

Evaluation of the bioequivalence of two formulations containing the combination of 400 mg of acetaminophen (paracetamol), 4 mg of phenylephrine and 4 mg of chlorpheniramine in capsules: open-label, three-way crossover study, partially replicated in healthy volunteers of both sexes

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This study was carried out in order to compare the relative bioavailability of two different formulations containing 400 mg of acetaminophen + 4 mg of phenylephrine hydrochloride + 4 mg of chlorpheniramine maleate, Test formulation (Cimegripe[®]) and Reference formulation (Resfenol[®]) in 84 healthy volunteers of both sexes under fasting conditions. The study was conducted in a single dose, randomized, open-label, crossover 3-way and partially replicated. The tolerability was evaluated by the monitoring of adverse events and vital signs, results of clinical and laboratory tests. Plasma concentrations were quantified by validated bioanalytical methods using the ultra-performance liquid chromatography coupled to tandem mass spectrometry. The C_{max} , T_{max} , AUC_{0-t} , AUC_{0-inf} , $T_{1/2}$ and Kel pharmacokinetic parameters were calculated from these obtained concentrations. The 90% confidence intervals were constructed for the ratio reference/test from the geometric average of the C_{max} and AUC parameters which were comprised between 80% and 125%. Only the C_{max} parameter of the phenylephrine was applied the scaled average bioequivalence due to the intraindividual coefficient of variation > 30% obtained, thus extending the acceptance limits of the interval. It can be concluded that the two formulations were bioequivalent in terms of rate and absorption extent and thus interchangeable.

Keywords: Acetaminophen. Phenylephrine. Chlorpheniramine. Bioequivalence. Partially replicated crossover. Ultra-high performance liquid chromatography. Mass spectrometry.

INTRODUCTION

Over-the-counter medications do not need a prescription in order to be bought, they stay at pharmacy and drugstore counters, making self-medication easier. In general, medicines for the treatment of common cold and flu symptoms are freely mass-marketed to all the population (Eccles, 2014).

Acetaminophen (paracetamol) is a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of *p-aminophenol* derivatives, with analgesic and antipyretic activity (Kalantzi *et al.*, 2006; Guzman *et al.*, 2016). Its analgesic action consists in inhibiting the synthesis of prostaglandins inside the central nervous system (CNS), besides acting as a peripheral blocker of pain impulse generation. However, its antipyretic effect is due to the inhibition of the hypothalamic thermal regulation center, causing peripheral vasodilation, sweating and heat dissipation (Anderson *et al.*, 1998; Sebben *et al.*, 2010). It is a drug rapidly absorbed by

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the gastrointestinal tract, and its absolute bioavailability ranges from 62% to 89%, reaching the maximum plasma concentration of about 0.17 to 1.2 hours after its intake. Acetaminophen is distributed in most tissues and its binding to plasma proteins is negligible at therapeutic concentrations. Its half-life elimination is reported between 1.0 to 4.3 hours (Kalantzi *et al.*, 2006; Guzman *et al.*, 2016).

Phenylephrine hydrochloride is an alpha-adrenergic agonist, being vasoconstriction, redistribution of local blood flow and reduction of nasal mucosa edema some of the main effects of these receptors activation, therefore it is indicated for the temporary relief of nasal congestion (Resfenol, 2014; Gelotte, Zimmerman, 2015). The most peak plasma concentration is obtained from 0.5 to 2.0 hours after oral administration, then it is a quickly absorbed drug. It rapidly distributes itself into peripheral tissues and undergoes first-pass metabolism producing a biphasic pharmacokinetic profile. The half-life elimination period is short, about 2.5 hours and the oral bioavailability is relatively low, about 38% (Gelotte, Zimmerman, 2015).

Chlorpheniramine maleate is an antihistamine that acts as the first generation H1 receptor antagonist, the most potent in the alkylamines group. Used as an antiallergic, it is slowly absorbed from the gastrointestinal tract, its peak plasma concentration is from 2.5 to 6.0 hours after oral administration. About 70% of the chlorpheniramine present in the circulation is bound to plasma proteins, consequently it is widely distributed throughout the body (Resfenol, 2014). The half-life elimination reported in the literature ranges from 18 to 43 hours (Yasuda *et al.*, 2002).

