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Comparative Bioavailability and Pharmacodynamic Aspects of Cyclobenzaprine and Caffeine in Healthy Subjects and the Effect on Drowsiness Intensity

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Abstract

A specific, fast and sensitive LC–MS/MS assay was developed for the determination of cyclobenzaprine in human plasma using imipramine as the internal standard (IS). The limit of quantification was 0.05 ng/mL and the method was linear in the range of 0.05 to 50 ng/mL. The cyclobenzaprine and IS retention times were 2.74±0.2 min and 2.69±0.2 min, respectively.

Method intra-batch precision and accuracy ranged from 2.90 to 9.72%, and 91.63 to 107.33%, respectively. Inter-batch precision ranged from 3.37 to 10.27%, while Inter-batch accuracy ranged from 96.13 to 106.10%. The analytical method was applied to evaluate the pharmacokinetic and relative bioavailability of two different pharmaceutical formulations containing cyclobenzaprine, one test tablet containing 10 mg of cyclobenzaprine plus 60 mg of caffeine (Miosan®/cafeine) and the reference Miosan® containing only 10 mg of cyclobenzaprine, manufactured by the same pharmaceutical company. In addition to the pharmacokinetic analysis, a pharmacodinamic evaluation of the drowsiness intensity during the confinement periods was conducted in order to evaluate the caffeine effect. This study evaluated 34 subjects in a randomized, 2-period crossover study with 14 days washout period between doses.

Based on the 90% confidence interval of the individual ratios (test formulation/reference formulation) for C_{max} and AUC_{inf}, it was concluded that the test formulation is bioequivalent to the reference Miosan® with respect to the rate and extent of absorption of cyclobenzaprine and that caffeine had no effect on the relative pharmacokinetic parameters. However, based on the Stanford point analysis, the combination of Miosan® with caffeine in the same tablet formulation significantly decreased the drowsiness intensity observed during the confinement periods.

Keywords: Cyclobenzaprine; Caffeine; Pharmacokinetics; HPLC; Mass spectrometry; Bioavailability

Introduction

Cyclobenzaprine hydrochloride is a centrally acting muscle relaxant that has been widely used over the past 30 years for relief of muscle spasm associated with acute, painful musculoskeletal conditions (Borenstein et al., 2003; Browning et al., 2001; Katz et al., 1988; Winchell et al., 2002). Disposition studies in humans and laboratory animals have previously shown that cyclobenzaprine hydrochloride is well absorbed (Hucker et al., 1978), is widely distributed among body tissues (Hucker et al., 1978), is subject to enterohepatic circulation (Wang et al., 1996), and is extensively metabolized via both oxidative and conjugative pathways (Hucker et al., 1978).

It is generally prescribed at a dose of 10 mg three times daily and its bioavailability is 33-55% (4, 5). Cyclobenzaprine pharmacokinetics are linear for doses from 2.5 to 10 mg despite the slight deviations from linearity observed at the 5 mg dose (Winchell et al., 2002). Like other tricyclic antidepressants, cyclobenzaprine is also prescribed off-label as a sleep-aid. The sedative effects of cyclobenzaprine are likely due to its antagonistic effect on histamine H1, serotonin 5-HT2A, and muscarinic acetylcholine receptors. Indeed, some studies have confirmed the improvement in sleep quality. For example, a meta-analysis of five published, randomized controlled trials suggests

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that cyclobenzaprine improves global functioning of patients with fibromyalgia with a modest improvement in sleep quality (Tofferi et al., 2004). In other study conducted with 120 fibromyalgia patients, those receiving Cyclobenzaprine (10 to 40 mg) over a 12-week period had significantly improved quality of sleep and pain score (Bennett et al., 1988).

The plasma concentration of cyclobenzaprine has been determined by methods using performance liquid chromatography with UV detection (HPLC-UV) (Constanzer et al., 1995) and HPLC method coupled to tandem mass-spectrometric detection (Constanzer et al., 1995; Darwish et al., 2009).

