Research article

Biomedical Chromatography

Received: 13 May 2015, Revised: 11 July 2015, Accepted: 3 August 2015 Published online in Wiley Online Library: 4 November 2015

(wileyonlinelibrary.com) DOI 10.1002/bmc.3579

Assessment of pharmacokinetic interaction between piracetam and l-carnitine in healthy subjects

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ABSTRACT: A rapid, sensitive and specific method for quantifying piracetam in human plasma using Piracetam d-8 as the internal standard (IS) is described. The analyte and the IS were extracted from plasma by one-step precipitation of protein using an acetonitrile (100%). The extracts were analyzed by high-performance liquid chromatography coupled with electrospray tandem mass spectrometry (HPLC-MS/MS). The method had a chromatographic run time of 3.8 min and a linear calibration curve over the range 0.5–50 μg/mL ($r > 0.99$). This LC-MS-MS procedure was used to assess the bioavailability of two piracetam formulations: piracetam + l-carnitine (Piracar®; 270/330 mg tablet) and piracetam (Nootropil®; 800 mg tablet) in healthy volunteers of both sexes. The geometric means with corresponding 90% confidence interval (CI) for test/reference percentage ratios were 88.49% (90% CI = 81.19 – 96.46) for peak concentration/dose and 102.55% (90% CI = 100.62 – 104.51) for AUC_{inf}/dose. The limit of quantitation of 0.5 μg/mL is well suited for pharmacokinetic studies in healthy volunteers. It was concluded that piracetam (Piracar®; 270/330 mg tablet) has a bioavailability equivalent to the piracetam (Nootropil®; 800 mg tablet) formulation with regard to both the rate and the extent of absorption. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: tandem mass spectrometry; LC/MS/MS; bioavailability; pharmacokinetics

Introduction

In recent years, a broad spectrum of diseases has been associated with changes in mitochondrial DNA (mtDNA), including hereditary diseases and mitochondrial dysfunctions associated with aging and neurodegenerative diseases associated with aging, for example, Parkinson and Alzheimer's disease (Maruszak *et al*., 2006). Also included are medical conditions in mtDNA mutations associated with mitochondrial myopathies and a group of neuromuscular disorders caused by damage to mitochondria (Ozawa, 1995; Pitceathly and McFarland, 2014). Although there is no specific treatment for mitochondrial myopathies, certain regimes have been recommended to alleviate the symptoms of these diseases, such as physical therapy and therapy with vitamins, for example, riboflavin with coenzyme Q and carnitine (DiMauro and Mancuso, 2007; Tarnopolsky and Raha, 2005).

Piracetam (2-oxo-pyrrolidone) was the first drug of the racetam group to be identified, and it is typically used to treat dementia caused by Alzheimer's and other neurodegenerative diseases associated with aging, decreased cognitive ability and memory (Malykh and Sadaie, 2010). One of the most important characteristics of piracetam is its ability to improve cerebral energy, ATP, especially under stress conditions (Grau *et al*., 1987; Malykh and Sadaie, 2010; Nickolson and Wolthuis, 1976; Tacconi and Wurtman, 1986). Piracetam prevents mitochondrial changes and improves the function of mitochondria during development of traumatic brain edema (Ve and Kovaleva, 1998). Piracetam has the ability to improve mitochondrial dysfunction associated with oxidative stress and/or aging (Keil *et al*., 2006). The time to reach peak concentration (T_{max}), $T_{1/2}$= ln (2)/ke and peak concentration (C_{max}) are 0.92 ± 0.44 h, 2.64 ± 1.14 h and 15.75 ± 2.56 μg/mL after oral administration of a single dosage of 50 mg/kg piracetam to rats ($n = 6$), respectively (Wang *et al*., 2010). The pharmacokinetic parameters of 800 mg piracetam tablet in 18 healthy male volunteers for $T_{1/2}$, T_{max}, C_{max} and AUC_{inf} were 4.40 ± 0.179 h, 2.33 ± 0.105 h, 14.53 ± 0.282 μg/mL and 59.19 ± 4.402 μg h/mL, respectively (Barkat *et al*., 2014).