The combination of acetaminophen, phenylephrine and chlorpheniramine is often used as active ingredients in medications for the symptomatic treatment of flu and common cold due to its pharmacological activities described above (Eccles *et al.*, 2014; Khoshayand *et al.*, 2010; Picon *et al.*, 2013). Although, they have been available for over 50 years and have been used by millions of people, the pharmacokinetic data for phenylephrine and chlorpheniramine have been limited or nonexistent (Atkinson *et al.*, 2015, Gelotte, Zimmerman, 2015, Yasuda *et al.*, 2002).

Bioequivalence was defined as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered

at the same molar dose under similar conditions in an appropriately designed study. Bioequivalents. Two pharmaceutical products are therapeutically equivalent if they are pharmaceutically equivalent and after administration in the same molar dose their effects, with respect to both efficacy and safety, will be essentially the same as can be derived from appropriate studies bioequivalence. Therapeutically equivalent drug products are interchangeable (Brasil, 2002).

Pharmacokinetic parameters were calculated from the plasma concentrations of healthy volunteers at predetermined times and the analytes were quantified by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UPLC - MS/MS).

In Brazil, relative bioavailability tests are required for the registration of similar medicinal product which use the bioequivalence criterion. The drug that meets the requirements of pharmaceutical equivalence, dissolution profile, bioequivalence and quality is considered a therapeutic equivalent of the reference medicine and can be interchangeable with it, presenting the same performance in the body, with the same efficacy and safety (Brasil, 2003; Porta, Chang, Storpirtis, 2005).

Thus, the objective of this study was to evaluate the relative bioavailability/bioequivalence for the registration of the similar drug acetaminophen 400 mg + phenylephrine hydrochloride 4 mg + chlorpheniramine maleate 4 mg (Cimegripe®, test product) produced by Cimed Indústria de Medicamentos Ltda., Brazil versus acetaminophen 400 mg + phenylephrine hydrochloride 4 mg + chlorpheniramine maleate 4 mg (Resfenol®, reference product), produced by Kley Hertz S.A. Indústria e Comércio, Brazil. Unlike the traditional (crossover 2x2) average bioequivalence, mostly used along the majority of studies of this nature, the proposed design is the 3x3 crossover with the replicated reference drug, such a design is applied well to drugs with high variability and is suitable for bioequivalence studies.

SUBJECTS AND METHODS

Subjects and Ethics

The study included healthy volunteers of both sexes, aged 18 to 49 years old (mean \pm sd; 32.44 \pm 8.51) and body mass index (BMI) between 18.75 and 29.62 kg/m² (mean \pm SD, 24.34 \pm 2.66). 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, urine 1, 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 (ICH, 1996; Brasil, 1997; Brasil, 2013; Declaration of Helsinki, 2013).

The project 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: 748,540). 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 Instituto Cláudia Marques de Pesquisa e Desenvolvimento Ltda. (ICMP&D), Pouso Alegre, Brazil.

Study design

The study planning was developed to include healthy volunteers of both sexes under fasting conditions, wherein the drugs administration were in a single dose, randomized, open-label, crossover with 2 treatments, 3 sequences, 3 periods and partially replicated. The study design was 3x3 with the reference replicated medication, generating the following sequences: RTR, RRT and TRR.

The administration of two capsules of each product (test and reference) was initiated, regarding the list of randomization, at 07:00 am, after a fasting period of 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 10 hours (supper) after

the drug intake in each treatment. The volunteers did not have alcoholic beverage, coffee, drinks containing xanthenes, or foods outside the prescribed diet during the study. Each confinement period lasted 26 hours on average and the washout period between these administrations was of seven days.

Tolerability

For tolerability monitoring purposes, the volunteers were supervised throughout the study in order to detect adverse events. The volunteers were questioned about the adverse events occurrence at the time of confinement, prior to the products administration under investigation and during the study follow-up in each period. In addition, all subjects were advised to report any adverse event occurrence immediately to the team. During hospitalizations, blood pressure, pulse and body temperature were monitored at predefined times. At the end of the study, the results of the laboratory tests (except serology) and ECG were repeated and evaluated during the medical appointment in a way that was comparable to those obtained during the volunteer selection phase.

