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Bioequivalence study between two formulations of betamethasone dipropionate and betamethasone disodium phosphate injectable suspension in healthy adults

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Abstract

Objective: To evaluate bioequivalence between two formulations of 5,0 mg/mL betamethasone dipropionate + 2,0 mg/mL betamethasone disodium phosphate injectable suspension in healthy adults under fasting condition. **Methods:** The study was an open label, randomized, single dose, 2x2 crossover study in 36 healthy adult subjects under fasting conditions. Betamethasone concentrations in human plasma were determined using a validated liquid chromatography coupled to a tandem mass spectrometer detector method. **Results:** Statistical analysis has determined confidence intervals, power of the test and p-value for sequence effect to the pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. The geometric mean ratio (90%CI) of the test drug/reference drug for betamethasone were 87.74% to 92.23% for C_{max}, 93.95% to 98.91% for AUC_{0-t} and 94.36% to 99.95% for $AUC_{0-\infty}$. Power of the test was 100% for all parameters and p-value for sequence effect were 33.39% for C_{max}, 19.98% AUC_{0-t}, and 24.32% for $AUC_{0-\infty}$. **Conclusion:** Reference and test formulations are statistically bioequivalent and, therefore, interchangeable, according to the local and international criteria, since confidence intervals for C_{max} and AUC_{0-t} ratios were within 80% and 125%, according to Anvisa resolution RE nº 1170/2006.

Keywords: betamethasone, bioavailability, bioequivalence, HPLC, LC-MS/MS, injectable suspension.

Estudo de Bioequivalência entre duas formulações de suspensão injetável de diproprionato de betametasona e fosfato dissódico de betametasona em adultos saudáveis

Resumo

Objetivo: Avaliar a bioequivalência entre duas formulações de dipropionato de betametasona 5,0 mg/mL + fosfato dissódico de betametasona 2,0 mg/mL em suspensão injetável em adultos saudáveis em jejum. **Métodos**: Foi realizado um estudo aberto, aleatório, de dose única, cruzado 2x2 em 36 indivíduos adultos saudáveis em jejum. As concentrações de betametasona no plasma humano foram determinadas utilizando um método validado de cromatografia líquida acoplada a um detetor espetrômetro de massas. **Resultados**: A análise estatística determinou intervalos de confiança, poder do teste e valor de p para efeito de sequência para os parâmetros farmacocinéticos C_{max}, AUC_{0-t} e $AUC_{0-\infty}$. A razão média geométrica (IC90%) do medicamento teste/fármaco de referência para a betametasona foi de 87,74% a 92,23% para C_{max}, 93,95% a 98,91% para AUC_{0-t} e 94,36% a 99,95% para $AUC_{0-\infty}$. O poder do teste foi de 100% para todos os parâmetros e o valor de p para o efeito de sequência foi de 33,39% para C_{max}, 19,98% para AUC_{0-t} e 24,32% para $AUC_{0-\infty}$. **Conclusões:** As formulações de referência e teste são estatisticamente bioequivalentes e, portanto, intercambiáveis, de acordo com os critérios locais e internacionais, uma vez que os intervalos de confiança para as razões C_{max} e AUC_{0-t} ficaram dentro dos limites de 80% e 125%, de acordo com a resolução RE nº 1170/2006 da Anvisa.

Palavras-chave: betametasona, biodisponibilidade, bioequivalência, HPLC, LC-MS/MS, suspensão injetável.

Introduction

A bioequivalence study is designed to compare the rate and extent of systemic absorption of a generic drug in relation to the reference drug, with the objective of verifying that both have equivalent pharmacokinetic profiles. These studies are conducted under controlled conditions, with parameters including the area under the curve (AUC), maximum plasma concentration (C_{max}), and time to reach C_{max} (Tmax). The primary objective is to demonstrate that the generic drug has equivalent bioavailability to the reference product, thereby ensuring comparable therapeutic efficacy and safety. Bioequivalence studies are essential for the regulatory approval of generic drugs, ensuring that they can be interchanged with the reference product without compromising clinical treatment[1].

Corticosteroids actions have been historically described glucocorticoids (which reflect their regulation activity of carbohydrates metabolism) and mineralocorticoids (which reflect their regulation activity of the electrolyte imbalance). Corticosteroids exert a wide range of effects, including alterations in the metabolism of carbohydrates, proteins, and lipids. Additionally, they contribute to the maintenance of the hydroelectric balance and the preservation of the normal functioning of the cardiovascular system, immune system, kidneys, musculoskeletal system, endocrine system, and nervous system. These are grouped according to their relative power in the retention of Na+, effects on the metabolism of carbohydrates and anti-inflammatory effects[2].

