Effect of Hyperlipemic Food on the Comparative Bioavailability of Two Bupropion Formulations after Administration of a Single Oral Dose of 150 mg in Healthy Human Volunteers

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Abstract

A specific, fast and sensitive LC–MS/MS assay was developed for the determination of bupropion and its metabolite hydroxybupropion in human plasma using lidocaine as the internal standard. The limit of quantification was 3.13 ng/ml for bupropion and 7.81 ng/ml for hydroxybupropion. The method was linear in the studied range of 3.13 – 400.00 ng/ml for bupropion and 7.81 – 1000 ng/ml for hydroxybupropion. This analytical method was applied to a comparative pharmacokinetic study, in which seventy eight volunteers (39 men and 39 female) aged between 18 and 50 years received a single oral dose of 150 mg of reference and test bupropion formulation, in an open, two-period, balanced randomized, crossover protocol. Group 1 received the medication without any additional meal. Group two received a hyperlipemic meal 30 min before the medication. Based on the 90% confidence interval of the individual ratios for C_{max} and AUC_{0-inf}, it was concluded that the test formulation is bioequivalent to the reference formulation with respect to the rate and extent of absorption of both bupropion and hydroxybupropion and that food intake before the drug administration had no effect in the relative pharmacokinetic parameters. However, the hyperlipemic meal significantly increased the bupropion absorption.

Introduction

Bupropion, a norepinephrine/dopamine reuptake inhibitor (NDRI) (Stahl et al., 2004), has been available in the United States for the treatment of major depressive disorder since 1989. It has no appreciable affinity for postsynaptic receptors, including receptors of histamine, alpha or beta-adrenergics, serotonin, acetylcholine, or dopamine (Stahl et al., 2004). The mechanism of action of bupropion lacks any significant serotonergic component, which may account for its low risk for sexual adverse events (AEs), which is 4- to 6-fold greater with selective serotonin reuptake inhibitors (SSRIs) (Clayton et al., 2002; Stahl et al., 2004). The antidepressant efficacy of bupropion has been demonstrated in comparisons with placebo in both short (Croft et al., 1999; Fabre et al., 1983; Lineberry et al., 1990) and in

long-term relapse-prevention studies (Weihs et al., 2002). It is considered a second-generation antidepressant agent that is also used in the management of smoking cessation (Johnston et al., 2002).

It is rapidly absorbed in the gastrointestinal tract after oral administration of the immediate release (IR) tablet, with T_{max} values of 1.3 to 1.9 hours (mean, 1.5 hours) (Findlay et al., 1981). Absorption of bupropion has been found to be nearly 100% (Schroeder, 1983). However, systemic bioavailability of the parent drug has been found to be <100% due to first-pass metabolism (Schroeder, 1983). Food does not appear to impair absorption (Jefferson et al., 2005). Bupropion binding to human plasma protein is 82% to 88% (Findlay et al., 1981).

Bupropion is extensively metabolized in the liver after oral administration (Schroeder, 1983) to hydroxybupropion (OH-BUP), the primary active metabolite of bupropion (Laizure et al., 1985a; Laizure et al., 1985b), formed via hydroxylation of the tert-butyl group and the amino alcohol isomers (Johnston et al., 2002) and subsequent formation of the morpholinol ring (Suckow et al., 1997; Welch et al., 1987). C_{max} values of OH-BUP are 4- to 7-fold those of bupropion, whereas total exposure to OH-BUP (based on AUC) is 10-fold that of the parent drug. Approximately 0.5% of bupropion is excreted in the urine as unchanged drug (Findlay et al., 1981).

To allow for the correct comparison of newer bupropion formulations with reference ones, it is necessary to have a sensitive assay capable of simultaneous quantification of bupropion and hydroxybupropion. Available HPLC assays using UV detection for simultaneous quantification of bupropion and metabolites have poor sensitivity or very long chromatographic

run time (Cooper et al., 1984; Loboz et al., 2005). Few methods describing the quantification of bupropion in human plasma using mass spectrometric detection have been reported (Borges et al., 2004; Coles et al., 2007; Hsyu et al., 1997; Kustra et al., 1999; Palovaara et al., 2003; Stewart et al., 2001). Because of the high selectivity and sensitivity of LC-MS/MS, this method has been currently accepted as the method of choice for the determination of organic molecules from complex biological matrices (Kerns et al., 2006; Marzo et al., 2007; Maurer, 2007; Zhong et al., 2001).

Here, we describe a fast, sensitive and specific LC-MS/MS method for the simultaneous quantification of bupropion and hydroxybupropion in plasma samples using lidocaine as internal standard. This method was applied to evaluate the comparative bioavailability and the effect of hyperlipemic food on the bupropion and hydroxybupropion bioavailability in 78 healthy volunteers divided in two study groups. One group received a hyperlipemic meal 30 min before the drug administration. All volunteers received a single 150 mg tablet orally corresponding to the test bupropion or reference formulation Zyban®, following an open, two-period, balanced randomized, crossover protocol.