Sample collection and processing

The definition of the number of collections and the interval from obtaining the blood samples to constructing the concentration curves versus time were based on the pharmacokinetic profile of the drugs: acetaminophen, phenylephrine hydrochloride and chlorpheniramine maleate. We used the collection schedule truncated in 72 hours for the chlorpheniramine drug that has half-life greater than 24 hours. Before the administration of the investigational products, baseline blood sampling was performed and blood samples were collected at the following times: 0.16, 0.33, 0.50, 0.66, 0.83, 1.0, 1.25, 1.50, 1.75, 2.0, 2.33, 2.66, 3.0, 3.50, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0, 24.0, 48.0 and 72.0 hours in 7.5 ml tubes containing heparin as anticoagulant. Plasma was immediately separated by centrifugation at 3500 rpm for 10 minutes at 4 °C and stored in cryogenic tubes at -70 °C (ultra-freezer) until its analysis time.

Plasma samples analysis

Three different bioanalytical methods were developed and validated according to RDC n° 27/12 (ANVISA, 2012) for the plasma quantification of

the active components of acetaminophen (AMP), phenylephrine (PHEN) and chlorpheniramine (CHL) by liquid chromatography coupled to tandem mass spectrometry based on previously published methods (Feng *et al.*, 2013; Li *et al.*, 2010; Moreno *et al.*, 2010; Ptáček *et al.*, 2007; Sebben *et al.*, 2010).

The analytical run contained samples of quality controls (QCs), calibration standards, and unknown samples from one or more study volunteers. The samples analysis for each analyte in the biological matrix was completed within the time period for which the stabilities were determined. All samples from the same volunteer were analyzed in the chromatographic run. In the routine use of the validated analytical method, its precision and accuracy were regularly monitored through QCs samples to ensure satisfactory performance continuity. The QCs samples were incorporated at appropriate

intervals depending on the total number of run samples, always on an equal number of replicates of each high, middle and low levels (HQC, MQC and LQC).

Chromatographic analyses were performed on a liquid chromatograph of the Acquity UPLC System (Waters Corporation, MA, USA) coupled to a mass spectrometer (MS/MS), Mass Spectrometer Acquity TQ Detector (Waters Corporation, MA, USA) for acetaminophen and chlorpheniramine drugs and coupled to the Xevo TQS equipment (Waters Corporation, MA, USA) for phenylephrine analysis. An electrospray ionization (ESI) with multiple reaction monitoring (MRM) mode was used to monitor the precursor-product ion transitions and triple quadrupole (Tandem Quadrupole) analyzer using the MassLynx™ software (Waters Corporation, MA, USA) in version 4.1. Table I shows the methods description used for the samples analysis.

TABLE I - Bioanalytical methods for the drugs acetaminophen, phenylephrine and chlorpheniramine