Several analytical methods based on high-performance liquid chromatography (HPLC) in human plasma (Barkat *et al*., 2014;

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Abbreviations used: mtDNA, mitochondrial DNA; SRM, selected reaction monitoring.

Curticapean and Imre, 2007; Louchahi *et al*., 1995; Siddiqui *et al*., 2014), solution (Siddiqui *et al*., 2014) and human urine (Curticapean and Imre, 2007) samples have been used for piracetam quantification, including powder X-ray diffraction and Raman and near-infrared spectroscopy for quantification of binary polymorphic mixtures of piracetam (Croker *et al*., 2010). There are two methods in rat plasma by liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS): one has been validated for piracetam (Wang *et al*., 2010) and the other for metabolite only (Sahu *et al*., 2013).

Carnitine is present in muscle of vertebrate animals and it is involved in the transfer of fatty acids across the mitochondrial membrane process (Georges *et al*., 2000). Primary carnitine deficiency is a medical condition that prevents the body from using fat for energy production (Georges *et al*., 2000). It is also known that acetyl l-carnitine plays a role in neurodegenerative diseases such as Alzheimer's (Gavrilova *et al*., 2011; Traina *et al*., 2008) and Parkinson's(Kidd,2000; Beal, 2003).

There is a close correlation between the inhibition of mitochondrial electron transport resulting from treatment with l-carnitine and the prevention of permeability transition in mitochondria isolated hepatocyte culture (Pastorino *et al*., 1993). The ability of l-carnitine to protect hepatocytes in culture is a consequence of its ability to prevent mitochondrial permeability transition. Therefore, both anoxia-induced cell death and electron transport inhibition are associated with mitochondrial-related changes, independent mechanisms of maintaining membrane potential and cellular ATP reserves (Pastorino *et al*., 1993).

The aim of this study was to compare pharmacokinetic profiles of healthy volunteers of both sexes to evaluate the comparative bioavailability of two piracetam formulations [test formulation, piracetam + l-carnitine (Piracar®; 270/330 mg tablet) and reference formulation, piracetam (Nootropil®; 800 mg tablet)]. Piracetam plasma levels were measured by LC-MS-MS in human plasma using Piracetam d-8 as an internal standard.

Experimental

Calibration standards and quality control

Stock solutions of piracetam and internal standard (Piracetam d-8) were prepared in acetonitrile–water (50:50 v/v) at concentrations of 1 mg/mL. Calibration curves of piracetam were prepared by spiking blank plasma at concentrations of 0.5, 5, 10, 18, 26, 34, 42 and 50 μg/mL and the analysis was carried out in duplicate for each concentration. The quality control samples were prepared in blank plasma at concentrations of 1.25, 12.5, 25 and 40 μg/mL (QCA, QCB1, QCB2 and QCC, respectively). The spiked plasma samples (standards and quality controls) were extracted from each analytical batch along with the unknown samples.

Chemicals and reagents

Piracetam was provided by the Northeast Pharmaceutical Group Co. Ltd, China, lot number DY030120440. Piracetam d-8 was obtained from CDN Isotopes, Canada, lot number C327P2. Acetonitrile (HPLC grade), formic acid (88%; analysis grade) and ammonium acetate were purchased from Mallinckrodt (St Louis, MO, USA). Ultrapure water was obtained from an Elga UHQ system (Elga, UK). Blank human blood was collected from healthy, drug-free volunteers. Plasma was obtained by centrifugation of blood treated with the anticoagulant sodium heparin. Pooled plasma was prepared and stored at approximately −20°C until needed.

Drug analysis

Blood samples (7 mL) from a suitable antecubital vein were collected by an indwelling catheter into heparin containing tubes at 0, 0.173, 0.33, 0.5, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 18 and 24 post-dosing. The blood samples were centrifuged at approximately 2000***g*** for 1 min at 4°C, and the decanted plasma stored at −20°C until assayed for piracetam contents. Piracetam and the internal standard (IS; Piracetam d-8) were extracted from volunteers' plasma samples and quantified by LC-MS-MS with positive electrospray ionization using selected reaction monitoring (SRM) at the Galeno Research Unit (Campinas, Brazil).

