmann P. Population pharmacokinetic analysis of the active product of dipyrone. Int J Clin Pharmacol Ther. 2010 Dec;48(12):791-7.

Freitag FG, Cady R, DiSerio F, Elkind A, Gallagher RM, Goldstein J, Klapper JA, Rapoport AM, Sadowsky C, Saper JR, Smith TR. Comparative study of a combination of isometheptene mucate, dichloralphenazone with acetaminophen and sumatriptan succinate in the treatment of migraine. *Headache*. 2001 Apr;41(4):391-8.

Lyris E, Tsiakatouras G, Angelis Y, Koupparis M, Spyridaki MH, Georgakopoulos C. Metabolism of isometheptene in human urine and analysis by gas chromatography-mass spectrometry in doping control. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005 Dec 5;827(2):199-204. Epub 2005 Oct 19.

Guzmán NA, Molina DR, Núñez BF, Soto-Sosa JC, Abarca JE. Bioequivalence and Pharmacokinetic Evaluation Study of Acetaminophen vs. Acetaminophen Plus Caffeine Tablets in Healthy Mexican Volunteers. *Drugs R D*. 2016 Dec;16(4):339-345.

BRASIL. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Resolução – RDC nº 17, de 02 de março de 2007. *Diário Oficial da União, Brasília, 05 de março de 2007*.

BRASIL. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Resolução – RDC nº 58, de 10 de outubro de 2014. *Diário Oficial da União, Brasília, 13 de outubro de 2014*.

BRASIL. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Resolução – RDC nº 37, de 03 de agosto de 2011. *Diário Oficial da União, Brasília, 05 de agosto de 2011*.

International Conference of Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonized Guidelines. Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2) Guideline for Good Clinical Practices. Available in https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf. Accessed 27 Dec 2017).

World Medical Association. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*. 2013;310(20):2191–2194.

BRASIL. CONSELHO NACIONAL DE SAÚDE. Resolução n° 466, de 12 de Dezembro de 2012. Diário Oficial da União, Brasília, 13 de Junho de 2013.

BRASIL. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Resolução – RDC n° 27 de 17 de maio de 2012. Diário Oficial da União, Brasília, 22 de maio de 2012.

Lyris E, Tsiakatouras G, Angelis Y, Koupparis M, Spyridaki MH, Georgakopoulos C. Metabolism of isometheptene in human urine and analysis by gas chromatography-mass spectrometry in doping control. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2005 Dec 5;827(2):199-204. Epub 2005 Oct 19.

Ojha A, Rathod R, Padh H. Bioanalysis. Quantification of 4-methylaminoantipyrine, the active metabolite of dipyron, in human plasma 2009 May;1(2):293-8.