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RESEARCH ARTICLE

BIOEQUIVALENCE STUDY BETWEEN TWO FORMULATIONS OF TRAMADOL HYDROCHLORIDE AND ACETAMINOPHEN COATED TABLETS IN HEALTHY MALE AND FEMALE SUBJECTS UNDER FASTING CONDITIONS

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Abstract

Objective: To evaluate bioequivalence between a new formulation of Tramadol Hydrochloride/Acetaminophen and Ultracet® coated tablets in healthy subjects under fasting conditions.

Methods: The present study was conducted as an open-label, monocentric, randomized, 2 x 2 crossover study on 40 healthy subjects in a state of fasting. The objective of the study was to compare the pharmacokinetic profiles of two formulations of tramadol hydrochloride and acetaminophen-coated tablets. The concentrations of the analytes in human plasma were measured using a validated UPLC-MS/MS method.

Results: The geometric mean of the test/reference ratio, confidence intervals, and power of the test for the pharmacokinetic parametersC_{max} and AUC_{0-t}, were determined by statistical analysis, in accordance with the Anvisa's guidelines. The geometric mean ratio (90% CI) of the test drug/reference drug for acetaminophen was found to be 82.69% to 100.00% for Cmax and 95.36% to 100.34% for AUC0-t. The results for tramadol hydrochloride were 89.01% to 99.25% for Cmax and 94.86% to 100.71% for AUC0-t. The power of the test was greater than 98%.

Conclusion: Both formulations demonstrated bioequivalent profiles, indicating that they are interchangeable in accordance with the Brazilian criteria. This is evidenced by the fact that the confidence intervals for Cmax and AUC0-t ratios were within 80% and 125%, respectively, as stipulated in Anvisa resolution RE nº 1170/2006.

Introduction:-

Pain, which can affect quality of life and productivity, is a challenging global public health problem. Since pain is often caused by multiple factors, a single analgesic agent may not be effective. The combination of two or more agents at a fixed dose can provide additional benefits and effectively treat pain while reducing toxicity due to lower doses[1].

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Tramadol is a synthetic analogue of codeine with weak opioid antagonist properties. Its analgesic effects may be attributed to the inhibition of norepinephrine and serotonin reuptake. Following oral administration, the elimination half-life for Tramadol is estimated to be six hours, with a similar half-life for its active metabolite. Pain relief begins one hour after ingestion, reaches its maximum intensity within two to three hours, and lasts approximately six hours. The recommended dosage is 400 mg. The most prevalent adverse effects include nausea, vomiting, dry mouth, dizziness, sedation, and headache. [2].

Acetaminophen was first employed in the field of medicine by von Mering in 1893. However, it only gained widespread recognition after 1949, when it was identified as the principal active metabolite of both acetanilide and phenacetin. Acetaminophen enhances the pain threshold, providing analgesic effects against pain caused by a multitude of etiologies. It has become an efficacious alternative to acetylsalicylic acid as an antipyretic analgesic. The drug is well-tolerated and has a low incidence of gastrointestinal side effects. However, an acute overdose of this medication may cause serious liver damage. Peak plasma concentrations are reached within 30 to 60 minutes, and the half-life in plasma is approximately 2 hours after therapeutic doses[2]. There is substantial evidence to suggest that the analgesic effect of acetaminophen is central in nature, deriving from the activation of descending serotonergic pathways. However, its principal site of action may still be the inhibition of PGE2 synthesis. The precise molecular mechanism of acetaminophen's action remains unclear, although there is a possibility that it may relate to the production of reactive metabolites by the peroxidase function of COX-2. Such metabolites may potentially exhaust glutathione, a cofactor of enzymes such as PGE synthase[3].

The combination of agents, such as Tramadol and Acetaminophen, which have complementary mechanisms of action and target multiple sites, may potentially provide superior analgesia for various types and sources of pain[4]. Tramadol and acetaminophen are often co-administered to control moderate to severe pain due to their rapid onset and long-lasting analgesic effect [5]. Fixed-dose combination trials, such as Tramadol/Acetaminophen, are promising for the treatment of chronic pain syndrome. They provide safe and efficient pain relief with good tolerability and security profiles [1].The association of Tramadol Hydrochloride and Acetaminophen is indicated for the treatment of moderate to severe pain classified as acute, subacute, and chronic [6].