ITENS	AMP	PHEN	CHL
Internal standard	50 µL venlafaxine (50 µg/mL)	25 µL propranolol (10 ng/mL)	25 µL bromodiphenhydramine (300 ng/mL)
Biological matrix	100 µL of plasma	500 µL of plasma	200 µL of plasma
Extraction	1.50 mL ethyl acetate	1.25 mL of ethyl ether: dichloromethane (70:30; v:v)	1.00 mL of ethyl acetate: hexane (1:1; v:v)
Column	Waters Acquity UPLC BEH C8 1.7 µm (2.1 x 50.0) mm	Waters Acquity Cortecs UPLC HILIC 1.6 µm (2.1 x 75.0) mm	Waters Acquity UPLC BEH C18 1.7 µm (2.1 x 50.0) mm
Guard cartridge	VanGuard UPLC BEH C8 1,7 µm (2.1 x 5.0) mm	VanGuard UPLC BEH HILIC 1.7 µm (2.1 x 5.0) mm	VanGuard UPLC BEH C18 1.7 µm (2.1 x 5.0) mm
Oven temperature	35°C ± 5 °C	30°C ±5 °C	35°C ± 5 °C
Transition	AMP 152.10 > 110.10 IS 278.24 > 121.07	PHEN 168.13 > 150,10 IS 260.10 > 116,14	CHL 275.16 > 230.10 IS 334.10 > 165.15
Wash solution	Strong Needle Wash: methanol : Ultrapure water (90:10, v/v) Weak Needle Wash: metanol: Ultrapure water (70:30, v/v) + 0,05% ammonium hydroxide Seal Wash in: Ultrapure Water : methanol ((90:10, v/v)	Strong Needle Wash: acetonitrile Weak Needle Wash: acetonitrile: acetonitrile: 20 mM ammonium formate solution + 0.1% acetic Acid (90:10, v/v) Seal Wash in:Ultrapure Water : methanol (90:10, v/v)	Strong Needle Wash: methanol Weak Needle Wash: methanol: Ultrapure Water(80:20, v/v) Seal Wash in: Ultrapure Water : methanol (90:10, v/v)

(continuing)

TABLE I - Bioanalytical methods for the drugs acetaminophen, phenylephrine and chlorpheniramine

ITENS	AMP	PHEN	CHL
Mobile fase	methanol (HPLC level) : Ultrapure water + 0,05% de ammonium hydroxide (70:30, v/v), separate channels	acetonitrile (HPLC level): acetonitrile (HPLC grade): 20 mM ammonium formate solution + 0.1% acetic acid (89:11, v/v), separate channels	methanol (HPLC level) : 10mM ammonium formate solution (80:20, v/v), separate channels
Flow of mobile fase	0.300 mL/min	0.350 mL/min	0.250 mL/min
Injection volume	2.0 µL	7.5 µL	4.0 µL
Auto-injector temperature	5°C ± 4 °C	5°C ± 3 °C	10°C ± 3 °C
Linearity	300 a 15000 ng/mL	50 a 20000 pg/mL	400 a 30000 pg/mL
Retention time	PCT: 0.48 min IS: 1.40 min	PHEN: 3.61 min IS: 2.22 min	CHL: 0.69 min IS: 0.85 min
Limit of quantification	300 ng/mL	50 pg/mL	400 pg/mL
Low quality control	900 ng/mL	150 pg/mL	1200 pg/mL
Mediam quality control	7500 ng/mL	10000 pg/mL	15000 pg/mL
High quality control	11500 ng/mL	1500 pg/mL	22500 pg/mL

AMP: acetaminophen; PHEN: phenylephrine; CHL: chlorpheniramine; IS: internal standard.

Pharmacokinetic and Statistical Analysis

All pharmacokinetic and statistical analyses were performed using Phoenix WinNonlin 6.4 software version (Pharsigh Corporation, NC, USA). The following pharmacokinetic parameters were determined after the quantification of plasma concentrations performed in the analytical stage and the non-compartmental kinetic model application:

C_{max} = Maximum plasma concentration detected after each treatment (Test and Reference).

T_{max} = Time corresponding to maximum plasma concentration.

AUC_{0-t} = Area under the concentration curve versus time from zero to the last experimentally determined concentration.

AUC_{0-inf} = Area under the concentration curve versus time from zero to infinity.

$T_{1/2}$ = Terminal elimination half-life..

K_{el} = Constant rate of terminal phase.

The pharmacokinetic parameters employed in the relative bioavailability/bioequivalence analysis were C_{max} and AUC. C_{max} was obtained directly from the plasma concentration versus time curve and the AUC was calculated using conventional trapezoidal rule. The traditional criterion of average bioequivalence

was used, thus, a 90% confidence interval (CI) was constructed for the difference of log-transformed data averages of test drugs and reference, for C_{max} and AUC_{0-t} parameters. The antilogarithm of the obtained CI constituted the 90% CI for geometric averages ratio of these parameters: (C_{max} test/C_{max} ref and AUC_{0-t} test/AUC_{0-t} ref). The formulations were considered statistically bioequivalent if these ranges were between 80% and 125% limits.