Methods

Clinical Study

Test products

The validated HPLC-MS/MS method was applied to evaluate the comparative bioavailability of two 150 mg bupropion tablets (test formulation against the reference Zyban®).

Subjects

Seventy eight volunteers (39 men and 39 female) aged between 18 and 50 years and within 15% variation in the ideal body weight were selected for the study. The volunteers were divided in two groups based on a randomization list generated by the statistical software R 2.2. After specifying the sequences and gender of volunteers the list is then generated automatically by the program. Group 1 was composed of 42 subjects (21 men and 21 female) and designated to take part in the tablet formulations comparative bioavailability study without any special meal prior the drug administration. Group 2 contained 36 volunteers (18 men and 18 female) assigned to take the 150 bupropion tablet after taken a special meal. The group 1 consisted of individuals with the following characteristics (mean ± SD): 29.9 ± 5.9 years (range 18-40 years), IMC 23.6 ± 2.2 kg (range 19.5 - 28.2 Kg), height between 1.5 and 1.9 m (1.7 ± 0.1 cm) and weighing between 44.4 and 88.0 kg (65.5 ± 11.0 kg). The group 2 consisted of individuals with the following characteristics: 29.1 ± 6.0 years (range 19-43 years), IMC 23.4 ± 2.0 kg (range 19.1 - 27.9 Kg), height between 1.5 and 1.9 m (1.7 ± 0.1 cm) and weighing between 43.5 and 82.0 kg (65.0 ± 8.3 kg).

All volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, and hematological diseases, as assessed by general physical examination, ECG, and the following laboratory tests: blood glucose, urea, uric acid, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, L-γ-glutamyl-transferase (γ-GT), total bilirubin, albumin and total protein, triglycerides, total cholesterol, hemoglobin, hematocrit, platelet count, total and differential white cell counts, feces parasitological examination and routine urinalysis. All subjects were negative for HIV, HBV (except for serological scar) and HCV. All female volunteers were negative for pregnancy test (βHCG).

The study was conducted in accordance 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 ethics committee and all participants provided written, informed consent.

Study design

The study was conducted in an open, randomized, single crossover balanced design with 14 days washout period between doses. During each period, the volunteers were hospitalized at 8:00 p.m. having an evening meal at 8:30. After an overnight fast the group 1 received the medication starting at 7:00 a.m without any additional meal. Group two received a hyperlipemic meal (46.6% of lipids) containing 864.6 Calories just 30 min before the medication. All volunteers received a single 150 mg tablet orally corresponding to the test bupropion or reference formulation Zyban®. Water (200 ml) was given immediately after drug administration in both studies. All volunteers were required to remain fasting until four hours after dose when a standard meal was provided after five (lunch), eight (coffee break), twelve hours after dosing (evening meal). No other food was permitted during the "in-house" period. Liquid consumption was permitted *ad libitum* six hours before and two hours after drug but 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.

For the bupropion quantification blood samples (6 ml) from a suitable antecubital vein were collected by indwelling catheter into heparin containing tubes before and 0:30h, 1h, 1:30h, 2h, 2:20h, 2:40h, 3h, 3:20h, 3:40h, 4h, 4:30h, 5h, 6h, 7h , 8h, 10h, 14h, 24h, 48h, 72h, 96h and 120h. For the hydroxybupropion quantification samples were collected before and 1h, 2h, 3h, 4h, 5h, 5:30h, 6h, 6:30h, 7h , 8h, 9h, 10h, 12h, 18h, 24h, 36h, 48h, 72h, 96h, 144h and 192h. All blood samples were centrifuged at approximately 2000 x *g* for 10 min at 4ºC and the plasma stored at -20°C until assayed for bupropion and hydroxybupropion content.

Analytical Method

Chemicals and materials

Bupropion (batch# 0410153) was obtained from Solmag S.P.A. (Italy). hydroxybupropion (batch# 010901) was synthesized by the Chemistry Institute of the University of São Paulo (São Pualo, Brazil). Lidocaine (batch# 1016) was obtained from the Chemical Center from Fundação Oswaldo Cruz (Rio de Janeiro, Brazil) as Chemical Reference Standard. All reagents and solvents were HPLC grade. Human Plasma samples (normal, hyperlipemic and hemolyzed) came from distinct drug free subjects and were obtained from the São Paulo Hospital Blood bank (São Paulo, Brazil).