Sample preparation

Frozen human plasma samples were thawed at room temperature and centrifuged at 2000***g*** for 5 min. The supernatant plasma (20 μL) was dispensed into glass test tubes (nonsiliconized) followed by 50 μL of the internal standard solution (Piracetam d-8 at 50 μg/mL). Samples were vortex-mixed for 10 s. The acetonitrile (100%, 500 μL) was added and the samples were vortex-mixed for 40 s. The sample was centrifuged at 2000***g*** for 5 min. The upper organic layer was transferred to a PCR plate using automatic pipettes with disposable plastic tips, capped and placed into autosampler rack.

Chromatographic conditions

An aliquot (7.5 μL) of each plasma extract was injected into an Inertsil HPLC CN-3-5u analytical column (4.6 × 250 mm i.d.). The compounds were eluted by the mobile phase [acetronitrile–H_2O (72/28 v/v) + 10 mm ammonium acetate + 0.1% formic acid] at a flow rate of 1100 μL/min. Under these conditions, typical standard retention times were 2.8 min for piracetam and Piracetam d-8, and back-pressure values of approximately 70 bar were observed. The temperature of the autosampler was maintained at 8 ± 2°C and the run-time was 3.8 min.

Mass-spectrometric conditions

A Waters Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray source using a crossflow counter-electrode run in positive mode (ES^+) was set up in SRM, monitoring the transitions *m/z* 143.2–126.0 and *m/z* 151.2–134.0, for piracetam and IS, respectively. The turboionspray capillary voltage was set at 3.2 kV. For both piracetam and internal standard, the following optimized parameters were obtained: time, collision energy and cone were 0.250 (s), 7 (eV) and 15 (V), respectively. Data acquisition and analyses were done using the software MassLynx (v 3.5).

Linearity

Linearity was determined to assess the performance of the method. A linear least-squares regression with a weighting index of 1/*x* was performed on the peak area ratios of piracetam and IS vs piracetam concentrations of the eight plasma standards (0.5, 5, 10, 18, 26, 34, 42 and 50 μg/mL) in duplicate to generate a calibration curve.

Matrix effect

Blank samples (normal, lipemic and haemolyzed) of plasma were extracted and added to the analyte and IS. Each sample was obtained by a factor matrix standard (FMS) IS according to the following formula: FMS = (response of the analyte in matrix/internal standard response matrix)/(response of the analyte in solution/response of the internal standard solution). The coefficient of variation of FMS concerning all samples must be <15%.

Precision and accuracy

Within- and between-run precisions were determined as the coefficient of variation, CV (%) = 100(SD/*M*) and the accuracy (%) as 100(*M*/*T*), where *M* is the mean, SD is the standard deviation of *M* and *T* is the theoretical concentration.

Stability

Stability quality control plasma samples (1.25 and 40.0 μg/mLfor piracetam) were subjected to short-term (13 h 22 min) room temperature, three freeze–thaw (−20–25°C) cycles, 418 days long term at −20°C and 42 h 8 min autosampler (8°C) stability tests. Subsequently, the piracetam concentrations were measured compared with freshly prepared samples. The significance of the results obtained was analyzed by Student's *t*-test ($p < 0.05$).

Clinical protocol

Thirty-two healthy volunteers of both sexes between 18 and 50 years of age and within 15% of their ideal body weight were selected for the study, having their health status previously accessed by clinical evaluation (physical examination, ECG) and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, γ -GT, total bilirubin, albumin and total protein, triglyceride, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts and routine urinalysis. All subjects were negative for HIV, HCV and HBV (except for serological scar).

