

Validation of a Selective Method for Determination of Paroxetine in human plasma by LC-MS/MS

Massaroti, P.; Cassiano, N. M.; Duarte, L. F.; Campos, D. R.; Marchioretto, M. A. M.; Bernasconi, G.; Calafatti, S.; Barros, F. A. P.; Meurer, E.C.; Pedrazzoli, J.

Clinical Pharmacology and Gastroenterology Unit, São Francisco University Medical School, Av. Sao Francisco de Assis 218, 12916-900, Bragança Paulista, SP, Brazil.

Received April 6, 2005, Revised June 22, 2005, Accepted June 22, 2005, Published Aug 17, 2005,

ABSTRACT

Purpose. A sensitive, robust, and selective liquid chromatographic – tandem mass spectrometric method (LC-MS/MS) was developed and validated for paroxetine quantification in human EDTA plasma. **Methods.** Sample preparation was based on liquid-liquid extraction using a mixture of ethyl acetate/hexane (50/50; v/v) to extract the drug and internal standard from plasma. Chromatography was performed on a C-18 analytical column and the retention times were 1.6 and 1.7 for paroxetine and fluoxetine (IS), respectively. The ionization was optimized using ESI(+) and selectivity was achieved by tandem mass spectrometric analysis using MRM functions, 330.0 → 70.0 and 310 → 43.9 for paroxetine and fluoxetine. **Results.** Analytical curve ranged from 0.2 to 20.0 ng/mL. Inter-day precision and accuracy of the quality control (QC) samples were < 15% relative standard deviation (RSD). Analyte stability during sampling processing and storage were established. **Conclusion.** Validation results on linearity, specificity, accuracy, precision as well as application to the analysis of samples taken up to 120 h after oral administration of 20 mg of paroxetine in 28 healthy volunteers were found to be of good performance in bioequivalence study.

Correspondence to: Eduardo Cesar Meurer, UNIFAG, Universidade Sao Francisco, Sao Francisco de Assis Ave. 218, 12.916.900, Braganca Paulista, SP, Brazil.
E-mail: eduardo.meurer@saofrancisco.edu.br

INTRODUCTION

Paroxetine (5-(4-*p*-Fluorophenyl-3-piperidyl-methoxy)-1,3-benzodioxole, CAS – 61869-08-7) is a phenylpiperidine compound that acts as a potent and selective serotonin reuptake inhibitor (SSRI)(1). Its action appears to account for the antidepressant activity observed with this class of drugs (2) that is safe and effective for treatment of depressive and obsessive-compulsive disorders (3). This drug bioavailability is not affected by food or antacids; it means half-life of 24 hours is consistent with once-a-day dosing (4), and undergoes a first pass metabolism which reduces the bioavailability at therapeutic doses to about 30-60%. Maximum blood levels are reached 2 to 8 hours after oral administration. In the plasma 95% of the drug is bounded to protein. Paroxetine is eliminated after transformation in the liver into pharmacologically inactive metabolites. Although clinical practice has not reported problems of the drug interactions so far, comedications with tricyclic antidepressants should be avoided. The most frequent side effects of paroxetine concern nausea and somnolence (5).

Different methods dedicated to the determination of Paroxetine in biological fluids have already been reported. Such methods had used gas chromatography (GC) with nitrogen and MS detections (6) or liquid chromatography (LC) with UV (7) or mass spectrometric analysis (8-10). To our knowledge, this is the low solvent consuming and best recovery mass spectrometric analysis method applied to bioequivalence so far reported.

This paper describes a validated method combining liquid-liquid extraction, reversed-phase LC and MS/MS detection to perform the selective determination of Paroxetine. Tandem mass spectrometry was selected in order to improve the selectivity and sensitivity of the method of determination. The LC conditions, the type of extractor solvent and the MS/MS optimization were investigated in order to select the most appropriate operating conditions. The validation of the method was performed considering parameters such as linearity of the chromatographic response, precision and accuracy that meets the accepted criteria for bioanalytical method validation (11), and employed in bioequivalence study of two paroxetine 20 mg paroxetine tablet formulations (standard and reference).

EXPERIMENTAL

Chemical

Paroxetine (lot number R40285) and fluoxetine (lot number F-1) were obtained from Zydus Cadila Healthcare Limited and U.S. Pharmacopeia, respectively. Acetonitrile and methanol (HPLC grade), n-hexane were purchased from Mallinckodt (St Louis, MO, USA) while formic acid, ethyl acetate, sodium hydroxide p.a. from Merck (Darmstadt, Germany). The water was purified using a Milli-Q system (Millipore Corporate Headquarters, USA).