Caffeine is the most widely used stimulant to counteract the effects of sleepiness. Consumption from all sources can be estimated to be approximately 210–238 mg/day/ person in Canada and the US (Barone et al., 1996). Caffeine can be used to increase alertness and performance especially in low action situations such as monotonous highway driving or after sleep deprivation. Moderate doses of caffeine reduce reaction time on performance tasks, improve subjective alertness, and diminish self-reported fatigue and sleepiness (Landolt et al., 2004; Reyner et al., 2000; Van Dongen et al., 2001; Wyatt et al., 2004).

The main objectives of this study were to evaluate the relative bioavailability between the reference Miosan® (10 mg of ciclobenzaprine) and the test Miosan®/caffeine (10 mg of ciclobenzaprine + 60 mg of caffeine) formulations, both manufactured by Apsen Farmacêutica S/A (São Paulo, SP, Brazil), and analyze the effect of caffeine in reducing or eliminating the associated side effects of drowsiness caused by cyclobenzaprine. This paper also describes a simple, rapid, sensitive and robust method combining high-performance liquid chromatography (HPLC) and positive electrospray tandem mass spectrometry (HPLC-ESI$^+$/MS/MS) for the cyclobenzaprine quantification in human plasma samples.

Methods

Subjects

Eligible subjects were healthy men or women aged 18 to 50 years with a body weight within 15% of their ideal weight for height (IMC 23.3 ± 1.9 kg, mean ± SD, range 19.7 - 26.6 Kg). The group comprised 34 subjects (31.4 ± 6.5 years, range 21-44 years), height between 1.52 and 1.83 m (1.67 ± 0.1 m), weighing between 50.0 and 85.0 kg (64.1 ± 8.0 kg). Subjects were excluded if they had participated in any investigational trial within the previous 4 months; were pregnant or lactating; had a history of substance abuse or recent excessive alcohol consumption; or had hypersensitivity to cyclobenzaprine or any of its excipients. Subjects also were excluded if they were currently using any regular medication within 14 days prior to the first intended dose, except oral contraceptives; or if they tested positive for hepatitis B, hepatitis C, or HIV. Subjects were excluded if they exhibited any concurrent or recent medical condition that might interfere with their ability to participate in the study (eg, hyperthyroidism, uncontrolled cardiac problems, urinary retention, glaucoma, recent surgery); or if they were hospitalized within 8 weeks before the beginning of the study.

Written informed consent was obtained from all subjects before screening.

Study Protocol and Procedures

This was a randomized, 2-period crossover study with 14 days washout period between doses conducted in Campinas, SP, Brazil and in compliance with the provisions of the Declaration of Helsinki (1964), Tokyo (1975), Venice (1983), Hong Kong (1989), Somerset West (1996), Edinburgh (2000) revisions and the Resolutions No.196/96 and 251/97 of National Health Council – Health Ministry, Brazil. The clinical protocol was also approved by the State University of Campinas Independent Ethic's committee and all participants provided written informed consent.

During each period, the subjects were hospitalized at 6:00 p.m. having an evening meal at 8:30. After an overnight fasting period, the medication started at 7:00 a.m. All doses were administered with 200 mL of water after a 4-hour fast. All subjects received a single tablet orally corresponding to the test formulation Miosan®/caffeine (MC) formulation containing 10 mg of ciclobenzaprine + 60 mg of caffeine or the reference formulation Miosan® (M) containing only 10 mg of ciclobenzaprine, both produced by Apsen Farmacêutica S/A (São Paulo, SP, Brazil).

All subjects were required to remain fasting at least for four hours after dose when a standard meal was provided after five (lunch), eight (snack) and twelve hours after dosing (evening meal). No other food intake was permitted during the "in-house" period. Liquid consumption was permitted *ad libitum* six hours before and two hours after drug but caffeine and/or xanthine-containing drinks including tea, coffee, and cola were prohibited. Food was also xanthine-free. Smoking was prohibited during the "in-house" period. All subjects were requested to stay in the clinic for a 24h period after drug administration.

Venous blood samples (7.5 mL each) for the determination of plasma cyclobenzaprine concentrations were collected from an indwelling catheter or by direct venipuncture of an antecubital vein into tubes containing heparin as the anticoagulant. Samples collected for cyclobenzaprine quantification (test and reference formulations) were collected at the following times: 30 min before dosing and 0.33, 0.67, 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 14, 24, 48, 72, 96, 120, and 144 hours after dosing. Blood samples were placed on ice until centrifuged. Plasma was separated by centrifugation at 3000 rpm for 10 minutes at 2°C. The plasma was subsequently stored frozen at less than -5°C until analyzed. After the evaluations at 48 hours, subjects were discharged from the study center and were asked to return to the site for the collects at 72, 96, 120 and 144 hours.