Betamethasone, a synthetic glucocorticoid, exhibits metabolic, immunosuppressive, and anti-inflammatory properties. It binds to intracellular glucocorticoid receptors and subsequently attaches to DNA, altering genetic expression. This process induces the synthesis of anti-inflammatory proteins while inhibiting the production of specific inflammatory mediators. Consequently, it leads to a widespread reduction in chronic inflammation and autoimmune responses[3].

Glucocorticoids are employed in the treatment of a number of chronic inflammatory diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, and autoimmune disorders. These conditions are associated with an increase in the expression of inflammatory genes[4].

One of the first clinical applications of betamethasone was reported in 1972,[5] obtaining the desired effect in the fetus maturation with one single injection per day of a suspension with equal amounts of phosphate and betamethasone acetate[6].

The injectable suspension, which contains betamethasone dipropionate and betamethasone disodium phosphate, is indicated for the treatment of various clinical conditions, including musculoskeletal and soft tissue disorders. These include rheumatoid arthritis, osteoarthritis, bursitis, ankylosing spondylitis, radiculitis, spondylitis, coccyx pain, sciatica, lumbago, torticollis, exostosis, and fasciitis. Furthermore, it is indicated for allergic conditions such as chronic bronchial asthma, allergic rhinitis due to pollen, angioneurotic edema, allergic bronchitis, seasonal or perennial allergic rhinitis, hypersensitivity to the drug, serum sickness, and insect bites. In addition, it is employed in the treatment of dermatological conditions, including atopic dermatitis, circumscribed neurodermatitis, contact dermatitis, severe sun dermatitis, hives, hypertrophic lichen planus, necrobiosis lipoidica associated with diabetes mellitus, alopecia areata, discoid lupus erythematosus, psoriasis, keloids, pemphigus, dermatitis herpetiformis, and cystic acne. Moreover, it is utilized in the treatment of collagen-related disorders, including systemic lupus erythematosus, scleroderma, dermatomyositis, and polyarteritis nodosa. It is also employed in the palliative treatment of leukemia and lymphoma in adults and acute leukemia in infants[7].

In the study published by He,[8] Chinese adults were administered intramuscular betamethasone containing 2 mg betamethasone disodium phosphate and 5 mg betamethasone dipropionate; C_{max} 14.5 ng/mL and T_{max} 2.8 hours average values were found.

Betamethasone disodium phosphate (BSP) and betamethasone dipropionate (BDP) may be hydrolyzed by phosphatase as both fast-release phosphate prodrug and esterase enzymes and extended-release dipropionate prodrug to betamethasone active pharmaceutic ingredients (BOH), betamethasone 17-monodipropionate(B17P) and betamethasone 21-monodipropionate (B21P). Due to the above, Chen9 defends the importance and utility of determining BSP, BPD and their metabolites in human plasma.

Common adverse reactions related to the use of Diprospan® informed in the product insert are: insomnia, dyspepsia, hunger increase and increase of the infections' incidence. Rare adverse reactions related to the central nervous system include depression, seizures, dizziness, headache, mental confusion, euphoria, personality disorder and changes in humor[7].

The objective of this study was to evaluate whether the test formulations manufactured by Eurofarma Laboratórios S/A reach equivalent plasma levels to those of the reference product Diprospan®, and therefore be considered bioequivalent pursuant to the legislation in force in Brazil at the time of the study[1, 10-12].

Methods

Study formulations

The test drug, 5.0 mg/mL betamethasone dipropionate + 2.0 mg/mL betamethasone disodium phosphate injectable suspension was manufactured by Eurofarma Laboratórios S/A. The test drug used in the study was Diprospan® (5.0 mg/mL betamethasone dipropionate + 2.0 mg/mL betamethasone disodium phosphate, injectable suspension), manufactured by Brainfarma Indústria Química e Farmacêutica S/A and registered with the Brazilian National Health Surveillance Agency (Agência nacional de Vigilância Sanitária - ANVISA) by Comed Indústria de Cosméticos e Medicamentos S.A.

Study subjects

After taking part in a presentation in order to explain the particulars of the study and after having their inquiries clarified and having decided to willingly take part in the study, each subject signed the Informed Consent Form (ICF) previously approved by the Research Ethics Committee of the University of São Francisco, São Paulo, under the registry number CAAE 18017119.1.0000.5514, and conducted by UNIFAG.