The study began with 32 volunteers and finished with 30 volunteers. Two volunteers dropped out of the study for personal reasons. The volunteers ($n = 30$) had the following clinical characteristics (according to gender and expressed as mean ± SD [range]): *males* – age, 32.06 ± 8.90 [18–48]; height, 1.72 ± 0.07 m [1.59–1.87]; body weight, 74.69 ± 9.86 [52–100]; *females* – age, 31.75 ± 7.71 [22–49]; height, 1.61 ± 0.06 [1.51–1.73]; body weight, 60.28 ± 7.62 [48–73]. All subjects provided written informed consent and the University of São Paulo approved the clinical protocol (no. 550.543). 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), Edimburgo (2000), Washington (2002), Tóquio (2004) and Seoul (2008) revisions. After screening and a wash-out period (of at least 2 weeks), individuals who qualified were confined for 2 periods of approximately 36 h each.

The following formulations were employed: Piracar® (piracetam + l-carnitine) 270/330 mg tablet (test formulation manufactured by Biolab Sanus Farmacêutica Ltda; lot no. 2070260, expiration date 05/2014) and Nootropil® (piracetam) 800 mg tablet (standard reference formulation from Sanofi Aventis Farmacêutica Ltda, lot no. 246722, expiration date 09/2014).

The study was a single-dose, two-period randomized design with at least a 7 day washout period between doses. During each period, the volunteers

Table 1. Accuracy and precision data for piracetam from the pre-study validation in human plasma

Intra-batch ($n = 7$)					
Nominal concentration (μg/mL)	1.25	12.5	25	40	80
Intra-batch arithmetic mean (μg/mL)	1.32	12.8	24.5	42.1	42.7
Intra-batch precision (CV, %)	7.7	3.8	3.5	4.4	5.5
Intra-batch accuracy (%)	105.6	102.5	97.9	105.3	106.7
Inter-batch ($n = 21$)					
Nominal concentration (μg/mL)	1.25	12.5	25	40	80
Inter-batch arithmetic mean (μg/mL)	1.26	13.25	25.3	41.7	41.9
Inter-batch precision (CV, %)	7.2	4.2	4.8	4.1	4.9
Inter-batch accuracy (%)	100.7	106	101.2	104.4	104.7

Table 2. Stability tests for piracetam: post-processing stability test (μg/mL; $n = 5$)

	Reference values	Values after 42 h 08 min	Reference values	Values after 42 h 08 min
	1.25		40	
Arithmetic mean	1.24	1.25	43.7	42.9
CV (%)	4.1	4.9	3.8	4.8
Accuracy (%)	99.2	100	109.3	107.3
Variation (%)	−0.80		1.86	

Table 3. Stability tests for piracetam: freeze-and-thaw stability test (μg/mL; $n = 5$)

	Reference values	Values after three cycles	Reference values	Values after 3 cycles
	1.25		40	
Arithmetic mean	1.24	1.39	43.7	43.2
CV (%)	4.1	4.7	3.8	5.4
Accuracy (%)	99.2	111.2	109.3	108
Variation (%)	−10.79		1.16	

Table 4. Stability tests for piracetam

Short-term stability test (μg/mL; $n = 5$)				
	Reference values	Values after 13 h 22 min	Reference values	Values after 13 h 22 min
	1.25		40	
Arithmetic mean	1.24	1.29	43.7	43.2
CV (%)	4.1	3.7	3.8	6.8
Accuracy (%)	99.2	103.2	109.3	108
Variation (%)	−3.88		1.16	
Long-term stability test (μg/mL; $n = 5$)				
	Reference values	Values after 418 days	Reference values	Values after 418 days
	1.25		40	
Arithmetic mean	1.24	1.21	43.7	45.4
CV (%)	4.1	0.9	3.8	3.1
Accuracy (%)	99.2	96.8	109.3	113.5
Variation (%)	−2.42		3.89	