Sample Analysis

Plasma concentrations of cyclobenzaprine were determined by a validated high-performance liquid chromatography (HPLC) method with tandem mass-spectrometric detection in an Applied Biosystems API 5000 (Concord, Ontario, Canada) using ESI+ (positive Electron Spray Ionization). Standard solutions for the analytes were prepared using cyclobenzaprine HCl (European Pharmacopeia, Batch# 1) and imipramine as the internal standard (USP, Batch# 1). Aliquots of 50 µL of plasma samples were mixed with 400 µL of internal standard working solution (Imipramine 10 ng/mL) in acetonitrile and vortex-mixed for 15 seconds. Next, samples were centrifuged at 5000 rpm for 3 min-

utes. The supernatant was transferred to a clean tube and 600 µL of AcN-H_2O) (1:1) were added. After homogenization, 100 µL were transferred to a 96-well plate for injection.

Chromatographic separation was performed by an Agilent model 1100 HPLC system (Santa Clara, CA, USA) using a C18 4.0 x 3 mm Phenomenex (Torrance, CA, USA) guard column and a Agilent Zorbax Eclipse XDB (Santa Clara, CA, USA) C8 150 x 4.6 mm (5µm) column, under a flow of 0.80 mL/min.

The mass spectrometer equipped with electrospray ionization (ESI) source was operated in the positive ion mode (ES+) and multiple reactions monitoring (MRM) mode. The tuning parameters were optimized for cyclobenzaprine and imipramine by infusing the standard solution of each compound into the stainless steel sample capillary of the electrospray source. The capillary voltage was set to 4000 V and Nitrogen was used as drying gas for solvent evaporation at 600°C. The collision energies were 57 and 55 eV for cyclobenzaprine and imipramine, respectively. Based on the full scan MS/MS spectrum of each drug, the most abundant ions were selected and the mass spectrometer was set to monitor the transitions of the precursors to the product ions. The mass transitions monitored were 276.3 > 215.1 for cyclobenzaprine and 281.2 > 193.0 for imipramine, respectively. The dwell time was set to 225 msec for both transitions.

The standard calibration curves were constructed using the peak area ratios of cyclobenzaprine and IS versus the cyclobenzaprine nominal concentrations of the eight plasma standards (0.05, 0.10, 0.50, 1.00, 5.00, 10.00, 30.00 and 50.00 ng/mL) in duplicate. Linear least-square regression analysis, with weighting factor of $1/x^2$, was performed to assess the linearity, as well as to generate the standard calibration equation: $y = ax + b$, where y is the peak–area ratio, x the concentration, a the slope and b is the intercept of the regression line. In addition, a blank (non-spiked sample) and a zero plasma sample (only spiked with IS) were run to demonstrate the absence of interferences.

Pharmacokinetic Analysis

Bioequivalence between the two formulations was assessed by calculating individual test/reference ratios for the peak of concentration (C_{max}), area under the curve (AUC) of plasma concentration until the last concentration observed (AUC_{last}) and the area under the curve between the first sample (pre-dosage) and infinite (AUC_{inf}). C_{max} and the time taken to achieve this concentration (T_{max}) were obtained directly from the curves. The areas under the cyclobenzaprine plasma concentration versus time curves from 0 to the last detectable concentration (AUC_{last}) were calculated by applying the linear trapezoid rule. Statistical calculations were defined at the level of $P \leq 0.05$ and bioequivalence for test and reference formulations was concluded as the 90.0% confidence interval for C_{max}, AUC_{last} and AUC_{inf} within the range of 80.0–125.0% defined by both the Food and Drug Administration (FDA) and the National Sanitary Surveillance Agency (ANVISA). The software used included Winnonlin® 2.0, MS Excel® 2003, Graph Pad Prism v 4.

Pharmacodynamic Aspects

The comparison of the pharmacodinamic aspects after administration of both test (MC) and reference (M) formulations was studied by the evaluation of the drowsiness intensity during the confinement.