The scaled bioequivalence average can be applied only to highly variable drugs, where the intraindividual coefficient of variation is greater than 30% (CV_{wr} > 30%), as it was the case with phenylephrine. Due to the lack of information regarding chlorpheniramine drug the stepped bioequivalence average could also be applied to this drug as long as the conditions established above were satisfied. According to the World Health Organization (WHO, 2017) for highly variable drugs, it is recommended a three-way partial replication, where the reference drug is administered twice, and to use the scaled bioequivalence technique that allows the IC of 90% for C_{max} parameter if high intra-subject variability for C_{max} after replicate administrations of the reference product was > 30%. If this were the case, the acceptance criteria for C_{max} could be extended to the maximum of 69.84 - 143.19%.

Thus, a 3x3 design (three periods and three sequences) with the replicated reference product was generated, providing the sequences: Reference/Test/Reference (RTR), Reference/Reference/Test (RRT) and Test/Reference/Reference (TRR).

The widening extent of the interval for C_{max} is defined based on the intra-subject variability seen in the bioequivalence study using scaled bioequivalence average according to $[U, L] = \exp[\pm k \text{ sWR}]$, where U is the upper limit of the acceptance range, L is the lower limit of the acceptance range, k is the regulatory constant set to 0.760 and sWR is the standard deviation of the log-transformed values of the reference product C_{max} (WHO, 2017).

RESULTS

Subjects and Tolerability

The study was completed with 77 volunteers (38 men and 39 women), which are part of the statistical analyses. Of the seven volunteers who dropped out of the study, four did not attend the second period of

confinement and three did not attend the third period for personal reasons.

The most common adverse event reported by 11 volunteers was headache. Only 3.77% of the events reported by the volunteers were classified with possible relation with the drugs under study. Of the reported adverse events, 52.83% occurred in the reference drug administration and 47.17% in the test formulation administration, of which 86.79% were of mild intensity.

Both formulations were well tolerated, no serious adverse events were reported or observed during the study. No clinical and laboratory parameters presented clinically relevant alterations following physical examination, vital signs, electrocardiogram and laboratory tests.

Method validation and plasma samples analysis

We used three different quantification methods, one for each drug. The methods were validated by evaluating selectivity, linearity, accuracy, precision, short and long stabilities and matrix effect. In order to determine precision and accuracy of the methods, five replicates of each QC's sample were evaluated. In this study short and long stability tests under ANVISA requirements were performed. The range of the coefficients of variation (CVs) of the data set was used to express the results obtained. Table II summarizes the results.

The concentration calculations of the volunteers' samples were performed according to the calibration curve constructed by acetaminophen, phenylephrine and chlorpheniramine analytes and obtained using MassLynx™ software (Waters Corporation, MA, USA) from the calibration standards. These functions are calculated by the ratio between the peak areas of the analytes under study and respective peaks of internal standards.

TABLE II - Parameters obtained in validations of the bioanalytical methods for determination acetaminophen, phenylephrine and chlorpheniramine

Parameters	AMP	PHEN	CHL
Linearity	300 – 15000 ng/mL	50 – 20000 pg/mL	400 – 300000 pg/mL
Straigh-line equation	$y = 0.000323x + 0.0143$	$y = 0.000193x + 0.00284$	$y = 0.0000606x + 0.00368$
Linear correlation coefficient	$r = 0.998363$	$r = 0.999450$	$r = 0.999009$
Precision intra-day	0.8 – 10.5%	0.5 – 12.9%	1.2 – 7.8%
Precision inter-day	2.3 – 6.9%	2.7 – 8.5%	3.2 – 6.0%
Accuracy intra-day	– 9.7 – 11.8%	– 16.1 – 0.4%	– 14.5 – 6.3
Accuracy inter-day	– 9.2 – 6.6%	– 8.1 – 2.0%	– 10.0% – 3.8%
Short-term stability			
Autosampler samples, 12h at 5°C	– 13.6 – 7.0% (12h at 5°C)	– 8.3 – 1.0%	– 3.9 – 0.4
Room sample, 6 h at 25°C	– 11.0 – 9.8%	– 13.0 – 3.8%	– 6.6 5.3
4 Freeze-thaw cycles	– 11.7 – 6.9%	– 13.0 – 5.2%	– 4.3 4.5
Long-term stability			
Plasma sample (storage at -70 °C)	– 11.0 – 6.9% (122 days)	– 13% – 2.8% (70 days)	– 4.3 – 6.0 (107 days)
FMN (Normalized matrix factor for analyte and IS) AMP: acetaminophen; phen: phenylephrine; Chl: chlorpheniramine	3.5%	10.0%	5.1%