Study Population and Selection Criteria

Bioequivalence studies typically involve the recruitment of healthy adult participants to establish a uniform group with comparable traits, such as age and body mass index (BMI). This strategy reduces variability caused by factors like body composition, metabolic differences, or prior health conditions, which is particularly important in parallel study designs. Consequently, it allows for more reliable conclusions regarding the bioequivalence of the products being evaluated.

Furthermore, all participants were required to provide a negative result for both HIV-1 and HIV-2 in serum tests, as well as demonstrate the absence of any indications of infection with hepatitis B or C viruses. A physical examination was conducted for each individual. The health status of each individual was determined based on the results of a comprehensive clinical assessment, which included a personal interview, a full physical examination (measuring blood pressure, heart rate, weight, height, temperature, and respiratory rate), diagnostic procedures such as a 12-lead electrocardiogram (ECG), and laboratory tests. The tests included a complete blood cell count, metabolic and liver function assessments (alanine and aspartate aminotransferase levels), biochemistry (glucose, blood urea nitrogen, creatinine), serological tests for hepatitis B, hepatitis C, and HIV antibodies, urinalysis, and, for women, a pregnancy test. Candidates were excluded if any laboratory values fell significantly outside the reference ranges or if they had not completed all the required tests. Furthermore, individuals with a history of allergic reactions to nitazoxanide or similar medications, evidence of organ dysfunction, or a history of gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological, psychiatric, hematological disorders, diabetes, or glaucoma were excluded. Furthermore, individuals with a history of psychotropic drug use or consumption of more than two units of alcohol per day were excluded. In the 48 hours preceding the study, participants were disqualified if they had consumed alcohol, tobacco, or foods high in xanthines.

Thirty-six male and female healthy adult subjects between 18 and 49 years old were selected, with a body mass index (BMI) between 20.10 and 29.20 kg/m², according to the inclusion and exclusion criteria. There were no dropouts or withdrawals by any study subjects, reason for which the same 36 volunteers were considered for the statistical analysis.

Subjects were under fasting conditions for 9 hours before administration and for at least 4 hours after administration in each study period.

Study Design

Open label, randomized, crossover, 2x2, two-treatment, two-sequence, two-period study. The interval between periods (washout) was 14 days.

Drug administration

All subjects were administered, via intramuscular, in each period 01 mL injectable suspension of 5.0 mg/mL betamethasone dipropionate + 2,0 mg/mL betamethasone disodium phosphate of the test product (manufactured by Eurofarma Laboratórios S/A) or the reference product (Diprospan®), according to the randomization list.

Blood sampling

A total number of 22 blood samples were collected in each period in tubes with heparin anticoagulant.

Samples were collected before administration (zero time) and 0:20, 0:40, 1:00, 1:20, 1:40, 2:00, 2:20, 2:40, 3:00, 3:30, 4:00, 5:00, 6:00, 8:00, 10:00, 12:00, 14:00, 24:00, 36:00, 48:00 and 72:00 hours after the drug administration.

Biological samples processing

After collection, tubes with the blood samples were centrifuged at 3.000 rpm for 10 minutes. Plasma samples were separated and stored in a freezer at approximately-20ºC.

Quantification of Betamethasone in Plasma:

Method Validation

The bioanalytical method validation for the quantification of betamethasone through the liquid/liquid extraction and liquid chromatography coupled to mass spectrometry was conducted in compliance with the acceptance criteria for selectivity, lower limit of quantification (LLOQ) calibration curve, linearity, precision, accuracy, residual effect, matrix effect and stability tests in the solution and the biological matrix[10].

All parameters complied Anvisa's requirements, and the summary of the method was presented in the Table 1. One of the most important criteria was the accepted range for variations (±15% for regular concentrations, ie, >LLOQ and ≤ upper limit of quantification, and ±20% for LLOQ) from the nominal value. The accuracy was measured as a percentage of the nominal concentration within the limits of acceptance of 85%–115% for regular concentrations and 80%–120% for LLOQ. The precision was evaluated as a coefficient of variability (≤15% for regular concentrations and 20% for LLOQ). For selectivity, a discrimination level of 20% for LLOQ was used as the criterion, as well as 5 % for the internal standard.