The aim of this study was to determine, through a single-dose trial, whether the coated tablets of Tramadol Hydrochloride + Acetaminophen (brand name Gésico Duo) manufactured by EurofarmaLaboratórios S.A. are bioequivalent to the reference product ULTRACET® when administered to healthy adult subjects from both genders under fasting conditions.

Methods:-

Study Formulations

The test product,37,5 mg Tramadol Hydrochloride+ 325 mg Acetaminophen coated tablet, was manufactured by EurofarmaLaboratórios S/A, using a brand name Gésico Duo. The reference product used in the trial wasUltracet® (37,5 mg Tramadol Hydrochloride + 325 mg Acetaminophen coated tablet), manufactured by Janssen-Cilag Farmacêutica Ltda.

Study volunteers

Healthy adult male and female volunteers who were willing to participate in the study were selected based on the eligibility criteria outlined in the protocol. After fulfilling the protocol requirements, volunteers were provided with information about the study. Once their inquiries were clarified and they willingly agreed to participate, each subject signed the Informed Consent Form (ICF) that had been previously approved by the Ethics Research Committee of the Instituto de CiênciasFarmacêuticas (ICF) along with the study protocol. Forty study subjects were initially selected and randomized, with 20 male and 20 female participants. However, one subject was excluded, leaving a total of 39 participants who completed all stages of the study.

Study Design

This is a monocentric, open-label, crossover, randomized, prospective, two-treatment, two-sequence, two-period bioequivalence study conducted on 40 healthy male and female subjects aged between 18 and 50 years under fasting conditions.

Drug administration

Prior to and following each administration of the dose, the study subjects were required to fast for a minimum of eight hours and a maximum of four hours, respectively. This was in accordance with the guidelines issued by ANVISA – Agência Nacional de VigilânciaSanitária, which stipulate that this study must be conducted under fasting conditions[7].

During each study period, the subjects were orally administered a single dose of either the test product or the reference product (37.5 mg Tramadol + 325 mg Acetaminophen) while seated and with 200 mL of room temperature water under fasting conditions. A washout period of 7 days was applied.

Blood sampling

A total of twenty-six blood samples, each containing 9.8 mL (two 4.9 mL tubes), and eighteen blood samples, each containing 4.9 mL, were collected using lithium heparin anticoagulant. Additionally, two blood samples, each containing 15 mL, were collected for method validation purposes. Blood samples were collected at two specific times for pharmacokinetic evaluation, one for Acetaminophen, resulting from sample collection at time 0. 0:05, 00:10, 00:15, 00:20, 00:25, 00:30, 00:40, 00:50, 01:00, 01:15, 01:30, 01:45, 02:00, 02:30, 03:00, 04:00, 06:00, 08:00, and 12:00 for the first substance, and at 0. Samples were collected at 0.00 h before and after administration at various timepoints: 00 h before and after administration at timepoints 00:30, 01:00, 01:15, 01:30, 01:45, 02:00, 02:30, 03:00, 04:00, 06:00, 08:00, 12:00, 24:00, and 36:00 for Tramadol.

Biological Samples Processing

Subsequent to the collection of the blood samples, they were subjected to processing in a refrigerated centrifuge at 3000 rpm for a period of five minutes at a temperature of approximately 4°C. The resulting plasma was then transferred to two distinct cryogenic tubes that had been previously identified and stored at a temperature below -20°C.

Tramadoland Acetaminophen quantification in plasma

Method validation

The bioanalytical method was validated to quantify Tramadol and Acetaminophen in human plasma samples simultaneously. Metoprolol and Acetaminophen-d4 were the substances used as internal standards, respectively. The method used lithium heparin plasma for extraction through protein precipitation and liquid chromatography coupled with mass spectrometry (UPLC-MS/MS). The validation was performed according to the acceptance criteria for selectivity, calibration curve, precision, accuracy, residual effect, matrix effect, and stability tests in both solution and biological matrix.

The samples were stored at a temperature of six degrees Celsius in the autosampler. Each injection was made with a volume of 5 µL, and the retention times were as follows: 2.10 minutes for Tramadol, 2.20 minutes for Metoprolol, 1.11 minutes for Acetaminophen, and 1.11 minutes for Acetaminophen-d4. The running time was 3.20 minutes.

The method demonstrated a linear response in the range of concentrations of Tramadol from 1000 ng/mL to 4000 ng/mL, as well as in that of Acetaminophen from 25 ng/mL to 10000 ng/mL. The linearity was determined using the equation $y = ax + b$ [1/x], where "y" represents the response, "x" represents the analyte concentration, and "1/x" represents the chosen weight.