Initially, the residual interference between the two periods of confinement was evaluated; then, the direct effect of the treatment over drowsiness intensity was determined. Finally, the time correlation with the formulation administration was also evaluated using three different time observations. The intensity of the effect was quantified based on the Stanford Sleepiness Scale at 1:30h (T1), 3:30h (T2) and 5:00h (T3) after drug administration. In order to verify the residue interference between periods, the mean values obtained from the Stanford Sleepiness Scale on T1, T2 and T3 were calculated for each group in each confinement period. The residue effect over confinement was calculated using the ANOVA test with two factors (group and confinement), considering that the factor confinement contained repeated measures.

In addition, a brief survey questionnaire consisting of a few questions about the drowsiness effect in each study period was applied. In this case, the comparison between the formulations was performed using the chi-square analysis to study the response proportion for each treatment. The analysis were performed using the software Minitab 15.0 with a significance level of 5% (α=0.05).

Results

Method Validation

The simplest regression method for the calibration curves of the cyclobenzaprine was $Y = a + bx$ from 0.05 to 50 ng/mL and the correlation coefficient ranged from 0.9951 to 0.9983 (mean = 0.9974).

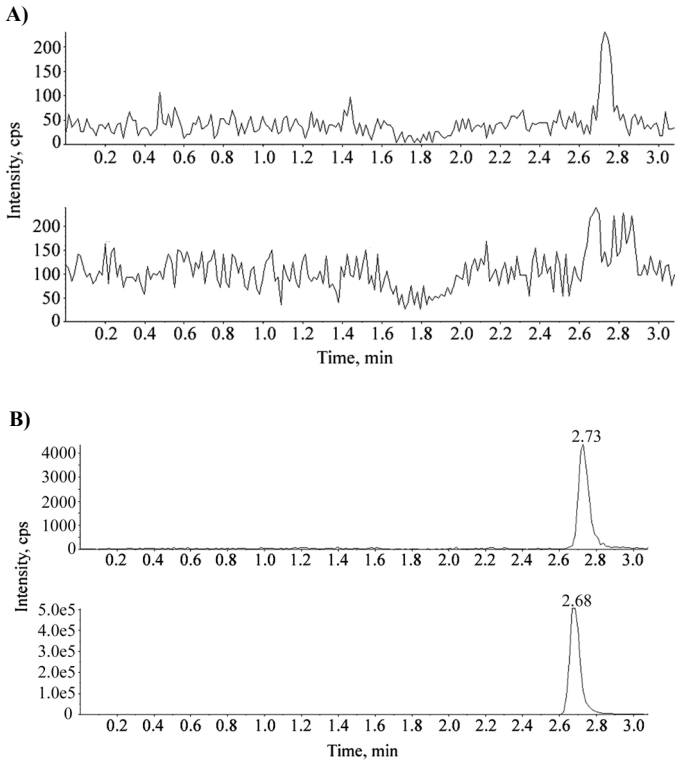

Figure 1: Cyclobenzaprine and imipramine chromatograms after extraction from human plasma. The mass transitions monitored for the analytes were 276.3 > 215.1 for cyclobenzaprine (upper panel) and 281.2 > 193.0 for imipramine (lower panel). The analysis was performed in extracted blank plasma (a) or in spiked blank plasma at cyclobenzaprine LLOQ (0.05ng/mL) concentration (b).

The chromatograms obtained from LLOQ (0.05 ng/mL) and extracted blank plasma are presented in Figure 1. The cyclobenzaprine and IS retention times were 2.74±0.2 min and 2.69±0.2 min, respectively. The signal-to-noise ratio was higher than 7.

In the case of cyclobenzaprine and its IS there was no significant ion suppression in the retention times where they are eluted. There was no suppression when the analysis was performed using blank normal plasma (Figure 2).

After analysis of the three QC levels (0.15, 20 and 40 ng/mL), cyclobenzaprine showed the mean recovery (values ± CV(%), n=5) of 82.27 ± 14.97% and the IS recovery was 92.43 ± 5.5%.