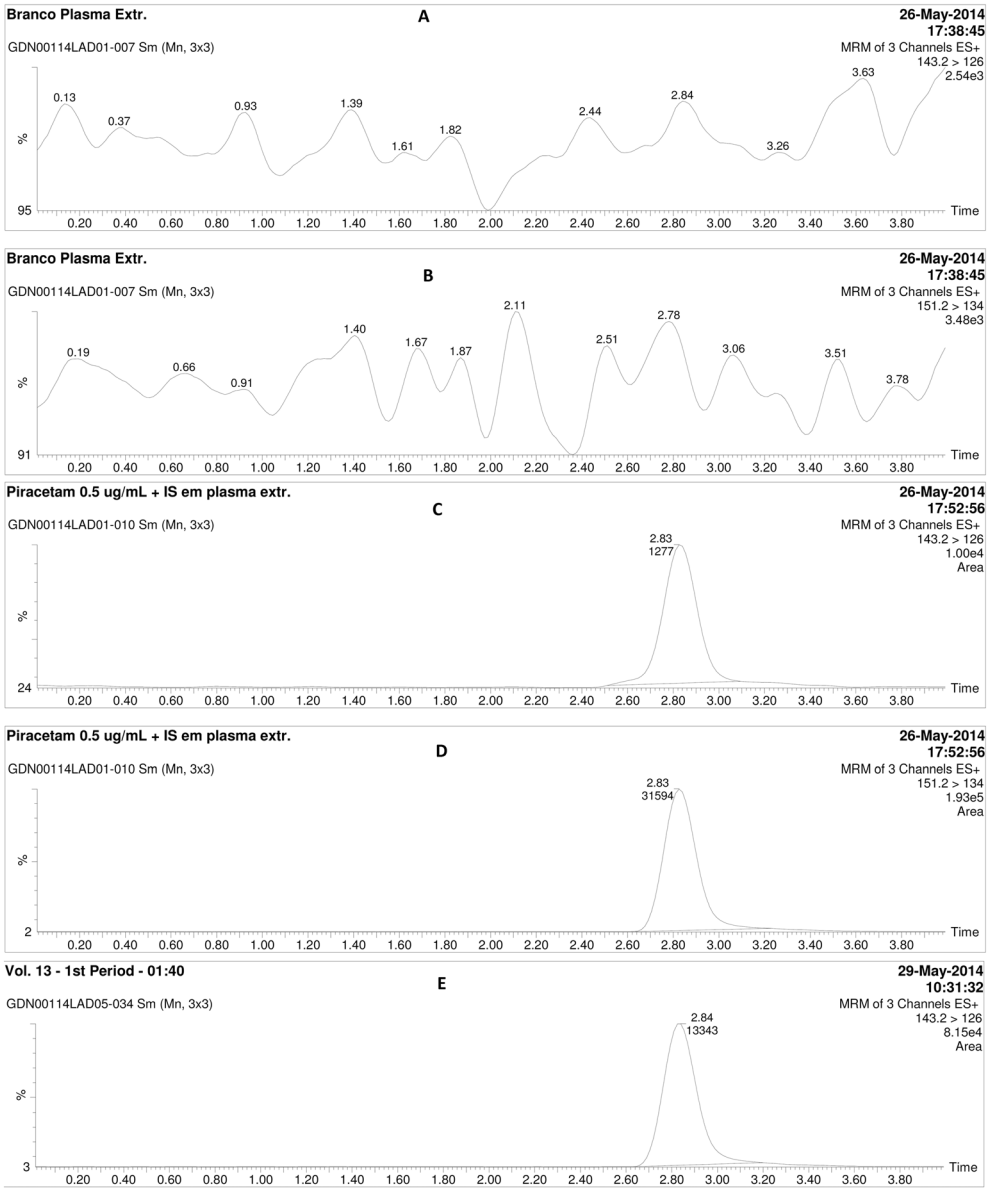

Figure 1. Selected reaction monitoring (SRM) chromatograms of blank normal human plasma: (A) piracetam; and (B) Piracetam d-8. SRM chromatograms of the LOQ: (C) piracetam; and (D) Piracetam d-8. (E) SRM chromatogram of a sample (vol. 13–1; period, 1:40).