Equipment and columns

The LC system used was an Agilent (Agilent Technologies, Inc., Palo Alto, CA) liquid chromatograph equipped with an isocratic pump (1100 series), an auto-sampler (1100 series) and a degasser (1100 series). Mass spectrometric analysis was performed using a Quattro Micro (triple-quadrupole) instrument from Micromass (Manchester, UK) working with ESI interface. The data acquisition and system controlling were obtained using MassLynx version 3.5 software from Micromass. Nitrogen was produced by an on-site nitrogen generator from Jun-Air.

The used stationary phase for paroxetine analytical run was C18 packed in a (50 x 2.0 mm) Polaris 5 μm particle size column from Varian[®]. All analytical runs were preceded by a Securityguard column packed with C18 from Phenomenex[®] (Torrance, CA, USA).

LC-MS/MS conditions

All chromatographic experiments were carried out in the isocratic mode at room temperature. The mobile phase for the chromatographic run was a solution of formic acid 0.1% in acetonitrile: water (6:4; v/v) pumped at a flow rate of 0.15 mL/min. The injection volume was 10 μL and the total run time is set for 2.6 min and typical standard retention times were 1.6 min for paroxetine and 1.7 min for fluoxetine.

Mass spectrometric analysis was performed using Quattro Micro equipment working with an ESI source in the positive ion mode. The conditions used for such analysis are: desolvation gas (N_2) flow-rate was 280 L/h, cone gas flow-rate was 70 L/h, the source and desolvation gas temperatures were

100°C and 350°C respectively and the ESI source tip voltage of 4.4 kV. The mass spectrometer generated the protonated molecules (MH^+) of m/z 330 and m/z 310 for paroxetine and fluoxetine (IS) respectively. These parent ions (MH^+) of m/z 330 for paroxetine and of m/z 310 for IS were selected using the first quadrupole analyzer (Q_1) and then dissociated into the second quadrupole used in rf/only mode (collision cell, q_2) with a collision energy of 30 eV for paroxetine and 10 eV for fluoxetine using Argon as collision gas. The product ions of m/z 70 for paroxetine and m/z 44 for IS were monitored via the third quadrupole mass analyzer (Q_3).

Standard solutions

Preparation of Calibration Standards

Stock solution of paroxetine was prepared by dissolving the drug in methanol obtaining a final concentration of 100 $\mu\text{g}/\text{mL}$. An aliquot of this solution was placed in a glass tube and the solvent was evaporated under a compressed air stream. The dried analyte was reconstituted using blank plasma to a final concentration of 1000 ng/mL and the solution was vortex-mixed for 15s. From this solution six calibration standard solution containing 0.2, 0.5, 1.0, 5.0, 10.0, 20.0 and three quality controls solutions at concentrations of 0.6, 8.0 and 16.0 ng/mL were prepared in blank plasma.

Aliquot (0.5 mL) of plasma standards were dispensed in into properly labelled eppendorff tubes and stored at -70 °C until required for assay. For each assay, one tube of each concentration is thawed immediately before sample extraction, giving enough volume for analyses.

Stock solution of fluoxetine internal standard was prepared dissolving the drug in methanol to a final concentration of 100 $\mu\text{g}/\text{mL}$. This solution was diluted with methanol to a final concentration of 500 ng/mL.

Sample preparation

The procedure of extraction was applied for all subject samples, analytical curve and quality control standards. All frozen human plasma samples were previously thawed to room temperature. In order to perform the sample extraction, 0.5 mL of sample (in human plasma) was dispensed in Eppendorff vials, after that added to this plasma 100 μL of 0.1 mol/L sodium hydroxide, 25 μL of 500ng/mL fluoxetine standard solution and vortex-mixed during 1 min.

Then 1000 μL of a ethyl acetate/hexane (50:50; v/v) was added to vials and vortex-mixed again during 10 min. The mixture were centrifuged at 14000 rpm, during 10 min, at 4°C, the upper organic phase (700 μL) was transferred to another Eppendorff vial and evaporated to dryness under a compressed air stream. The residues were reconstituted with 100 μL of mobile phase and 10 μL was injected.