Sample analysis

Standard stock solutions of bupropion and lidocaine were prepared, from separate weighting, in acetonitrile/water (50/50, v/v) at concentration of 100 µg/ml, transferred to a dark glass flask and kept at -20°C. Work solutions of bupropion and hydroxybupropion were prepared in blank plasma sample to obtain a final concentration of 400 ng/ml and 1000 ng/ml for bupropion and hydroxybupropion, respectively. Lidocaine work solution was prepared diluting the stock solution in acetonitrile/water (50:50, v/v) to obtain the final concentration of 2µg/ml. All calibration curve samples (non-zero samples), except blank plasma, were prepared by applying a series of appropriate dilutions of bupropion/hydroxybupropion work solution in blank plasma batches. Quality control samples were prepared in blank plasma at lower limit of quantification (LLOQ), quality control at low level (QCL), quality control at medium level (QCM) and quality control at high level (QCH). The final concentrations for these controls were 3.13, 8, 160, and 320 ng/ml for bupropion and 7.81, 20, 400 and 800 ng/ml for hydroxybupropion, respectively.

Aliquots of 450 µL of plasma samples were mixed with 50 µL of internal standard working solution (lidocaine 2µg/ml) and vortex-mixed for 15 seconds. An online solid phase extraction (SPE) was performed using the Prospekt2-Spark (Spark Holland, Emmen, Holland) extractor and Hysphere HD C18 column (Spark Holland), as follow. First, extraction cartridge was activated with 1000 µl of pure methanol pumped at 5 ml/min. Cartridges were then equilibrated with 1000 µl of Milli-Q water and 100 µl of human plasma samples were injected along with an additional 2000 µl of Milli-Q water. Cartridges were washed with 4000 µl of 10 mM acetic pumped at 3 ml/min. Samples were finally eluted with mobile. In this procedure, lidocaine was adopted as the internal standard (IS) because its structure and analytical characteristics are similar to those of bupropion and hydroxybupropion. Analytes and internal standard have partition coefficient (logP) about 2.5, which affords compatible solubility and extraction efficiency as well. All detection settings were similar for analytes and IS due to their analogue structures and ionization behaviors.

The high-performance liquid chromatography (HPLC system LC10ADVp, Shimadzu Scientific Instruments, Columbia, MD, USA) runs were performed on a Phenomenex Luna (Torrance, CA, USA) C18, 5µm analytical column (150 x 4.6 mm i.d.) coupled to a Phenomenex C18, 3 µm (4.0 x 3.0 mm i.d.) guard-column operating at room temperature. Bupropion, hydroxybupropion and lidocaine were eluted isocratically with a mixture of acetonitrile/20 mM ammonium acetate, pH 5.0 (40:60, v/v). A flow rate of 1.0 ml/min was used for sample analysis and the total run time was set to 4.0 minutes.

The mass spectrometer (model Quattro LC, Micromass, Waters/Micromass, Manchester, UK) 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 bupropion, hydroxybupropion and lidocaine by infusing the standard solution of each compound into the stainless steel sample capillary of the electrospray source.

The electrospray capillary potential was set to 4500 eV and Nitrogen was used as drying gas for solvent evaporation. The ESI source and drying gas temperatures were kept at 120°C and 250°C, respectively. The cone Voltages were kept to 20, 15 and 25V for bupropion, hydroxyproprion and lidocaine, respectively. The dwell time was 200 msec for all drugs. The collision energies were 12, 12, and 18eV for bupropion, hydroxyproprion and lidocaine, 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, as follows: m/z $240.0 \rightarrow 183.9$ for bupropion, m/z $256.0 \rightarrow 237.9$ for hydroxyproprion and m/z $235.5\ 86.3$ for lidocaine. Data acquisition and analysis were performed using the MassLynx software.

Method validation

The recovery was evaluated by calculating the mean of the response of six replicates of each quality control concentrations and dividing the extracted sample mean response by the unextracted (spiked mobile phase) sample mean of the corresponding concentration.

Precision and accuracy of this method were evaluated using three different batches of QCL, QCM and QCH of bupropion and hydroxybupropion, also including the lowest limit of quantification, LLOQ. For intra-batch assay precision and accuracy, six replicates of quality control samples at the three concentration levels were assayed all at once within a day to obtain %CV and accuracy values. The inter-batch assay precision and accuracy were determined by analyzing mean values of quality control samples from three plasma batches, yielding the corresponding inter-batches %CV and accuracy values.

All stability assays were performed using three replicates of plasma spiked with bupropion and hydroxybupropion at QCL, QCM and QCH concentrations. The percent of degradation was defined comparing sample concentration to the mean values obtained from fresh prepared ones at equivalent concentration. The following stability parameters were evaluated: Post-processing stability at room temperature for 24 h, three cycles of freeze–thaw, short term storage stability at room temperature for 6 h, long term storage stability at -20°C during 165 days, stock solution stability at room temperature for 6 h and at 4°C for 7 days.