were hospitalized at 06:00 p.m. Volunteers received specialized assistance and care during all treatment periods, which included a brief investigation of their conditions upon confinement and at time of discharge, in order to evaluate their adherence to the requirements of the clinical protocol. Standard meals were administered at 07:00 p.m. (dinner) and 10:00 p.m. (snack). After an overnight fast (approximately 8 h), they received a dose of three tablets of Piracar® (270/330 mg tablet) or one tablet of Nootropil® (800 mg tablet) at approximately 6:00 a.m. The formulations of piracetam were administered directly into the volunteer's mouth. Water (200 mL) was given immediately after the drug administration. All volunteers were required to remain fasting until 2:00 h after dose administration, when a xanthine-free standard breakfast was available. A xanthine-free standard meal was provided at 5 (lunch), 8 (afternoon snack), 12 (lunch) and 15 (dinner) h after dose. After the 24 h blood withdrawal, a standardized meal (breakfast) was served, and after a medical evaluation, volunteers were discharged. A standard meal (lunch) of rice, beans, vegetables and fried chicken plus a fruit as dessert was consumed. Morning, afternoon and evening snacks were also provided consisting of crackers, bread, jelly, cakes and apples. No other food was permitted during the 'in-house' period and liquid consumption was allowed *ad libitum* after lunch (with the exception of xanthine-containing drinks, including tea, coffee and cola). Systolic and diastolic arterial pressure (measured noninvasively with a sphygmomanometer), heart rate and temperature were recorded at specific time intervals.

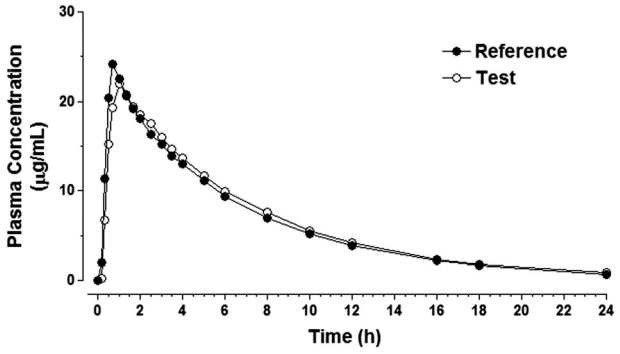

Figure 2. Piracetam plasma mean concentration vs time profile obtained after oral administration of piracetam.

Pharmacokinetic and statistical analysis

Comparative bioavailability between the two formulations was assessed by calculating individual test/reference ratios for the C_{max}, area under curve (AUC) of plasma concentration until the last concentration observed (AUC_{last}), the area under the curve of plasma concentration from zero until T_{max} ($AUC_{trunctmax}$) and the area under curve between the first sample (pre-dosage) and infinity (AUC_{0-inf}). The C_{max} and T_{max} were obtained directly from the curves. The AUC_{last} was calculated by applying the linear–log trapezoid rule. Extrapolation of these areas to infinity (AUC_{0-inf}) was done by adding the value C_{last}/k_e to the calculated AUC_{last} (where C_{last} = the last detectable concentration). The AUC_{inf}/dose and C_{max}/dose 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 comparative bioavailability as proposed by the US Food and Drug Administration.

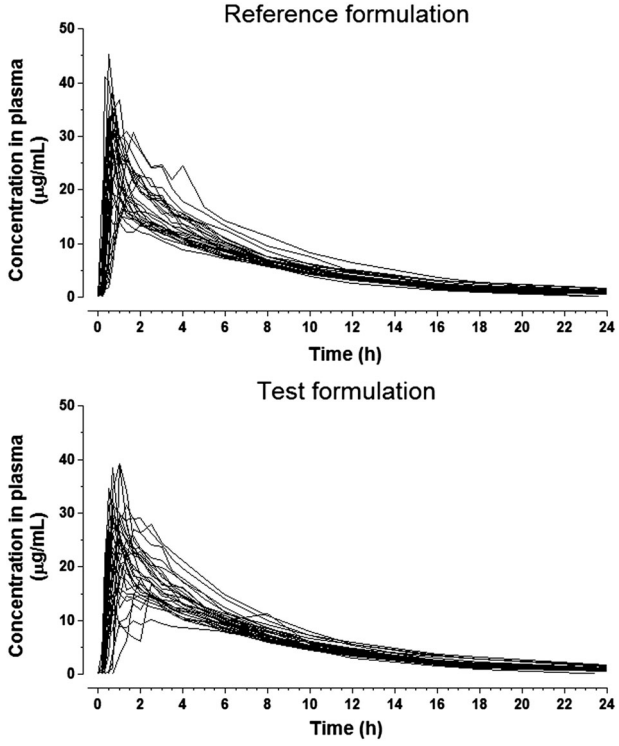

Figure 3. Piracetam plasma concentration vs time profile for each volunteer obtained after oral administration of piracetam.