Bioequivalence study

Twenty eight male volunteers aged between 18 and 50 years and index of corporal mass within 19 and minor of 30 kg/m^2 were selected for the study after assessment of their health status by clinical evaluation (physical examination, ECG) and the following laboratory tests: albumin, alkaline phosphatase, AST, ALT, blood glucose, creatinine, $\mu\text{-GT}$, total bilirubin, and total protein, triglyceride, total cholesterol, hemoglobin, hematocrit, total and differencial white cell counts, routine urinalysis and negative sorology for HIV, HBV and HCV. All the subjects gave written informed consent and the Universidade São Francisco Ethics Committee approved the clinical protocol. The study was conducted in accordance with the provisions of the Declaration of Helsinki (1964), Tokio (1975), Venice (1983), Hong Kong (1989), Somerset West (1996) and Edinburg (2000) revisions.

The volunteers possess the following clinical characteristics expressed as mean \pm SD (range): age 28.25 ± 6.03 years (18-42), height 1.73 ± 0.07 m (1.57-1.84), body weight 71.72 ± 6.97 kg (57-87). The study was a single-dose, two-way randomized crossover design with 13 days washout period between the doses.

During each period, the volunteers were hospitalized at 7,5 p.m. and had a light supper before the 10 p.m., and after an overnight fast they received (at ~ 7 a.m.) a single dose of paroxetine (20 mg of either formulation). Water (200 mL) was given immediately after the drug administration and the volunteers were then fasted for 4 h, after which period a standard lunch was served. After 7 hours was served a snack; After 12 hours, a evening meal was provided and 14 hours a supper was served. 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). At 0, 2, 4, 8, 12, 24 and 36 h after the dose

administration, systolic and diastolic arterial pressure (measured non-invasively with a sphygmo-manometer), heart rate and temperature were recorded. The hospitalar period was 36 h. The volunteers became to the unit to supply de blood samples at 36,0; 48,0; 72,0; 96,0 and 120,0 h post-dosing. The following formulations were employed: paroxetine (test formulation) and Aropax® (standard reference formulation from Glaxo SmithKline Brazil).

Blood samples (9 mL) from a suitable antecubital vein were collected by indwelling catheter into heparin-containing tubes at 0, 0,5; 1,0; 1,5; 2,0; 2,5; 3,0; 4,0; 4,5; 4,75; 5,0; 5,25; 5,5; 6,0; 8,0; 10,0; 12,0; 24,0; 36,0; 48,0; 72,0; 96,0 and 120,0 h post-dosing. The blood samples were centrifuged at ~ 2000 g for 10 minutes at room temperature and the plasma was stored at -70° until assayed for paroxetine content.

RESULTS AND DISCUSSION

Extraction conditions

Solid-phase extraction has been shown by Juan *et al.* (9) to be suitable for simultaneous determination of paroxetine and some other nontricyclic anti-depressants. However, a less expensive liquid-liquid extraction was found desirable.

Different kind of organic solvents and mixtures of solvents were used resulting in different polarities for extractor phases. The better extraction was the one using liquid-liquid extraction with ethyl acetate/hexane (50:50; v/v) a similar extraction to the one described by Zhu *et al* (8), who have shown good recoveries. The method was improved by replacing cyclohexane with hexane and adding a base. Another improvement of the extraction method was the use of 1000 μL extracting solvent instead 7000 μL used by Zhu which results is shorter evaporation time. The method reported herein is an environmental fair and cheaper method considering the amount of extracting solvent used.

The recovery from spiked plasma samples were calculated by comparing peak areas obtained from freshly prepared samples with those found by direct injection of methanolic solutions at the same concentration into the LC-MS/MS system, using the same auto-sampler). This extraction gave the best

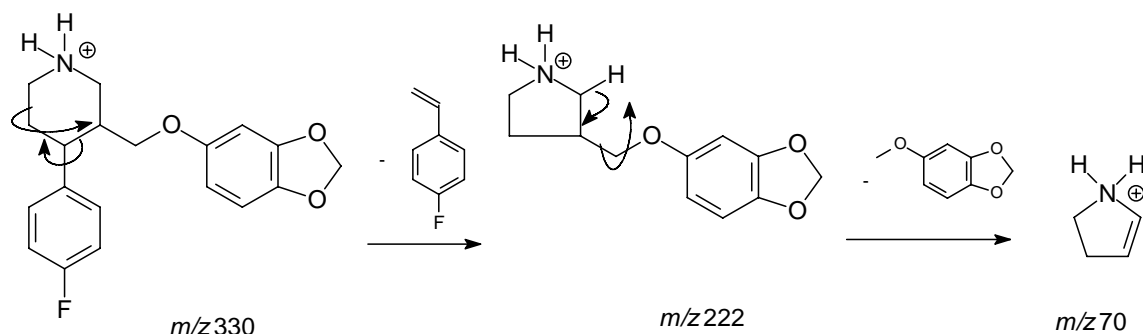


Figure 1: Collision induced dissociation (CID) with nitrogen at 30 eV of energy of the MH^+ ion of $m/z\ 330$.