The lower limit of quantification (LLOQ) of the method was set at 1,000 ng/mL for tramadol and 25.0 ng/mL for acetaminophen. Quality control samples were validated at 3,000 ng/mL, 160,000 ng/mL, and 320,000 ng/mL for tramadol and 75.0 ng/mL, 4000.0 ng/mL, and 8000.0 ng/mL for acetaminophen. The summary of bioanalytical method is shown in Table 1.

Stability

Stability tests were conducted on Tramadol and Paracetamol in plasma at concentrations of 3,000 ng/mL and 320,000 ng/mL, as well as 75.0 ng/mL and 8,000.0 ng/mL, respectively. Samples were subjected to short-term stability testing (16 hours at room temperature), post-process stability testing (181 hours in an autoinjector after completion of sample extraction), and also after three freeze-thaw cycles, and after 73 days of long-term storage. The results met the acceptance criteria established by regulations[8].

The analyte primary and working solutions, as well as the internal standards, met the acceptance criteria and demonstrated stability for 78 days when stored at refrigerated temperatures.

Standard solutions

The reference standards used were Tramadol Hydrochloride (European Pharmacopeia/France) and Acetaminophen (USP/USA) for analytes, while the internal standards used were Metoprolol Succinate (USP/USA) and Acetaminophen-d4 (Cerilliant/USA) for the preparation of standard solutions. The primary solutions for Acetaminophen and Acetaminophen-d4 were prepared in methanol, while Tramadol and Metoprolol primary solutions were prepared in methanol/water (50/50; v/v). The working solutions were prepared by diluting the original material with methanol and water in a ratio of 80:20 by volume. The prepared solutions were then stored at temperatures of between 2 and 8 degrees Celsius.

Quantification of compounds in biological samples.

A series of human plasma samples were extracted and simultaneously quantified using liquid chromatography coupled to mass spectrometry (LC-MS/MS) on the API5500 Q-TRAP (SCIEX) spectrometer, equipped with an electrospray ionisation source operating in positive mode. The analytes and internal standards were detected using MRM with m/z transitions. The following mass-to-charge ratios were observed for the compounds of interest: 264.0>58.0 for Tramadol, 268.2>116.1 for Metoprolol, 152.0>110.0 for Acetaminophen, and 156.0>114.1 for Acetaminophen-d4.

Software used

Analyst v1.5.2was used for calculating sample concentrations in the analyticalstep.

PhoenixWinNonlin^{TM}version6.3 andMicrosoftExcelwere used to perform the statistical analysis.

Table1:-Summary of the bioanalytical method.

Analyte	Tramadol	Acetaminophen
Internal Standard	Metoprolol	Acetaminophen-d4
Biological Matrix	Human Plasma	
Anticoagulant	Lithium Heparin	
Linearity	1.000 ng/mL to 400.000 ng/mL	25.0 ng/mL to 10000.0 ng/mL
Curve Equation	y = a + bx [1/x]	y = a + bx [1/x]
Lower Limit of Quantification (LLOQ)	1.000 ng/mL	25.0 ng/mL
Low Quality Control (LQC)	3.000 ng/mL	75.0 ng/mL
Medium Quality Control (MQC)	160.000 ng/mL	4000.0 ng/mL
High Quality Control (HQC)	320.000 ng/mL	8000.0 ng/mL
Post-processing Stability Time	181 hours	
Freeze/thaw cycles	3 cycles	
Short-term stability time	16 hours	
Long-term stability time	73 days	

Results:-

Study population

A total of 40 volunteers were recruited for the study. After screening, 39 healthy adult males and females, aged 18 to 50 years and with a BMI ranging from 18.5 to 28.6 kg/m², were enrolled. All subjects met the inclusion and exclusion criteria outlined in the protocol.

Pharmacokinetics and statistical analysis

Pharmacokinetic parameters $C_{max}$and AUC_{0-t}were set using softwarePhoenixWinNonlin^{TM}version6.3.

Pharmacokinetic parameters are shown in the Table 2.

The reference drug Ultracet® had an average C_{max} concentration of 5206.095 ng/mL for Acetaminophen and 129.135 ng/mL for Tramadol, both obtained in approximately 1.4 hours. The test drug had an average C_{max}

concentration of 4853.221 ng/mL for Acetaminophen, obtained in 1.0 hour, and 121.570 ng/mL for Tramadol, obtained in 1.5 hours.

Table 2:- Pharmacokinetic parameters (n=39).