Results and discussion

Method development

The method was linear, calculating the calibration curve by a least-squares regression for piracetam concentrations from 0.5 to 50 µg/mL (calibration curve equation: $0.0552910x + 0.0118509$, $r = 0.999739$).

The samples were analyzed in different matrices (normal, hemolyzed and lipemic) and there was no interference in the retention time for the analyte and internal standard. No significant matrix effect was observed (overall mean 1.2312072; overall CV 5.2%). The limit of quantification (LOQ), defined as the lowest concentration at which both the precision and accuracy were <20%, was 0.5 µg/mL. The within- and between-run precision and accuracy for the LOQ and QCs are summarized in Table 1.

The stability tests (Tables 2–4) indicated no significant degradation under the conditions described above, including in the post-processing stability test (42 h 8 min; 8°C), freeze–thaw test (three cycles; −30–5°C) 418 days long term at − 20°C and short-term stability at room temperature (13 h 22 min). The mass chromatograms are shown in Fig. 1, in which the retention time of both piracetam and Piracetam d-8 was 2.80 ± 0.3 min.

Bioanalytical performance

This is the first LC-MS-MS method developed for measuring piracetam in human plasma. Piracetam has been determined in human plasma (LOQ 0.01–3 µg/mL, retention time 5.1–6 min, liquid–liquid

Table 5. Mean pharmacokinetic parameters obtained from 30 volunteers after administration of piracetam formulations

Variable	Unit	*N*	Median	SD	Minimum	Maximum	CV (%)
				Reference			
$AUC_{\%\ extrap}$	(%)	30	4.54	2.05	1.96	13.03	45.14
AUC_{inf}	([µg*hr]/mL)	30	155.63	27.14	117.45	235.91	17.44
AUC_{last}	([µg*hr]/mL)	30	148.54	26.01	112.16	223.74	17.51
C_{last}	(µg/mL)	30	0.92	0.32	0.51	1.74	34.37
C_{max}	(µg/mL)	30	28.96	7.37	13.90	45.20	25.44
k_e	(1/hr)	30	0.14	0.03	0.08	0.19	18.57
$T_{1/2}$	(hr)	30	5.21	1.08	3.57	9.11	20.68
T_{max}	(hr)	30	0.87	0.42	0.33	2.00	47.95
Test							
$AUC_{\%\ extrap}$	(%)	30	4.48	2.38	2.05	12.32	52.99
AUC_{inf}	([µg*hr]/mL)	30	161.07	24.53	123.20	217.97	15.23
AUC_{last}	([µg*hr]/mL)	30	153.98	24.59	117.37	211.04	15.97
C_{last}	(µg/mL)	30	0.89	0.31	0.51	1.71	34.79
C_{max}	(µg/mL)	30	26.02	7.04	14.70	39.10	27.06
K_e	(1/hr)	30	0.14	0.02	0.09	0.19	16.56
$T_{1/2}$	(hr)	30	5.26	0.88	3.64	7.69	16.77
T_{max}	(hr)	30	1.11	0.58	0.50	2.50	52.19

AUC, Area under the concentration–time curve; C_{max}, peak concentration; K_e, elimination rate constant; $T_{1/2}$, Half-life time; T_{max}, time to reach C_{max}.