Pharmacokinetic parameters and Bioequivalence evaluation

The average plasma concentration-time profiles of 77 healthy volunteers in single dose oral administration with 800 mg of acetaminophen, 8 mg of phenylephrine and 8 mg of chlorpheniramine in gel capsules (two) are shown in Figures 1A, 1B and 1C, respectively. The curves demonstrated similar absorption, distribution and elimination for both products: reference (R) and test (T).

The main pharmacokinetics parameters were summarized in Table III. Table IV reports the confidence intervals for the test/reference ratio (90% CIs) of log-transformed C_{max}, AUC_{0-t}, AUC_{0-inf}.

The CV_{wr} for phenylephrine in C_{max} parameter obtained in the study was equal to 57.29%, and thus it was applied to scaled bioequivalence. Table V shows the ratio between the geometric averages, confidence intervals, variation coefficient of the reference medication and the acceptance limits extension for phenylephrine in C_{max} parameter.

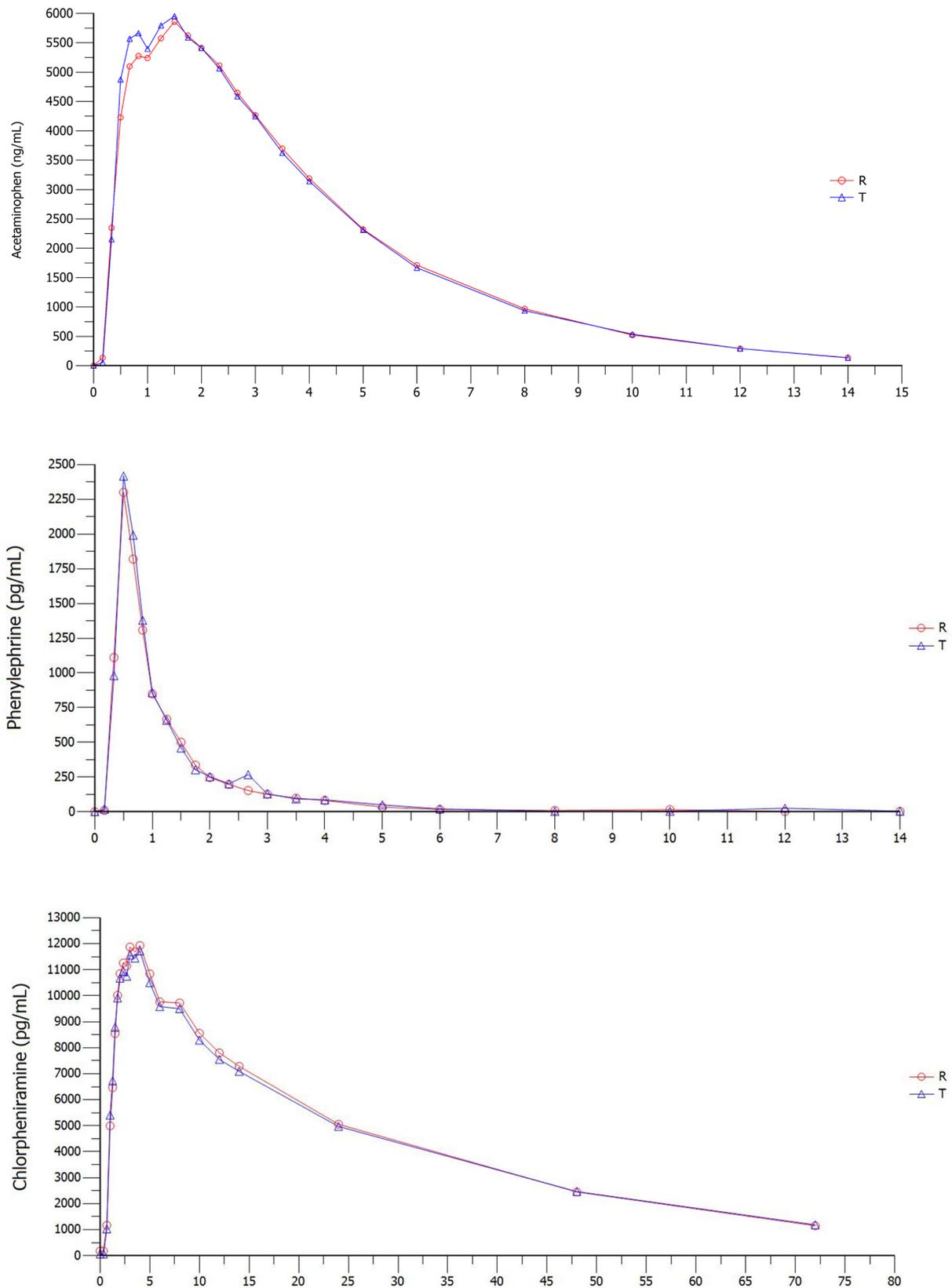


FIGURE 1 - Average plasma concentrations versus time profile obtained after a single oral administration of drugs containing acetaminophen (A), maleate of phenylephrine (B) and chlorpheniramine (C) in healthy volunteers.

TABLE III - Pharmacokinetics parameters of acetaminophen, phenylephrine and chlorpheniramine after single dose in healthy volunteers (n = 77), concerning test/reference treatments