Sample preparation

Prior to extraction, the plasma samples were thawed at room temperature. A 100-microliters portion of human plasma was transferred to a microcentrifuge tube, followed by the addition of 50 microliters of the cyclobenzaprine working solution and 2.0 milliliters of organic solvent. Following a one-minute vortex mixing period, the samples were subjected to centrifugation at 2,000 × g for one minute at 4°C. The mixture was frozen in an ethanol bath with dry ice, and the upper liquid organic phase was transferred to a clean glass tube and evaporated to dryness under nitrogen flow at 40 degrees Celsius. The resulting dry residues were reconstituted in 100 microliters of mobile phase by vortex mixing for 20 seconds, and then, the solution was transferred to an HPLC vial for injection of 10 microliters into the chromatographic system (Agilent Binary pump and Pal System autosampler at 4°C). The mobile phase consisted of 85/15 (v/v) acetonitrile-water mixture, flowing at a rate of 1 ml/min through a Phenomenex Phenil Column. The mass spectrometer, SCIEX API 5000, was equipped with an electrospray ionization source operating in negative mode. Monitoring was conducted on the fragmentations 361>292 m/z for betamethasone and 329>280 for prednisolone (internal standard).

The Lower Limit of Quantification (LLOQ) set for the method was 0.1000 ng/mL and the validated quality control samples were 0.3000 ng/mL, 20.0000 ng/mL and 40.000 ng/mL. Most relevant parameters can be seen in Table 1.

Table 1. Summary of the bioanalytical method validated parameters and stability timeframes assured

Parameter	Description
Analytical technique	LC-MS/MS
Analyte	Betametasone
Internal standard	Prednisolone
Biological matrix	Human Plasma
Anticoagulant	Heparin
Linearity	0,1000 ng/mL to 50,0000 ng/mL
Equation of the curve	y = a + bx (1/x^2)
Lower limit of quantification (LIQ)	0,1000 ng/mL
Low quality control (QCL)	0,3000 ng/mL
Medium quality control (QCM)	20,0000 ng/mL
High quality control (QCH)	40,0000 ng/mL
Stability Test	Timeframe
Post-processing stability time	114 h
Freezing and thawing cycles	5 cicles
Short-term stability time	15 h
Long-term stability time	84 days

Stability

Stability tests were conducted in plasma in concentrations of 3.000 ng/mL and 40.0000 ng/mL and they complied with the acceptance criteria when the samples were subjected to 15 hours at room temperature (short-term stability), 114 hours after finishing sample extraction (post-processing stability) 5 freeze-and-thaw cycles and 84 days of long-term stability stored at-20ºC.

Standard solutions and reagents

Reagents used included purified water obtained using Millipore purification system, methanol HPLC grade (Merck), analytical grade ethyl ether (Merck) and ammonia solution 30% (Sigma Aldrich).

Betamethasone reference standards were used as analyte and prednisolone as internal standard for the preparation of the primary internal solutions in pure methanol.

Compounds quantification in biological samples

Compounds were extracted from plasma samples and quantified by means of liquid chromatography coupled with mass spectrometry (LC-MS/MS) using API 5500 (MDS Sciex), spectrometer, equipped with negative electrospray ionization source and detecting analyte and internal standards using MRM with m/z transitions 361.1 > 292.1 and 329.1 > 280.1, respectively.

Statistical Analysis

The principal objective of the statistical analysis of bioequivalence was to ascertain whether the pharmacokinetic measures fall within the predefined equivalence margins, which were set at 80-125% for the ratio of geometric means based on log-transformed data.

A hypothesis test was employed in conjunction with the ANOVA results to evaluate whether the pharmacokinetic parameters of the test drug formulation are statistically equivalent to those of the reference formulation. The test is centered on a comparison of the means of the log-transformed pharmacokinetic parameters, including C_{max}, AUC_{0-t}, and AUC_{0-Inf}, as well as the secondary outcomes.

The hypotheses tested in bioequivalence studies were structured as follows:

Null hypothesis (H_0): There is a significant difference between the test and reference formulations, i.e., the ratio of the pharmacokinetic parameters between the two was not within the acceptable bioequivalence range (80-125%).

Alternative hypothesis (H_1): The test and reference formulations are bioequivalent, indicating that the ratio of their pharmacokinetic parameters falls within the acceptable range (80-125%).

The hypothesis test was conducted to ascertain whether the 90% confidence intervals for the pharmacokinetic parameter ratios fell within the predefined equivalence limits (80-125%). If the confidence intervals are found to lie entirely within the specified range, the null hypothesis (H_0) is rejected, indicating that the two formulations are bioequivalent.