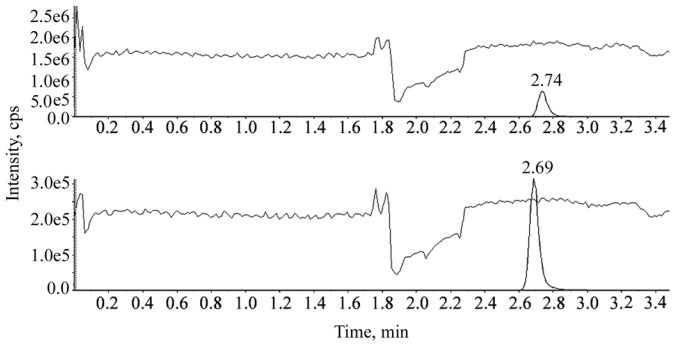

Figure 2: Ion suppression in normal blank plasma. The mass transitions monitored for the analytes were 276.3 > 215.1 for cyclobenzaprine (upper panel) and 281.2 > 193.0 for imipramine (lower panel).

Intra-batch precision and accuracy of the assay was measured for cyclobenzaprine at each QC level as presented in Table 1. These results were within the acceptance criteria for precision and accuracy, i.e., deviation values were within ±15% of the nominal values, except for the LLOQ which could show a ±20% deviation.

Stability tests indicated that there was no significant degradation of the stock solution. The variation between fresh and stored samples was -8.0 and 7.9% for cyclobenzaprine and IS respectively, after 1 day at 4.0 ± 2.0°C. In addition, the variations between fresh and frozen samples after 8 days were 1.4 and 3.7% for cyclobenzaprine and IS, respectively.

The stability of cyclobenzaprine was assessed in spiked human plasma and this analyte showed no significant degradation after 7h at room temperature, three freeze/thaw cycles, 48h post processing or 192 days at -20°C (Table 2).

Comparative Pharmacokinetic Study

Cyclobenzaprine was well tolerated at the administered doses and no significant adverse reactions were observed or reported. No clinically relevant changes were observed in any measured biochemical parameter. A total of 34 subjects finished the study. The mean cyclobenzaprine plasma concentration versus time curves obtained after a single oral dose of each formulation is shown in Figure 3. The plasma concentration of cyclobenzaprine

QC samples	Nominal concentration (ng/mL)	Intra-run accuracy[a] (%)	Inter-run accuracy[b] (%)	Intra-run precision[c] (%CV)	Inter-run precision[b] (%CV)
QC-LLOQ	0.05	91.63	96.13	9.72	10.27
QCL	0.15	100.29	101.78	4.62	7.22
QCM	20.0	107.33	106.10	2.90	3.37
QCH	40.0	106.36	104.18	6.14	4.92

[a](n=6), expressed as (found concentration / nominal concentration) x 100
[b]Values obtained from all 3 runs (n=18)
[c]n=6

Table 1: Accuracy and precision data for cyclobenzaprine quantification in human plasma.

	Initial mean conc. (ng/mL)	% CV	Final mean conc. (ng/mL)	% CV	Variation (%)
Freeze/thaw stability test (3 cycles)					
QCL	0.16	4.2	0.14	6.5	-10.9
QCH	45.38	2.5	38.64	3.5	-14.8
Short Term stability test (7h)					
QCL	0.16	3.9	0.16	3.6	-0.5
QCH	38.64	1.7	38.40	1.8	-0.6
Post Processing stability test (48h)					
QCL	0.16	3.9	0.16	4.4	1.8
QCH	38.64	1.7	38.26	1.7	-1.0
Long Term stability test (192 days, -20 °C)					
QCL	0.16	1.7	0.14	5.9	-13.5
QCH	42.54	3.9	42.24	3.8	-0.7

n=5 for each test.
QCL = 0.15 ng/mL; QCH = 40 ng/mL

Tabel 2: Stability tests of cyclobenzaprine in human plasma.