Drugs	AMP		PHEN		CHL	
	R (n = 154)	T (n = 77)	R (n = 154)	T (n = 77)	R (n = 154)	T (n = 77)
Pharmacokinetics parameters	(mean ± SD)		(mean ± SD)		(mean ± SD)	
C _{max} (ng/mL)	8116.689 ± 236.754	8347.459 ± 332.199	3.271 ± 0.195	3.470 ± 0.247	13.630 ± 0.318	13.359 ± 0.414
T _{max} (h)	1.314 ± 0.060	1.203 ± 0.082	0.779 ± 0.037	0.736 ± 0.045	3.024 ± 0.119	2.949 ± 0.150
AUC _{0-t} (ng*h/mL)	27367.318 ± 688.355	27454.247 ± 903.326	2.054 ± 0.079	2.157 ± 0.095	313.358 ± 9.652	309.393 ± 12.690
AUC _{0-inf} (ng*h/mL)	28918.267 ± 710.189	28992.062 ± 941.982	2.245 ± 0.083	2.385 ± 0.101	374.998 ± 16.427	368.447 ± 20.098
T _{1/2} (h)	2.598 ± 0.061	2.667 ± 0.083	1.835 ± 0.142	2.379 ± 0.396	21.961 ± 0.813	22.178 ± 1.099
K _{el}	0.286 ± 0.006	0.277 ± 0.008	0.618 ± 0.032	0.552 ± 0.037	0.036 ± 0.001	0.035 ± 0.001

AMP: acetaminophen; PHEN: phenylephrine; CHL: chlorpheniramine; R: product reference; T: product test; SD: standard deviation

TABLE IV - 90% Confidence intervals for the log-transformed parameters ratio test/reference (n=77)

Parameters	AMP		PHEN		CHL	
	Ratio %	90% CI	Ratio%	90% CI	Ratio %	90% CI
C _{max}	103.25	96.93 - 109.98	111.88	98.53 - 127.04	98.16	94.82 - 101.62
AUC _{0-t}	100.81	98.65 - 103.03	107.29	101.36 - 113.57	99.37	95.36 - 103.55
AUC _{0-inf}	100.63	98.58 - 102.73	108.15	102.41 - 114.22	99.65	94.68 - 104.88

AMP: acetaminophen; PHEN: phenylephrine; CHL: chlorpheniramine; CI: Confidence interval