The key pharmacokinetic parameters were assessed, including the elimination rate constant (Ke), half-life ($T_{1/2}$), time to maximum concentration (T_{max}), the maximum concentration (C_{max}), area under the curve from zero to the last measurable concentration (AUC_{0-t}), area under the curve extrapolated to infinity (AUC_{0-Inf}), and the percentage of the AUC that is extrapolated ($AUC_{\%Extrap}$). The Ke was calculated as the slope of the natural log of plasma drug concentration versus time during the elimination phase. Subsequently, $T_{1/2}$ was derived using the formula $T_{1/2}=\ln(2)/Ke$, which provides the time it takes for the drug concentration to decrease by half.

The maximum concentration (C_{max}) and time to maximum concentration (T_{max}) are directly measured from the plasma concentration-time curve, representing the highest concentration achieved and the time at which it occurs, respectively. The area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) and the area under the plasma concentration-time curve from time zero to infinity (AUC_{0-Inf}) are calculated using the trapezoidal rule, representing the total drug exposure over time. $AUC_{\%Extrap}$ is defined as the proportion of the total AUC extrapolated from the last measurable time point to infinity, expressed as a percentage. In order to ascertain whether the test and reference formulations were bioequivalent, a statistical analysis was conducted, whereby the 90% confidence intervals for the ratio of the pharmacokinetic parameters (C_{max}, AUC_{0-t}, and AUC_{0-Inf}) were calculated. If the confidence intervals fell within the 80-125% bioequivalence limits, the formulations were considered to be bioequivalent.

Software used

Analyst version 1.4.2. was used for calculating sample concentrations in the analytical phase

Phoenix/WinNonlin™ version 8.1 and Microsoft Excel version 2010 were used to perform the statistical analyses.

Results

The study was conducted between 2019 and 2020, then the regulations at the time of the study were used within this frame time and consequently cited in the references section. Once the drug has been registered, even if the regulations for conducting bioequivalence studies change, the product can be sold for use until its registration expires.

Pre-Study Validation of Bioanalytical Method

Prior to the quantification of the samples, a reliable, robust and reproducible method was validated, and its stability parameters were assessed according to the Anvisa's requirements complying the RESOLUÇÃO- RDC Nº 27, DE 17 DE MAIO DE 2012[10].

The method proved linear between concentrations of 0.1000 ng/mL to 50.0 ng/mL according to equation y = a + bx [1/x²], where "y" is the response, "x" is the analyte concentration and "1/x²" the selected weight for least square regression method.

The validation was conduct with required experiments subjecting to method performance tests with the following concentrations: Lower Limit of Quantification (LIQ = 0.1000 ng/mL), Low concentration Quality Control (QCL = 0.300 ng/mL), Medium concentration Quality Control (QCM = 20.0 ng/mL), High concentration Quality Control (QCH = 40.0 ng/mL), Dilution purposes Quality Control (QCD = 160.0 ng/mL).

The summarized results of method parameters evaluation are shown in the Table 2, including the assessment of Selectivity, Matrix effect, Linearity, Intra and Inter-batch Precision and Accuracy, with description of the conditions of each test and the criteria required by Legislation.

Study population

The study was completed with 36 healthy subjects (18 female and 18 male), between 19 and 49 years of age and a BMI between 20.1 and 29.2 kg/m². There were no dropouts or withdrawals in this trial. Therefore, the trial was completed with the same number of subjects planned by the protocol.

Pharmacokinetic and statistical analysis

The combination of ANOVA results and hypothesis testing provided a robust statistical framework to determine whether the test drug is bioequivalent to the reference drug based on pharmacokinetic parameter comparisons, and was in accordance with ANVISA's regulations[1, 11].

K_e, $T_{1/2}$, T_{max}, C_{max}, AUC_{0-t}, AUC_{0-inf} and $AUC_{\%Extrapolated}$ parameters were set using WinNonlin (Phoenix). Statistical analyses were performed with the Bioequivalence Wizard module, which automatically calculates intervals of confidence and estimates all additional statistics necessary for the study.

Maximum concentration C_{max} obtained for the reference product Diprospan® was 24.699 ng/mL in 1.9 hours. For the test drug, C_{max} of 22.679 ng/mL occurred in 2.2 hours.

Figure 1 shows average betamethasone concentrations (for both test and reference formulations) for the 36 study subjects along collection times.