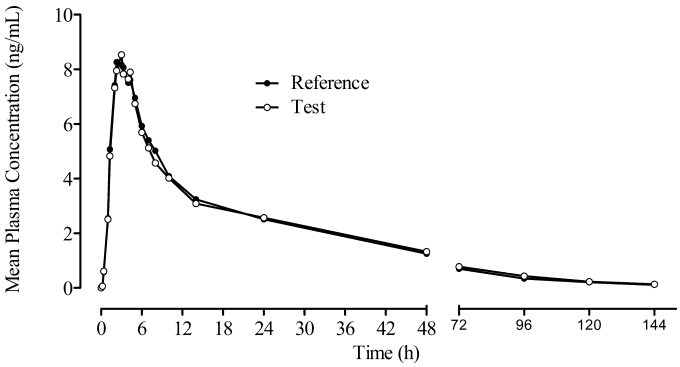

Figure 3: Cyclobenzaprine comparative pharmacokinetics. Mean cyclobenzaprine plasma concentration versus time curves obtained after a single oral dose of test (Miosan®/caffeine) and reference (Miosan®) formulations to 34 subjects.

	Test formulation		Reference formulation	
	Mean	SD	Mean	SD
C_{max} (ng/mL)	9.38	3.14	10.08	3.29
T_{max} (h)	3.04	1.03	3.25	1.05
$T_{1/2}$ (h)	26.46	6.44	26.08	7.63
AUC_{last} ([ng x h]/mL)	189.59	65.54	191.51	71.31
AUC_{inf} ([ng x h]/mL)	197.84	73.29	198.13	77.46

Table 3: Arithmetic mean pharmacokinetic parameters obtained from 34 volunteers after administration of the test formulation Miosan®/caffeine formulation containing 10 mg of ciclobenzaprine + 60 mg of caffeine or the reference formulation Miosan® containing 10 mg of ciclobenzaprine only.

	Parametric (n=34)			
Parameters	Geometric mean (%)	90% CI	Power (%)	CV (%)
AUC_{last} % ratio	98.90	93.17 – 104.99	99	14.60
AUC_{inf} % ratio	99.67	94.12 – 105.54	99	14.00
C_{max} % ratio	92.82	86.47 – 99.63	99	17.37

Table 4: Geometric mean of the individual AUClast, AUC0–inf and *C*max ratios (test/reference formulation) and the respective 90% CIs.

did not differ significantly after administration of both formulations (test formulation and the reference one).

Table 3 shows the values of the pharmacokinetic parameters and Table 4 summarizes the bioequivalence analysis for cyclobenzaprine formulations. Briefly, the geometric mean and respective 90% confidence intervals of cyclobenzaprine test/reference percent ratios were 92.82% (86.47 - 99.63%) for C_{max}, 98.90% (93.17 - 104.99%) for AUC_{last} and 99.67% (94.12 - 105.54%) for AUC_{inf}. These 90% confidence intervals for geometric mean ratios were within the acceptable limits (80 -125%) of bioequivalence. The test and reference formulations were considered bioequivalent.

Pharmacodynamic Aspects

The mean of the drowsiness score obtained in each period was 5.0 for period 1 and 5.1 for period 2. The ANOVA results showed no residue effect throughout the two confinement periods (p=0.716).

In each period, the group that received the test formulation

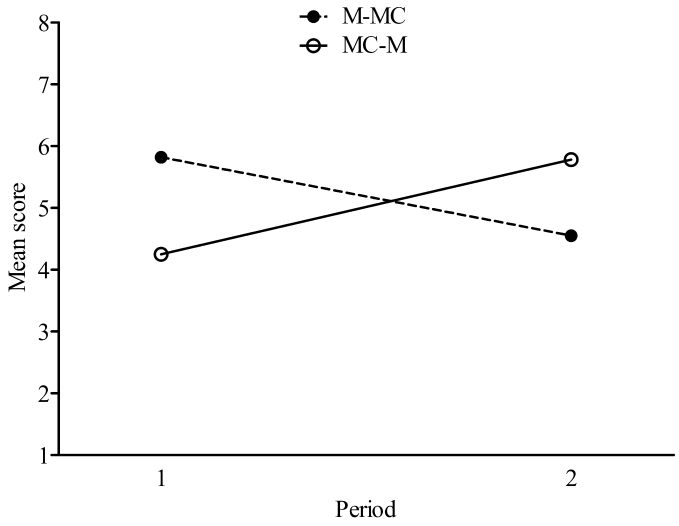

Figure 4: Mean drawsiness score during the confinement periods 1 and 2. Half of the subjects received the test formulation Miosan®/caffeine (MC) or the reference Miosan® (M) in the period 1 and inverted in the period 2. For the statistical purposes subjects were analyzed in two groups, accordingly to the formulation sequence adopted in the confinement periods: MC-M and M-MC.