Pharmacokinetic Analysis

The first-order terminal elimination rate constant (ke) was estimated by linear regression from the points describing the elimination phase in a log-linear plot. Half-life ($t_{1/2}$) was derived from this rate constant ($t_{1/2} = \ln(2)/ke$).

Bioequivalence between the two formulations was calculated for both oral suspension study and tablet formulation study by calculating individual test/reference ratios for the peak of concentration (C_{max}), area under curve (AUC) of plasma concentration until the last concentration observed (AUC_{0-t}), and the area under curve between the first sample (pre-dosage) and infinite (AUC_{inf}). The C_{max} and the time taken to achieve this concentration (T_{max}) were obtained directly from the curves. The areas under the bupropion plasma concentration vs. time curves from 0-to the last detectable concentration (AUC_{0-t}) were calculated by applying the linear trapezoid rule. Extrapolation of these areas to infinity (AUC_{inf}) was done by adding the value C_{last}/ke to the calculated AUC_{0-t} (where C_{last} = the last detectable concen-

tration). The AUC and C_{max} data for the two formulations were analyzed by ANOVA to establish whether the 90% CI of the ratios was within the 80 - 125% interval indicating bioequivalence as proposed by the US Food and Drug Administration. Parametric analyses of ln-transformed arithmetic means between test and reference formulations were also performed. The software used included SAS® and Microsoft Excel (v. 7.0).

Results

Clinical Observations

The post study clinical and laboratory evaluations showed no significant variations that could be attributed to treatment. Both formulations were well tolerated at the administered doses and no significant adverse reactions were observed or reported. No clinically relevant change was observed in any measured biochemical parameter. A total of 78 volunteers finished the study, 42 for group 1 and 36 for group 2. No dropped out was registered in both groups.

LC-MS/MS Analysis

Specificity or selectivity

All chromatograms were free from interferences at the retention times of bupropion, hydroxybupropion or lidocaine (Figure 1A). Under the conditions applied in this work, the retention times for bupropion, hydroxybupropion and IS were 2.80, 2.34 and 2.32 min, respectively (Figure 1B). Both compounds eluted as completely resolved peaks and no peak tailing was noticed enabling the use of either peak height or peak area in the calculation of standard curves. The absence of any peak in the normal, lipemic and hemolized blank plasma samples at the retention time of bupropion, hydroxybupropion and IS indicates the specificity and selectivity of the analytical method in plasma.
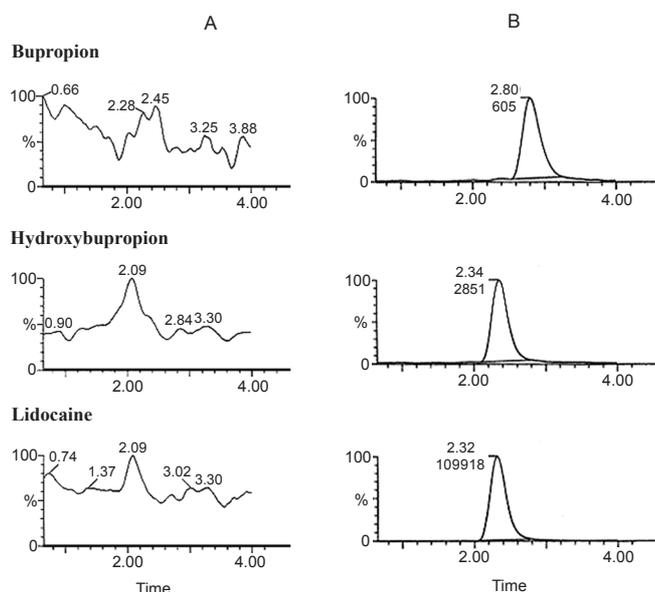

Figure 1: MRM chromatograms of human plasma samples spiked with bupropion, hydroxybupropion or internal standard (lidocaine). Panel (A), blank plasma sample monitored for bupropion, hydroxybupropion and lidocaine (IS) channels. Panel (B), plasma sample was spiked with bupropion at LLOQ concentration (3.13 ng/ml), hydroxybupropion at LLOQ concentration (7.81 ng/ml) and IS (lidocaine).

	Intra-run accuracy[a]	Inter-run accuracy[b]	Intra-run precision[c] (%CV)	Inter-run precision[b] (%CV)
Bupropion QC samples				
QC-LQ	111.7	112.9	3.5	1.8
QCL	91.7	94.3	6.1	4.0
QCM	91.2	92.4	5.9	3.9
QCH	99.1	100.1	5.0	8.7
Hydroxybupropion QC samples				
QC-LQ	86.3	99.1	8.3	14.3
QCL	92.4	95.2	5.7	8.5
QCM	97.6	98.6	2.3	1.0
QCH	99.5	98.9	2.7	1.3

[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: Summary of % intra- and inter-batch precision and accuracy results for the quantification of Quality Control (QC) samples of both bupropion and hydroxybupropion in human plasma.