Table 2. Summary of the bioanalytical method results for all evaluated parameters. Lower Limit of Quantification (LIQ = 0.1000 ng/mL), Low concentration Quality Control (QCL = 0.300 ng/mL), Medium concentration Quality Control (QCM = 20.0 ng/mL), High concentration Quality Control (QCH = 40.0 ng/mL), Dilution purposes Quality Control (QCD = 160.0 ng/mL)

Validation Parameter	Conditions	Results	Criteria
Selectivity	4 normal plasma samples, 2 hyperlipemic, 2 hemolized, and they with common concomitant drugs (caffeine, nicotine, pyridoxin, 4-methylamino antipirine, Dimenidrinate, Acetaminophen, Ondansetron, Escopolamine Buthylbromide)	No significant interference in analyte or internal standard channels, even though in the presence of common concomitant drugs	No significant interference in Analyte or Internal Standard response
Matrix effect	4 normal plasma samples, 2 hyperlipemic, 2 hemolized, and they with common concomitant drugs.	Without concomitants: MNF = 6.0% With concomitants: MNF = 4.2%	Matrix Normalyzed Factor ≤ 15%
Carry over	Blank sample after a sample at the highest concentration, compared to Lower Limit of Quantification	No significant interference in analyte or internal standard channels	No significant interference in Analyte or Internal Standard response
Calibration curve and linearity of instrumental response	6 plasma calibration curves, calculated individually with least square regression method, evaluating linearity and wighted	Linear model with 1/x^2 weighted: y = a + bx (1/x^2) R = 0.999	Use of the simplest mathematical model, with the simplest weighted factor, and with Correlation Factor R≥0.98
Intra-batch Precision	5 levels of Quality Controls, 6 replicate for each one, reported as coeficient of variation (CV%)	LIQ = 8.2% Mean of QCL, QCM, QCH = 4.6% QCD = 4.2%	≤ 20% for LIQ ≤ 15% for others
Inter-batch Precision	5 levels of Quality Controls, 6 replicate for each one, reported as coeficient of variation (CV%)	LIQ = 9.3% Mean of QCL, QCM, QCH = 5.1% QCD = 6.4%	≤ 20% for LIQ ≤ 15% for others
Intra-batch Accuracy	5 levels of Quality Controls, 6 replicate for each one, reported as coeficient of variation (CV%)	LIQ = 8.2% Mean of QCL, QCM, QCH = 4.6% QCD = 4.2%	≤ 20% for LIQ ≤ 15% for others
Inter-batch Accuracy	5 levels of Quality Controls, 6 replicate for each one, reported as coeficient of variation (CV%)	LIQ = 9.3% Mean of QCL, QCM, QCH = 5.1% QCD = 6.4%	≤ 20% for LIQ ≤ 15% for others

Figure 1. Obtained average betamethasone concentrations over time for each formulation. Maximum concentration Cmax obtained for the reference product Diprospan® was 24.699 ng/mL in 1.9 hours. For the test drug, Cmax of 22.679 ng/mL occurred in 2.2 hours.

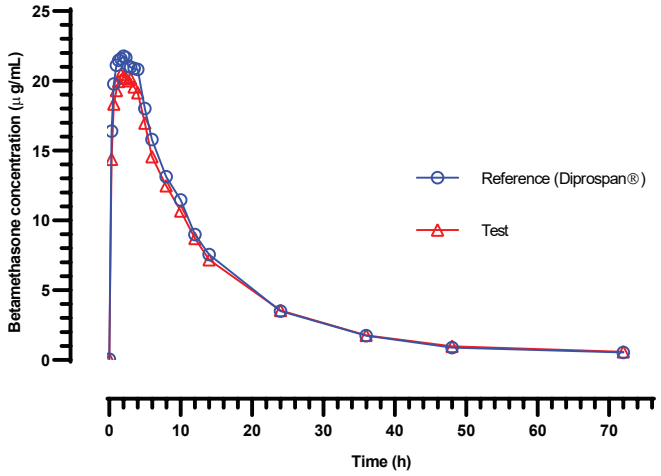

Table 3 shows the final results for pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf}, obtained from the plasma analyses of the 36 study subjects subjected to statistical analysis comparing the reference drug (Diprospan®) and the test drug (5.0 mg/mL betamethasone dipropionate + 2.0 mg/mL betamethasone disodium phosphate injectable suspension). All calculated pharmacokinetic parameters are showed in Table 4 and 5. Table 6 shows the individual geometric means for each formulation for the primary PK parameters, as well as the confidence intervals and p-values obtained in the analysis of variance for betamethasone.