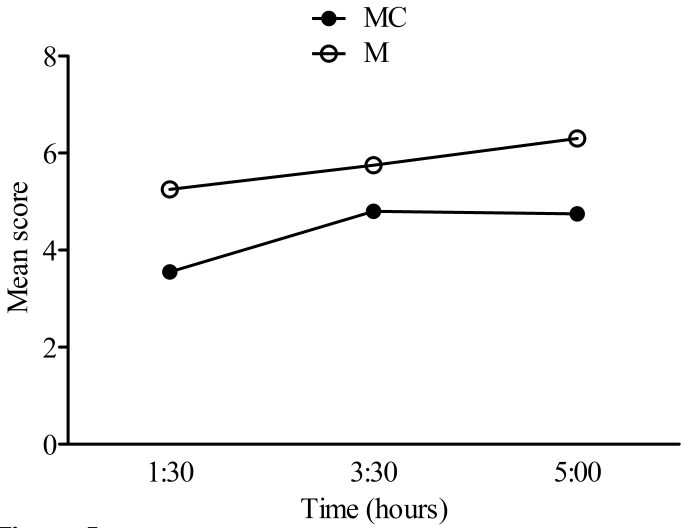

Figure 5: The effect of time on the mean drowsiness score. Half of the subjects received the formulation Miosan®/caffeine (MC) or the Miosan® (M) in the period 1 and inverted in the period 2. Results from period 1 and 2 were grouped for the same formulation and expressed as MC or M scores. Only the drowsiness score observed after 5:00h was significantly (p<0.001) more intense than that observed at 1:30h. The comparison between the closest time points (1:30 to 3:30h and 3:30 to 5:00h) showed no statistical significance.

(Miosan®/caffeine) showed a drowsiness score significantly (p<0,001) lower than that obtained with the group that received the reference Miosan® formulation (Figure 4). The mean score for the subjects group receiving the test formulation was 4.4, while the mean score for the reference was 5.8 Stanford points.

Using the multiple comparison analysis (Turkey method), it was determined that the drowsiness observed after 5:00h was more intense than that observed at 1:30h (p<0.001) (Figure 5). However, the comparison between the closest time points (1:30 to 3:30h and 3:30 to 5:00h) showed no statistical significance.

During the analysis about the subject perception of drowsiness, all 34 subjects were evaluated and 27 (79.4%) informed a more intense drowsiness during the administration of Miosan® formulation and only 6 (17.6%) informed a more intense drowsiness during the administration of Miosan®/caffeine formulation. One subject informed no difference between the periods. These results were statically significant after chi-square analysis (p<0,001).

Disucssion

The LC-MS/MS method described here for drug quantification is in accordance with both Food and Drug Administration (FDA) and the National Sanitary Surveillance Agency (ANVISA) requirements for pharmacokinetic studies.

The sample preparation method described in this work is based on a simple liquid–liquid extraction. Blank plasma samples from all 34 subjects showed a clear chromatogram in all cases. The extraction method was sufficient to providing a clean extracted and a reproducible quantification allied to the high selectivity of the MRM mode on LC–APPI-MS/MS spectrometer. This method offers advantages over those previously reported since it used a lower amount of plasma (50 µl), significantly lower than the 250 µl used by Darwish et al., (2009) and the 1000 µl used by Constanzer et al., (1995). In addition, our method is associated with a faster chromatographic run time (2.5 min) compared to the previously 15 min reported by (Constanzer et al., 1995).

The method provides excellent analytical performance for cyclobenzaprine extraction and proved to be appropriate for analyzing human plasma samples. The reported LLOQ 0.05 ng/mL is lower than the 0.1 ng/mL described by (Constanzer et al., 1995) and the 0.5 ng/mL method described by (Darwish et al., 2009). The reported analytical method has been successfully applied to human pharmacokinetic investigations and bioequivalence was confirmed by the 90% Confidence Interval for the ratios of the C_{max} and AUC_{last} values being within the acceptance range of 80–125%.