Linearity

Good linear relationships were found when the peak area ratios of bupropion and hydroxybupropion to the internal standard were plotted versus the bupropion and hydroxybupropion plasma concentration in all concentration applied to construct the standard curve. The simplest regression method for the calibration curves of the bupropion was $Y = a + bx$ from 3.13 to 400 ng/ml for bupropion and 7.81 to 1000 ng/ml for hydroxybupropion. Correlation coefficient was above 0.994 for all independent analytical runs. The LOQ for bupropion was 3 ng/ml and 7.81 ng/ml for hydroxybupropion at a signal to noise ratio over 10.

Method validation

The intra- and inter-days accuracy and precision values of the assay methods are presented in Table 1. Calculated intra-batch precisions ranged from 3.47 – 6.14% for bupropion and 2.26 – 8.34% for hydroxybupropion, while the inter-assay precisions ranged from 1.82 – 8.67% for bupropion and 0.96 – 14.30% for hydroxybupropion. The intra-assay accuracies ranged from 91.17 to 111.71% for bupropion and 86.32 to 99.52% for hydroxybupropion, while the inter-assay accuracies ranged from 92.36 to 112.89% for bupropion and 95.20 to 99.10% for hydroxybupropion.

The mean recoveries for bupropion in spiked blank plasma samples were found to be 58.43% (%CV 5.79) for LLOQ, 63.30% (%CV 13.31) for QCL, 64.17% (%CV 4.08) for QCM and 66.16% (%CV 2.69) for QCH. The mean recoveries for hydroxybupropion in spiked blank plasma samples were found to be 67.62% (%CV 4.55) for LLOQ, 75.250% (%CV 11.12) for QCL, 70.37% (%CV 4.76) for QCM and 74.95% (%CV 5.65) for QCH. Lidocaine mean recovery was found to be 91.49% (%CV 6.66).

Bupropion and hydroxybupropion did not suffer significant degradation in the stock solution or in spiked plasma samples.
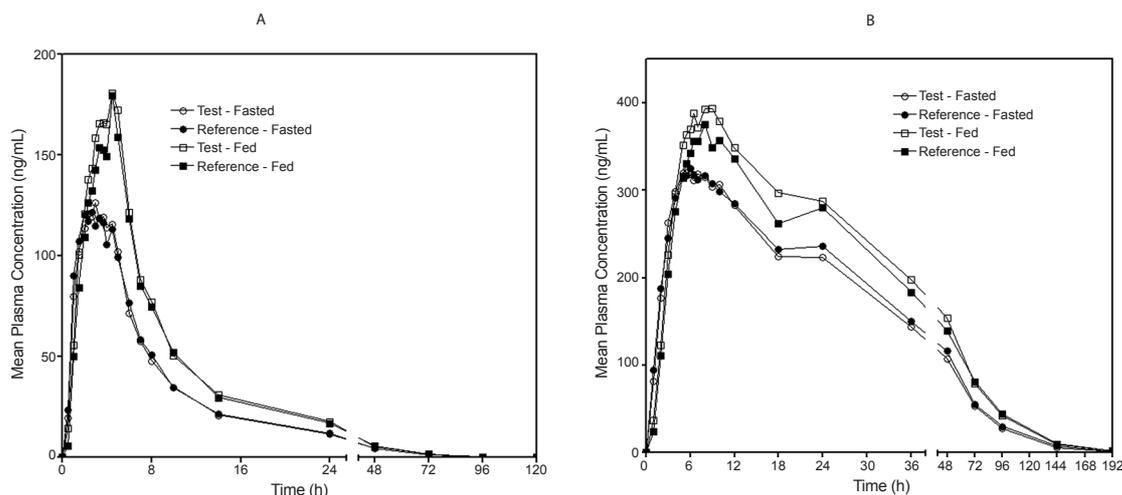
Figure 2: Mean bupropion and hydroxybupropion plasma concentration vs. time curve. A single oral dose administration of 150 mg bupropion tablet formulation (test or reference Zyban®) was given to 78 volunteers divided in two groups. Group 1 was composed of 42 subjects (21 men and 21 female) and received the bupropion tablet without any special meal prior the drug administration. Group 2 was composed of 36 subjects (18 men and 18 female) and received the bupropion tablet after taken a hyperlipemic meal prior the drug administration. Panel (A) shows the bupropion plasma concentrations and panel (B) shows the hydroxybupropion plasma concentrations.