Table 3. Summary of final results for C_{max}, AUC_{0-t} and AUC_{0-inf} parameters (n=36). C_{max} (Maximum concentration observed); ASC_{0-t} (Area Under the Curve from 0 to t); $ASC_{0-\infty}$ (Area Under the Curve from 0 to infinite).

Parameter	N	Ratio %	Lower Limit %	Higher Limit %	Test Power %	p Value (sequence effect) %
Cmax (ng/mL)	36	91.89	87.74	96.23	100.00	33.39
ASC0-t (ng.h.mL^{-1})	36	96.40	93.95	98.91	100.00	19.98
ASC0-∞ (ng.h.mL^{-1})	36	97.11	94.36	99.95	100.00	24.32

Tolerability and safety analysis

Twenty-three adverse events were reported in 19 subjects, out of which 95.65% were reported in the second period. The only reported adverse event in the first period was headache.

As to the relation with the drug, 34.78% of the events were possibly related to the drug and in 65.22% of the events the relation with the drug was improbable. Within adverse events possibly related to the drug, there were events of headache (4 subjects), elevated ALT (2 subjects), elevated AST (1 subject) and exanthema (1 subject).

Only 1 event were moderate (abdominal pain but with no cause relation with the drug) and the other 22 events were mild.

The number of adverse between the test and the reference drugs was very similar, that is, approximately 50% for each formulation. All adverse events were followed up and reported to healthy authority and Ethics Committee.

Table 4. Summary of final results descriptive calculated pharmacokinetic parameters (n=36) for Test formulation. Arithmetic Means are reported. Ke (Constant of elimination); $T_{1/2}$ (Halfe Life); T_{max} (Time to reach the maximum contcentration); C_{max} (Maximum concentration observed); ASC_{0-t} (Area Under the Curve from 0 to t); ASC_{0-inf} (Area Under the Curve from 0 to infinite); $AUC_{\%extrap}$ (percentage of the AUC that is extrapolated)

	Pharmacokinetic Parameters (N=36)						
	Ke 1/h	T1/2 h	Tmax h	Cmax ng/mL	ASC0 – t h*ng/mL	ASC0 – Inf h*ng/mL	ASC%Extrap h*ng/mL
Mean	0.049	15.97	2.236	22.68	313.563	327.993	4.126
Standard Error	0.002	1.31	0.183	0.794	10.167	11.444	0.38
Minimum	0.014	10.7	0.333	13.69	208.569	215.012	1.78
Maximum	0.065	48.63	4	31.21	433.42	464.188	10.423
CV %	27.72	49.22	49.07	20.99	19.455	20.934	55.303

Table 5. Summary of final results descriptive calculated pharmacokinetic parameters (n=36) for Reference formulation. Arithmetic Means are reported. Ke (Constant of elimination); $T_{1/2}$ (Halfe Life); T_{max} (Time to reach the maximum contcentration); C_{max} (Maximum concentration observed); ASC_{0-t} (Area Under the Curve from 0 to t); ASC_{0-inf} (Area Under the Curve from 0 to infinite); $AUC_{\%extrap}$ (percentage of the AUC that is extrapolated)

	Pharmacokinetic Parameters (N=36)						
	Ke 1/h	T1/2 h	Tmax h	Cmax ng/mL	ASC0 – t h*ng/mL	ASC0 – Inf h*ng/mL	ASC%Extrap h*ng/mL
Mean	0.055	14.3	1.949	24.7	326.071	338.616	3.405
Standard Error	0.003	1186	0.2	0.86	11.215	12.582	0.437
Minimum	0.017	9.53	0.333	13.24	185.535	187.092	0.833
Maximum	0.073	41.78	5000	34850	509174	565.894	11.249
CV %	27.35	49.75	61.42	20.9	20.636	22.294	77.043

Table 6. Geometric means, confidence intervals and p-values obtained in the analysis of variance for betamethasone. C_{max} (Maximum concentration observed); ASC_{0-t} (Area Under the Curve from 0 to t); ASC_{0-inf} (Area Under the Curve from 0 to infinite).