Drug	Ratio (test / reference)	Geometric Mean (%)	CI (90%)	Test Power (%)	p-value (sequence)
Acetaminophen	**C_{max}**	90.94	82.69 – 100.00	98.55	82.56
	AUC_{0-t}	97.82	95.36 – 100.34	100.00	62.26
	$AUC_{0-\infty}$	99.03	96.79 – 101.33	100.00	64.46
Tramadol	**C_{max}**	93.99	89.01 – 99.25	100.00	34.58
	AUC_{0-t}	97.74	94.86 – 100.71	100.00	43.78
	$AUC_{0-\infty}$	98.39	95.74 – 101.11	100.00	39.79

Figure 1 displays the average concentrations of Tramadol for the 39 study subjects over time and Figure 2 displays the average concentrations of Acetaminophen for both formulations over time.

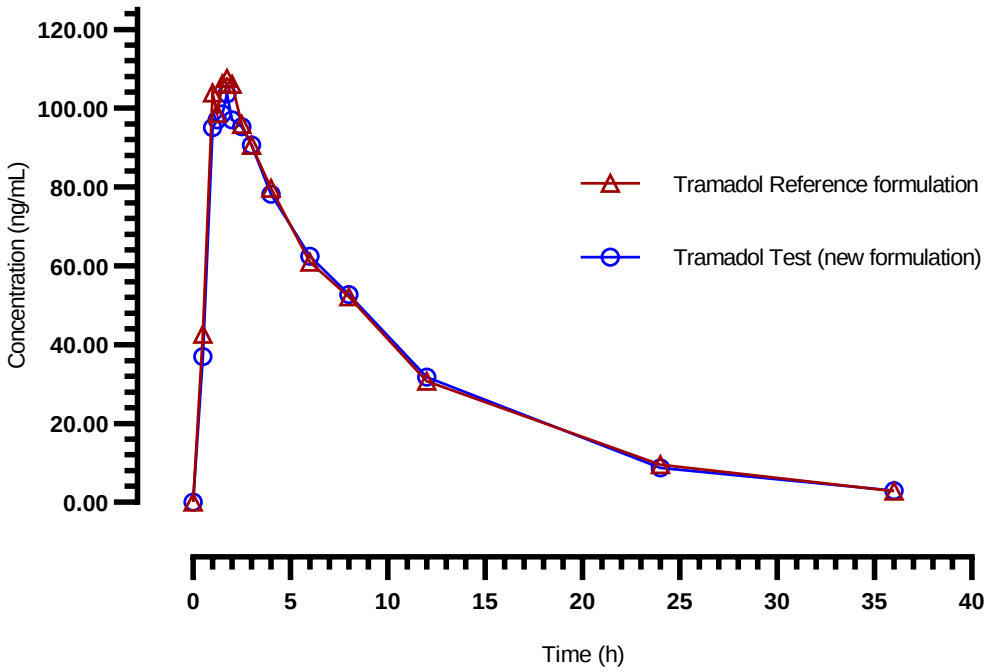

Figure1:- Average concentrations of Tramadol over time for each formulation.