In all stability experiments the variation observed between fresh and stored samples was inferior to 15%. For example, the stock solution stored for 7 days at 4°C showed that the variation between fresh and stored samples for bupropion were 0.3, -0.3 and 3.8% for low, median and high QC samples, respectively, while for hydroxybupropion the variations were -1.3, 1.6 and -1.4%. After the freeze and thaw assay, both bupropion and hydroxybupropion did not suffer significant degradation in spiked plasma. At the end of the third thaw cycle the variations between fresh and tested samples for bupropion were 10.7, -7.6 and 10.5% for low, median and high QC samples, respectively, while for hydroxybupropion the variations were 6.7, 3.4 and 11.2%.

Pharmacokinetic Evaluation

Applicability of this method was confirmed by analyzing true samples obtained after administration of 150 mg of bupropion to healthy volunteers. In addition to the bupropion quantification in plasma, the concentration of the main metabolite hydroxybupropion was also evaluated and used to determine de

relative bioavailability in both group 1 and group 2. The mean bupropion and hydroxybupropion plasma concentration vs time curves obtained after a single oral dose of each tablet formulation with or without a special meal are shown in Figure 2. In both group1 and 2, the plasma concentrations of both bupropion and hydroxybupropion did not differ significantly after administration of bupropion tablet for the test formulation compared to the reference Zyban®. However, volunteers who received a hyperlipemic meal 30 min before the drug administration showed a higher bupropion and hydroxybupropion concentrations compared to the fasted volunteers.

The mean pharmacokinetic parameters for bupropion and hydroxybupropion in Group1 are summarized in Tables 2, 3 and 6. Briefly, the geometric mean and respective 90% CI of bupropion test/reference percent ratios were 98.82% (91.54-106.67%) for C_{max} and 97.53% (93.10-102.17%) for AUC_{0-t}. In addition, the geometric mean and respective 90% CI of hydroxybupropion test/reference percent ratios were 99.85% (94.78-105.19%) for C_{max} and 95.58% (90.45-101.01%) for AUC_{0-t}.

Pharmacokinetics Parameters	Reference formulation		Test formulation	
	Mean	SD	Mean	SD
C_{max} (ng/ml)	152.55	48.84	149.99	42.90
T_{max} (h)	2.67	5.00	3.00	4.00
$T_{1/2}$ (h)	9.39	15.68	9.20	12.48
AUC_{0-t} ([ng x h]/ml)	1341.89	410.91	1315.21	436.54
AUC_{inf} ([ng x h]/ml)	1350.32	440.21	1317.75	460.86

Table 2: Arithmetic mean and 90% CI of the pharmacokinetics parameters of bupropion obtained from 42 volunteers after the administration of 150 mg bupropion tablet formulation of each test and reference formulations without any additional meal.

Pharmacokinetics Parameters	Reference formulation		Test formulation	
	Mean	SD	Mean	SD
C_{max} (ng/ml)	352.85	141.10	358.14	163.97
T_{max} (h)	6.50	8.00	6.00	6.00
$T_{1/2}$ (h)	26.23	23.93	25.32	25.21
AUC_{0-t} ([ng x h]/ml)	14055.86	5899.89	13509.83	5805.36
AUC_{inf} ([ng x h]/ml)	14328.97	6001.88	13749.97	5811.75

Table 3: Arithmetic mean and 90% CI of the pharmacokinetics parameters of hydroxybupropion obtained from 42 volunteers after the administration of 150 mg bupropion tablet formulation of each test and reference formulations without any additional meal.

Pharmacokinetics Parameters	Reference formulation		Test formulation	
	Mean	SD	Mean	SD
C_{max} (ng/ml)	211.26	76.30	222.76	78.67
T_{max} (h)	3.84	5.00	4.00	4.50
$T_{1/2}$ (h)	9.21	14.71	8.77	16.40
AUC_{0-t} ([ng x h]/ml)	1765.04	654.93	1838.82	721.17
AUC_{inf} ([ng x h]/ml)	1767.83	686.36	1841.80	747.48

Table 4: Arithmetic mean and 90% CI of the pharmacokinetics parameters of bupropion obtained from 36 volunteers after receiving a hyperlipemic meal followed by the administration of 150 mg bupropion tablet formulation of each test and reference formulations.

Pharmacokinetics Parameters	Reference formulation		Test formulation	
	Mean	SD	Mean	SD
C_{max} (ng/ml)	408.53	239.90	441.55	284.80
T_{max} (h)	8.00	9.00	8.00	21.00
$T_{1/2}$ (h)	27.38	35.61	26.77	48.25
AUC_{0-t} ([ng x h]/ml)	17092.18	11074.63	17932.06	11507.83
AUC_{inf} ([ng x h]/ml)	17486.79	11253.24	18281.56	11580.15

Table 5: Arithmetic mean and 90% CI of the pharmacokinetics parameters of hydroxybupropion obtained from 36 volunteers after receiving a hyperlipemic meal followed by the administration of 150 mg bupropion tablet formulation of each test and reference formulations.