Statistical results by primary PK Parameters for each formulation (n=36)			
	Cmax	**ASC0-t**	**ASC0-inf**
Geometric means calculated using the least squares method			
Reference (R)	24.1420	319.2575	330.6353
Test (T)	22.1829	307.7597	321.0938
The obtained 90% confidence intervals (shortest) for the ratio between treatments (transformed data)			
Ratio (T/R)	91.89	96.40	97.11
Lower Limit	87.74	93.95	94.36
Upper Limit	96.23	98.91	99.95
Power of the test *A Posteriori* (%)			
T/R	100.00	100.00	100.00
Mean Square Error (Error Variance)			
Inter-subject	0.0336	0.0367	0.0409
Intra-subject	0.0134	0.0042	0.0052
Coefficient of Variation (%)			
Inter-subject	18.49	19.33	20.44
Intra-subject	11.62	6.45	7.23
P-values obtained for ANOVA fixed effects (Sequential)			
Sequence	0.3339	0.1998	0.2432
Treatment	0.0039	0.0213	0.0946
Period	0.0244	0.2260	0.1773
P-values obtained for ANOVA fixed effects (Partial)			
Sequence	0.3339	0.1998	0.2432
Treatment	0.0039	0.0213	0.0946
Period	0.0244	0.2260	0.1773

Discussion

The study was adequately planned and conducted according to the principles of Good Clinical Practices and the legislations in force. The planned and included number of subjects who completed the trial was 36 healthy adults, consistent with the studies published by other authors[9, 13, 14].

Both formulations were well tolerated during the trial, and the number of adverse events was practically the same for both drugs. No serious adverse event was reported. Most adverse events were mild. Headache was the most frequent adverse event, as reported in another betamethasone study[15].

Pain in the injection site and insomnia reported by Salem[14] were not identified in this study.

The validated and used analytic method to quantify betamethasone in the samples of this study was the LC-MS/MS technique, which was also chosen by other authors[13, 16, 17].

Although Chen[9] states the importance of quantifying betamethasone and its metabolites, the drug quantification in its unchanged form was conducted according to the list released by ANVISA at the time of conduction of the study[12].

Maximum concentrations C_{max} obtained in this study, 24.699 ng/mL and 22.679 ng/mL, for both test and reference products, respectively, were consistent with the values obtained in the literature[8, 18-20].

Given that the ratios of C_{max}, AUC_{0-t}, and AUC_{0-inf} fell within the 80-125% interval, and therefore constituted proof of bioequivalence, it can be observed that sequence effects presented significant values. However, these effects can be disregarded if the the study employed a 2 x 2 single-dose crossover design involving only healthy volunteers; the drug in question was not an endogenous substance, the elimination period was deemed adequate, the pre-dose samples demonstrated no detectable drug levels, and the study met all scientific and statistical criteria, which were in accordance with current legislation[1].

Conclusion

This study was successfully conducted to evaluate the bioequivalence of Betamethasone in 36 human volunteers from both genders after intramuscular injection and under fasting conditions. The results demonstrated the bioequivalence between the test formulation (5.0 mg/mL betamethasone dipropionate + 2.0 mg/mL betamethasone disodium phosphate) and the reference formulation (Diprospan®). The test formulation (5.0 mg/mL betamethasone dipropionate + 2.0 mg/mL betamethasone disodium phosphate, injectable suspension, produced by Eurofarma Laboratórios S.A.) was compared to the reference formulation Diprospan® (5.0 mg/mL betamethasone dipropionate + 2.0 mg/mL betamethasone disodium phosphate, injectable suspension, manufactured by Brainfarma Indústria Química e Farmacêutica S/A).

The proposed bioanalytical method was found to be sensitive, robust, and reproducible, enabling the successful determination of plasma levels of the drugs in question. With plasma concentrations over time, it was possible to determine the main pharmacokinetic parameters, including C_{max}, AUC0-t, and AUC_{0-inf}. Based on the IC90, which demonstrated that the ratios of C_{max}, AUC_{0-t}, and AUC_{0-inf} were within the 80-125% interval as set forth in Resolution Anvisa RE 1170/2006, the test drug (manufactured by Eurofarma Laboratórios S/A) and the reference drug (Diprospan®) were determined to be bioequivalent and, therefore, interchangeable[1].

Ultimately, there is a critical need to advocate for the widespread adoption of generic medications as a financially prudent option within public healthcare systems. Bioequivalence studies have consistently shown that generic drugs can be substituted for their brand-name counterparts with confidence, thus guaranteeing the continuity of treatment efficacy.

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Conflict of interest

Excepting for CKA and VE that were associates of the sponsor, the authors declare that there are no conflicts of interest regarding this article.

Collaboration

CS and VMR curated the data, wrote the first draft, and made revisions. CLG validated the method and analyzed the samples.

CKA and VE managed the study. AMS and MAA conducted the clinical step of study. All authors reviewed and approved the final version.

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