After evaluation of the formulations effect on the drowsiness during the pharmacokinetics study, the ANOVA analysis of the drowsiness score obtained in each period showed that there is no residue effect through the two confinement periods (p=0.716), confirming that the results obtained in the second period does not depend on the formulation used in the first confinement period. However, when the direct effect of the treatment over drowsiness was evaluated to clarify the differences between the two formulations, it was observed that there is a statically significant difference (p<0,001) between the groups. In each period, the group that received the test formulation Miosan®/caffeine showed drowsiness score significantly lower than the one obtained with the group that received the reference formulation Miosan®.

In the analysis of the time effect the results showed no interaction between time and formulation (p=0.416), since both formulations presented a similar behavior during the time analyzed. However, a time effect was observed in both formulations, meaning that the analysis performed at the three time points showed different drowsiness levels (p<0.001). However, the statistic significance was observed only between the extreme time points (1:30 and 5:00h). The Stanford point's analysis was corroborated by the evaluation of the subject drowsiness perception. After a direct questioning, a clear statistical significance was obtained (p<0,001) since 79.4% of the subjects declared a more intense drowsiness during the administration of reference formulation containing only cyclobenzaprine and only 17.6% declared a more intense drowsiness during the administration of test formulation containing cyclobenzaprine and caffeine.

The effect of cyclobenzaprine effect on sleep physiology is well described in the literature. For example, Bennett et al., (1988) showed that in a study of 120 fibromyalgia patients, those receiving cyclobenzaprine (10 to 40 mg) over a 12-week period had significantly improved quality of sleep and pain score. There was also a reduction in the total number of tender points and muscle tightness. Reynolds et al., (1991) performed a crossover designed study to exam the overnight sleep physiology, pain, fatigue, and mood symptoms in 12 patients with fibromyalgia treated with cyclobenzaprine. Patients receiving cyclobenzaprine showed a significant decrease in evening fatigue and an significant increase in total sleep time. More recently, Tofferi et al., (2004) showed that cyclobenzaprine-treated patients were 3 times as likely to report overall improvement and to report moderate reductions in individual symptoms, particularly sleep.

In addition, caffeine is often used to counteract sleepiness generated by sleep deprivation, jet lag, and shift-work. Recently, Carrier et al., (2007) performed a study with thirty-four moderate caffeine consumers in both caffeine (200 mg) and placebo (lactose) conditions in a double-blind crossover design. Compared to placebo, caffeine lengthened sleep latency, increased stage 1, reduced stage 2 and slow-wave sleep in both groups. The authors also concluded that the main effects of caffeine are on daytime recovery sleep compared to nocturnal sleep. In an even more recent work, Carrier et al., (2009) studied the combined influence of age and caffeine and concluded that the sleep of middle-aged subjects are particularly vulnerable to the circadian waking signal and proposed that lower brain synchronization due to age and caffeine produces greater difficulty in overriding the circadian waking signal during daytime sleep and leads to fragmented sleep.

Michael et al., (2008) studied the effects of a capsule containing 200 mg of caffeine in twelve non-sleep-deprived participants. The author observed that caffeine reduced the Johns Drowsiness Scale score and reaction times, and these changes persisted for 3 to 4 h. They concluded that despite being well rested, administration of caffeine significantly increased alertness and enhanced performance.

Conclusion

This work describes a fast, sensitive and robust method to quantify cyclobenzaprine in human plasma in conformity with the requirements proposed by the US Food and Drug Administration for pharmacokinetic assays such as bioequivalence studies. The described method was successfully applied in a bioequivalence study of two cyclobenzaprine 10 mg tablet formulations using an open, randomized, two-period crossover design. Since the 90% CI for C_{max} and AUC ratios were all inside the 80–125% interval, it was concluded that the test formulation of cyclobenzaprine is bioequivalent to the reference formulation with respect to both the rate and the extent of absorption and that caffeine present in the test formulation had no effect on the cyclobenzaprine pharmacokinetics.

There is a statically significant difference between the effects of each formulation on the subject drowsiness. Since the drowsiness score was significantly lower when the formulation Miosan®/caffeine was administrated in comparison with the results obtained after administration of the Miosan® formulation, it was concluded that combination of Miosan® with caffeine in the same tablet formulation significantly decreases the drowsiness intensity.

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