Bupropion/Zyban®				
	Geometric mean (%)	90% CI	Power (%)	%CV
C_{max} % ratio	98.82	91.54-106.67	99.80	21.04
AUC_{0-t} % ratio	97.53	93.10-102.17	100.00	12.70
AUC_{inf} % ratio	97.16	92.62-101.92	100.00	13.06
hydroxybupropion/Zyban®				
	Geometric mean (%)	90% CI	Power (%)	%CV
C_{max} % ratio	99.85	94.78-105.19	100.00	14.25
AUC_{0-t} % ratio	95.58	90.45-101.01	99.99	15.11
AUC_{inf} % ratio	95.32	90.42-100.49	99.99	14.45

Table 6: Bupropion and hydroxybupropion geometric mean of the individual C_{max}, AUC_{0-t}, AUC_{0-inf} and ratios (test/reference formulation) and the respective 90% confidence intervals (CI) obtained from 42 volunteers after administration of each 150 mg bupropion tablet formulation without special meal.

The same pharmacokinetic parameters for Group2 study are summarized in Tables 4, 5 and 7. In this study, the geometric mean and respective 90% CI of bupropion test/reference percent ratios were 104.41% (95.81-113.79%) for C_{max} and 101.62% (93.92-109.96%) for AUC_{0-t}. Finally, the geometric mean and respective 90% CI of hydroxybupropion test/reference percent ratios were 105.40% (95.00-116.95%) for C_{max} and 105.89% (93.77-119.57%) for AUC_{0-t}.

In both groups of study, the results were found to be within the FDA acceptable range of 80 - 125% for evaluation of bioequivalence.

Bupropion/Zyban®				
	Geometric mean (%)	90% CI	Power (%)	%CV
C_{max} % ratio	104.41	95.81-113.79	96.55	21.83
AUC_{0-t} % ratio	101.62	93.92-109.96	99.62	19.97
AUC_{inf} % ratio	101.79	94.06-110.15	99.58	20.01
hydroxybupropion/Zyban®				
	Geometric mean (%)	90% CI	Power (%)	%CV
C_{max} % ratio	105.40	95.00-116.95	85.52	26.53
AUC_{0-t} % ratio	105.89	93.77-119.57	71.67	31.21
AUC_{inf} % ratio	105.52	93.67-118.88	74.71	30.59

Table 7: Bupropion and hydroxybupropion geometric mean of the individual C_{max}, AUC_{0-t}, AUC_{0-inf} and ratios (test/reference formulation) and the respective 90% confidence intervals (CI) obtained from 36 volunteers after administration of each 150 mg bupropion tablet formulation after receiving a hyperlipemic meal.

Discussion

A simple, rapid and specific method for simultaneously quantifying bupropion and hydroxybupropion in human plasma was fully validated. Pharmacokinetic studies require highly selective and sensitive analytical methods that provide high accuracy and precision for the determination of drug levels. The combination liquid chromatography/mass spectrometry is currently accepted as being a powerful means of determining organic molecules from complex biological matrices (Kerns et al., 2006; Marzo et al., 2007; Maurer, 2007). The selectivity and sensitivity of LC-MS/MS allowed analysis times to be reduced, such that sample preparation time often exceeds the analysis time of samples.

LC–MS and LC–MS/MS methods for bupropion and metabolite quantification had also been described in previous works (Borges et al., 2004; Coles et al., 2007; Hsyu et al., 1997; Kustra et al., 1999; Palovaara et al., 2003; Stewart et al., 2001). These methods allow for greater sensitivity than the published HPLC

methods and presented LOQ varying from 0.25 to 1.25 ng/ml for bupropion and 0.25 to 5 ng/ml for hydroxybupropion. The LOQ of 3.13 ng/ml for bupropion and 7.81 ng/ml for hydroxybupropion achieved in this work are only of intermediate sensitivity compared to the literature, but are adequate for pharmacokinetics studies and could be further improved by sample concentration if required.

Based on the assay procedure results, mainly specificity, accuracy and precision, the method described here for bupropion and hydroxybupropion quantification in human plasma agrees with the requirements for high sensitivity, specificity and high sample throughput to be applied in clinical studies such as bioequivalence or for the routine evaluation. In addition, the recoveries were consistent throughout the replicates as demonstrated by the %CV observed throughout the analysis of the six samples in each run. This assay has been tested in a clinical setting and allows for the accurate measurement of plasma bupropion and metabolite concentration time curves. The plasma concentrations observed in this study for bupropion and its major metabolite are consistent with the published literature (Hsyu et al., 1997; Palovaara et al., 2003; Stewart et al., 2001).