Table 6. Geometric mean of the individual AUC_{inf}/dose and C_{max}/dose ratios (test/reference), the respective 90% confidence intervals (CI) and CV

Piracar® [3 × (270/330 mg)]/ Nootropil® (800 mg; n = 30)	Piracetam			
	Parametric analysis			
	Geometric mean	90% CI	Power	Intra-subject CV
C_{max}/dose	88.49	81.19–96.46	0.9943	19.76
AUC_{inf}/dose	102.55	100.62–104.51	1.0000	4.32

extraction; Barkat *et al*., 2014; Curticapean and Imre, 2007; Louchahi *et al*., 1995; Siddiqui *et al*., 2014), in pharmaceutical syrup (LOQ 0.01 μg/mL, retention time 6 min, liquid–liquid extraction; Siddiqui *et al*., 2014) and urine (LOQ 100 μg/mL, liquid–liquid extraction) by HPLC. Piracetam has been determined in rat plasma (LOQ 0.1 μg/mL, retention time 1.8 min, liquid–liquid extraction, internal standard oxiracetam) by LC-MS-MS (Barkat *et al*., 2014). Our method has good sensitivity (LOQ of 0.5 μg/mL) for pharmacokinetic evaluation (C_{max} = 28.96 μg/mL; %LOQ/C_{max} 1.7%) in humans, has adequate selectivity with the use of a deuterated standard and can be carried out quickly (run time 2.8 min), permitting high throughput. Furthermore, this method involves a very simple liquid extraction.

Pharmacokinetic study

The piracetam was well tolerated at the administered doses and no significant adverse reactions were observed or reported. A total of 13 adverse events were reported during the study, nine of which were considered probably related to the administration of piracetam (headache, n = 8/hypotension, n = 1). The other adverse events were hypertriglyceridemia (n = 2) and low hematocrit (n = 2). The biochemical parameters presented no clinically relevant alterations.

The mean and individual piracetam plasma concentrations vs time profiles after oral dose [three tablets of Piracar® (270/330 mg tablet) or one tablet of Nootropil® (800 mg tablet)] of piracetam are shown in Figs. 2 and 3, respectively. Table 5 shows the mean pharmacokinetic parameters obtained from 30 volunteers after the administration of piracetam. Table 6 shows geometric mean of the individual AUC_{inf}/dose and C_{max}/dose (test/reference formulation), the respective 90% confidence intervals (CI power) and intra-subject CV for the 30 volunteers.

After oral administration of the piracetam tablet (800 mg) to the volunteers, the peak plasma concentrations (C_{max} 28.96 μg/mL) of piracetam were 2 (C_{max} 14.53 μg/mL) and 3 (C_{max} 8 μg/mL) times higher than the values reported in the literature for the same dose (800 mg; Barkat *et al*., 2014; Siddiqui *et al*., 2014). The T_{max} (0.87 h) of piracetam was 2.5 times less than the value reported in the literature (T_{max} 2.33 h) for the same (800 mg) dose (Barkat *et al*., 2014). The plasma concentrations of both studies were quantified by HPLC, the volunteers were Pakistani, and the reference formulation (Ceremin) was different (Barkat *et al*., 2014; Siddiqui *et al*., 2014). These factors may explain the observed differences in plasma concentration.

Conclusion

Since the 90% CI for AUC_{inf}/dose and C_{max}/dose ratios were within 80–125% (US Department of Health and Human Services *et al*., 2003), it was concluded that piracetam (Piracar®; 270/330 mg tablet) formulation manufactured by Biolab Sanus Farmacêutica Ltda has a bioavailability equivalent to that of the piracetam (Nootropil®; 800 mg tablet) formulation regarding both the rate and the extent of absorption.

This paper reports for the first time on a novel method to measure piracetam by LC-MS-MS in human plasma. This method offers advantages in terms of a simple liquid–liquid extraction without clean-up procedures, as well as a faster run time (3.8 min). The LOQ of 0.5 μg/mL is well suited for pharmacokinetic studies. The assay performance results indicate that the method is precise and accurate enough for the routine determination of the piracetam in human plasma.

Acknowledgments

This pharmacokinetic trial was funded by Biolab Sanus Farmacêutica Ltda. Jim Hesson of AcademicEnglishSolutions.com revised the English.

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