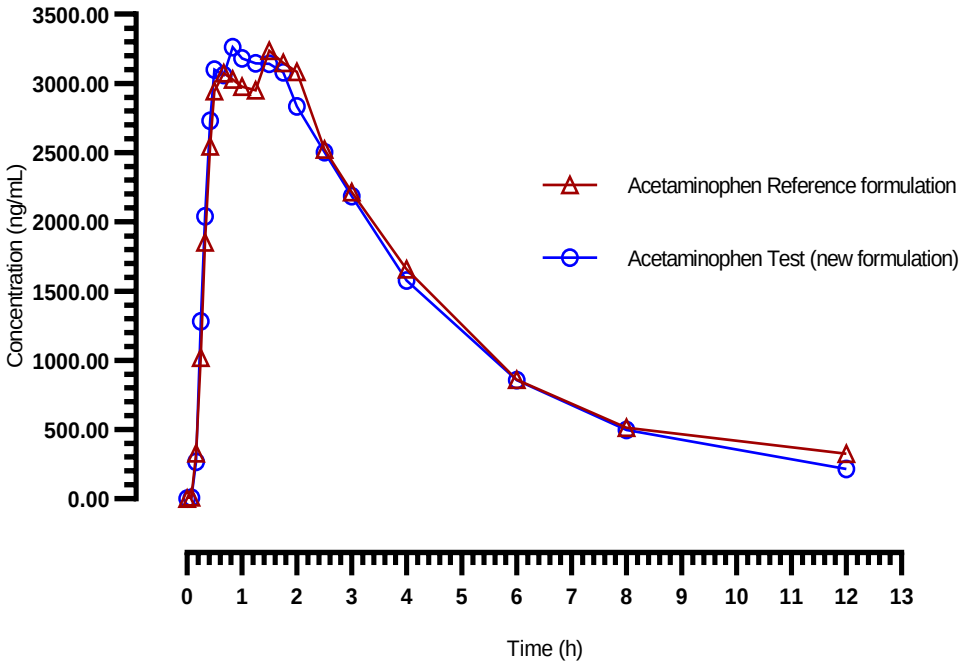

Figure2:- Average concentrations of Acetaminophen over for each formulation.

Tolerability/Safety analysis

During the study, 28 adverse events were reported. The most frequent events were lipothymia (5), leukocyturia (5), nausea (3), headache (2), and serum triglyceride increase (2). One subject reported a moderate intensity syncope. No serious adverse events were reported, with 24 events classified as mild and 4 as moderate.

Discussion:-

The study was conducted in accordance with a well-designed protocol and yielded pharmacokinetic parameters, including C_{max} and AUC_{0-t}. The 90% confidence interval values for the ratio between the geometric means of the test and reference products were found to be within the acceptable limit (80-125%) according to Brazilian legislation[9]. The number of healthy adult subjects planned for this study is consistent with previous research conducted by other authors [5, 10-12]. The sample size was proven to be adequate as the minimum power obtained for the test was 98.55%, exceeding the desired power of 80% recommended by Brazilian legislation[13].

Both formulations were found to be well tolerated during the clinical trial, with no serious adverse events reported. Adverse events suspected to be drug related were reported with the greatest frequency, including lipothymia, syncope, nausea, headache, dizziness, and hypotension. These observations align with those reported by other authors and as described within the reference drug manufacturer's product information leaflet[4, 6, 14, 15].

The washout period of 7 days appeared to be sufficient, as all baseline collection samples of the second period volunteers had a concentration below the Lower Limit of Quantification (LLOQ) for both drugs. The collection timepoints also seemed appropriate, as almost all collections - except the baseline collection - showed quantified values and the plasma curve was well represented, allowing for adequate statistical analysis.

The trial successfully employed the LC-MS/MS analytic technique to simultaneously quantify Tramadol and Acetaminophen in human plasma samples. This technique is consistent with other publications on separate drug quantifications[5, 16-18].

Tramadol was quantified in its unaltered form in accordance with the stipulations of Brazilian legislation[19].

Both the reference and test formulations exhibited a maximum plasma concentration (C_{max}) of 5206.095 ng/mL and 4853.221 ng/mL for Acetaminophen, and 129.135 ng/mL and 121.570 ng/mL for Tramadol, respectively. These values are consistent with those reported in the literature. [4, 5].

It is of the utmost importance to ensure a consistent supply of government-subsidized medicines in countries such as Brazil. This necessitates the establishment of long-term agreements with pharmaceutical companies to procure medications at reduced costs and the promotion of competition among market participants to guarantee affordable prices.[20].

Bioequivalence studies are of vital importance in the pharmaceutical industry, as they ensure that generic drugs are as effective as their branded counterparts[21, 22]. These studies assess whether generic drugs release their active ingredient into the bloodstream at a comparable rate to the original drug. They are cost-effective and save time compared to full clinical trials, thus promoting the development of generic formulations[21, 23, 24]. Regulatory agencies have established guidelines to ensure consistent and reliable evaluations. The availability of generic drugs encourages market competition, which frequently results in reduced prices for consumers and governments alike.

These considerations are consistent with the broader experience and observations within the field, reflecting both the current study and the extensive long-term experience of this group. They accurately represent the predominant strengths and limitations of bioequivalence studies for public health, including increased access to medications in Brazil and other emerging countries [21, 22].

Conclusion:-

It has been demonstrated that the formulations exhibit comparable absorption rates and extents, in accordance with the regulatory criteria set forth by the Brazilian authority, specifically in regard to the pharmacokinetic parameters Cmax and AUC0-t, where the 90% confidence interval falls between 80 and 125%. Consequently, the test formulation, which consists of 37.5 mg tramadol hydrochloride and 325 mg acetaminophen coated tablets manufactured by EurofarmaLaboratórios S/A, and the reference formulation Ultracet® coated tablets are bioequivalent and interchangeable.

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