Since 1997 the Food and Drug Administration approved bupropion as an aid for smoking cessation in subjects older than 18 years of age and many clinical trials have been performed to evaluate the effects of smoking in bupropion absorption and metabolization. Hsyu et al. (Hsyu et al., 1997), worked with thirty-four healthy volunteers between 18 to 45 years old. Pharmacokinetic parameters of the parent compound and metabolites did not differ dramatically between the adolescents and adults. However statistically significant gender-based differences were found (Hsyu et al., 1997), since adolescent females showed higher AUC and Cmax values for bupropion and its metabolites than adolescent males (Stewart et al., 2001).

In this work we used volunteers ranging from 18 to 50 years old and the effect of food intake before the drug administration was evaluated over the absorption and metabolization of bupropion. A pronounced difference was observed when comparing the group that received the hyperlipemic meal against the group without any meal before the tablet intake. The volunteers who received the hyperlipemic meal 30 min before the medication presented a higher concentration of both bupropion and its metabolite hydroxybupropion. In contrast, some reports in the literature describe that food does not appear to impair absorption (Jefferson et al., 2005). Based on the fact that both bupropion and hydroxybupropion showed higher concentrations without changing the ratio between them, our results clear indicate that the drug absorption was increased without any significant change in the rate of bupropion metabolization. In fact, if the metabolization rate is increased we would expect higher metabolite concentration but much lower bupropion levels.

Food can affect drug bioavailability either by interacting directly with drug substance or indirectly by altering drug release and subsequent absorption from the drug product. Fatty Food is able to slow the gastric emptying rate under normal physiological conditions. Consequently, the gastric residence time of the concurrently administered drug is prolonged. This may affect the pharmacokinetics of the drug in several ways. For those acid-labile drugs such as penicillins, erythromycin, and cephalospor-

ins, the extent of absorption is reduced because drug lost by hydrolysis is increased as a result of increased gastric residence time. On the other hand, for those drugs that are poorly or slowly soluble, the extent of absorption may be increased, because the residence time and fluid volume are increased producing better dissolution (Greenblatt et al., 1978). In particular, poorly water soluble drugs (e.g. griseofulvin, mebendazole and halofantrine), when taken with very fatty foods can increase bioavailability, possibly by the formation of solutions in the dietary oil.

The administration of the drugs propranolol, metoprolol, labetalol and hydralazine to non-fasted subjects significantly increases their bioavailability (Daneshmend et al., 1982; Melander et al., 1977a; Melander et al., 1977b). This effect is likely to be due to transient food-induced changes in drug absorption rate, splanchnic blood flow, plasma protein binding and activity of drug metabolizing enzymes, causing temporary reduction of first pass metabolism (Melander et al., 1988). Since bupropion presents a good solubility in water is unlikely that the increase in bupropion bioavailability is caused only by the interaction with fat. The combinations of fat with the prolonged time in a higher fluid volume environment are the most likely reasons for the results observed in this work. As stated before, our results do not indicate any change in metabolism rate.

None adverse effects related to drug intake were observed in this study. Vital signs of all volunteers were normal throughout the study. These results indicate that sustained-release bupropion at 150 mg was well tolerated in healthy volunteers after a single dose. The tolerability profile of bupropion is well documented, with thousands of patients participating in clinical trials and more than 40 million clinical-use exposures for all uses (Fava et al., 2005). The most common (incidence, >10%) adverse events associated with the use of bupropion have been found to be headache (27% vs 23% for placebo), dry mouth (16% vs 7%; P < 0.05), nausea (13% vs 8%; P < 0.05), and insomnia (11% vs 7%; P < 0.05) (Settle et al., 1999).

In spite of showing difference in the absorption level between group 1 and group 2, the respective 90% confidence intervals of the ratios of geometric means of C_{max}, AUC_{0-t} and AUC_{inf} values of bupropion and hydroxybupropion absorbed from both test and reference formulations were included in the 80% to 125% interval proposed by the FDA in both groups. The test tablet formulation was considered bioequivalent to the reference Zyban® in both fed and fasted studies.

Conclusion

A very simple and fast method involving the SPE protocol and LC-MS/MS analysis for the bupropion and hydroxybupropion quantification in human plasma using lidocaine as the internal standard was fully validated. This method is in agreement with the high sensitivity, specificity and high throughput analysis required for pharmacokinetic studies and hundreds of samples can be analyzed daily, while only small quantities of plasma and solvent are consumed. The method developed here was linear over the concentration ranges of 3.13 to 400 ng/ml for bupropion and 7.81 to 1000 ng/ml for hydroxybupropion.

The 90% CI for C_{max}, AUC_{0-last} and AUC_{0-inf} were within the 80-125% interval for both bupropion and hydroxybupropion.

The food intake before the drug administration had no effect in the relative pharmacokinetic parameters. It was concluded that test bupropion 150mg tablet formulation is bioequivalent to Zyban® 150 mg with respect to both the rate and extent of absorption.

Even having no interference in the relative pharmacokinetics parameters, the hyperlipemic meal applied 30 min before the tablet intake increased the mean bupropion absorption.

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