TABLE V - Ratio between geometric means, confidence intervals, reference coefficient of variation and extension of the acceptance limits for phenylephrine in parameter C_{max}

Ratio (%)	ABE 90% CI Lower	ABE 90% CI Upper	CV _{wr} (%)	Extended Lower Bound	Extended Upper Bound
111.88	98.53	127.04	57.29	69.84	143.19

ABE: Average Bioequivalence; CI: Confidence interval; CV_{wr}: coefficient of variation intra-subject of the reference product

DISCUSSION

The efficacy, safety and quality evidences of over-the-counter drugs in Brazil aim to ensure that individuals who choose self-medication have access to safe and effective medication.

The results obtained by Picon *et al.*, (2013) suggest that the fixed dose combination of acetaminophen, phenylephrine and chlorpheniramine may be an effective and safe alternative for the symptomatic treatment of common cold and flu. Combination products for multiple symptoms relief containing various active ingredients provide a safe, effective, economical and convenient way of treating multiple symptoms when used according to instructions. Both formulations were well tolerated and all volunteers who completed the study had no significant adverse events.

In the present study, we used three ultra-performance liquid chromatography methods coupled to mass spectrometry (UPLC - MS/MS) to quantify acetaminophen, phenylephrine and chlorpheniramine analytes in plasma and allowed us to evaluate the pharmacokinetic profiles of the active principles in bioequivalence studies. The results of the methods validation indicated that the selectivity, precision, accuracy, short term stability, long term stability and the methods matrix effect obeyed the criteria required by Brazilian law for bioanalysis application.

The analytical methods were simple and fast and no interferences were found from endogenous components of plasma or other sources. It can be seen that the coefficients of variation of the intra-day and inter-day precision did not exceed 15.0%. In the case of accuracy intra and inter-day, the values were within -15.0 and 15.0%. Except for phenylephrine which had the CV of -16.1% in the intra-assay accuracy because the reported values included the lower limit of quantification quality control (LLOQ). The LLOQ should not deviate by more than 20%. Therefore no stability related problems were found during sample analyses. No statistical differences were found in slope or intercept between human plasma samples of volunteers. The bioanalytical methods can also be used for pharmacokinetic and bioavailability studies.

The plasma concentrations of the samples were derived from the linear regression equation of the line using the 1/x² weighting, since it presented the smallest sum of the nominal values relative errors of the calibration standards versus values obtained by their

curve equation. The use of this mathematical model was decided due to the extensive range of concentrations used in the samples quantification. This model is the simplest and best accommodates the inherent variations to the experimental method.

The pharmacokinetic parameters calculated for both the test and the reference formulations were not significantly different, reflecting the comparable pharmacokinetic characteristics of two formulations. The parameters T_{max}, T_{1/2}, C_{max} and AUC_{0-t} for the three drugs which make up the formulations are in accordance with those established in the drug reference paper and in the consulted literature (Kalantzi *et al.*, 2006; Ptáček *et al.*, 2007; Lou *et al.*, 2010; Moreno *et al.*, 2010, Atkinson *et al.*, 2015; Gelotte, Zimmerman, 2015). Observing the statistical results of phenylephrine hydrochloride, it is clear, for the C_{max} parameter, that the upper limit of the 90% confidence interval is outside the 80% - 125% limits. With the use of a suitable design, for this study the RRT, RTR and TRR partially replicated and as the reference product variation coefficient was superior to 30% (CV_{wr} = 57.29%, obtained in the study in question) the scaled bioequivalence could be applied. Thus, the acceptance limits for this parameter were 69.84% - 143.19%. The statistical treatments adopted for it were suitable to guarantee the reliability of the bioequivalence results among the formulations. All 90% confidence intervals for C_{max} and AUC pharmacokinetic parameters are within the limits previously established in protocol.

CONCLUSION

The drugs acetaminophen, phenylephrine and chlorpheniramine were evaluated in both formulations according to the results obtained. The test formulation is statistically bioequivalent to the reference formulation in terms of rate and extent of absorption and therefore interchangeable.

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