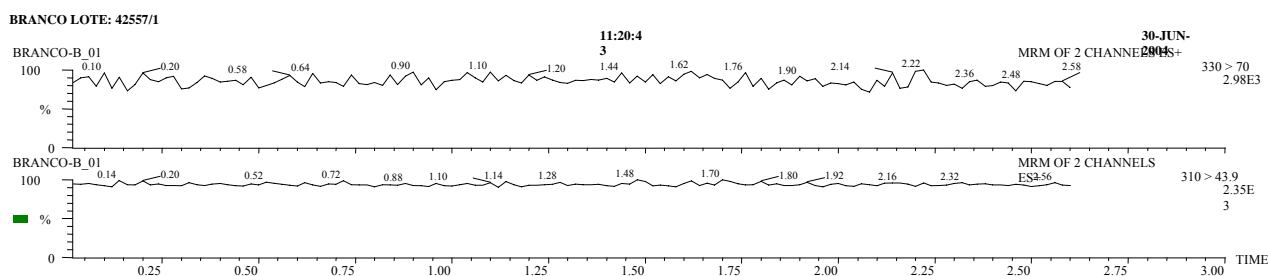


Figure 2: a) Normal Human Plasma Normal chromatogram, lot # 42557/1

recoveries and low ionic suppression as has been shown in the blank, hyperlipemic and haemolised matrix.

LC conditions

Paroxetine is a compound with both polar and apolar counterparts and this characteristic made it feasible to be handled using apolar C18 (ODS, octadecyl) stationary phase toward a reversed-phase chromatography. Previously Naidong *et al.*(10) have used a silica column with a very short run time. However, for bioequivalence studies that require a great number of sample injections, they used a C18 rather than the silica column.

To enhance the throughput capability, 50 mm column with a run time of 2.6 min was used. Previous chromatographic separation was not necessary due to the mass spectrometric separation into the two MRM channels that select specifically paroxetine and fluoxetine. The mobile-phase used was optimized for ionic response of paroxetine.

Mass Spectrometric Analysis — optimization of ESI(+)-MS/MS conditions

Among the different possible detection techniques that can be coupled to LC, mass spectrometry is the most suitable one for bioanalytical determinations due to its high selectivity and sensitivity (12). MS detection in LC became feasible in early 90's by the emergence of the atmospheric pressure ionization interfaces (API) such as atmospheric pressure chemical ionization (APCI) and Electrospray ionization (ESI) that expanded the analysis of mass spectrometry to more polar compounds including compounds of pharmacological interest (13).

In the present work, the MS optimization was performed using direct infusion of a methanolic solution of both paroxetine and fluoxetine (IS) into de ESI source of the mass spectrometer and parameters such as tip (ESI), extractor, and cone voltages were adjusted. Nebulizer and desolvation gases were optimized to obtain better spray shape resulting in better ionization and droplet drying to

form the protonated ionic paroxetine and fluoxetine (IS) molecules.

The most suitable collision energy was determined by observing the response obtained versus selectivity response for the fragment ion for each compound. The best collision energies set were 30 eV for paroxetine and 10 eV for fluoxetine obtaining fragments of m/z 70 and 44 from the protonated compounds of m/z 330 (paroxetine) and m/z 310 (fluoxetine). The selectivity of the used MRM channels were observed comparing both collision induced dissociation mass spectrum indicating that two different routes of fragmentation lead to different obtained fragment. Figure 1 depicts the proposed dissociation mechanism for protonated paroxetine toward two neutral losses.

Paroxetine protonation occurs mainly on nitrogen atom in the molecule forming MH^+ ion of m/z 330. The selective dissociation occurs by tandem 4-fluorostyrene and 5-methoxy-1,3-benzodioxole loss forming a very stable 2,4-dihidropyrrolidinium cation with the positive charge at the nitrogen atom close an double bond. Subsequently, chromatograms were obtained using MRM mode (Multiple Reactions Monitoring) that is, selecting parent ions dissociating them and finally analyzing the daughter selective ions reaching great selectivity and sensitivity of this operational mode.

The mobile phase was tested taking in account the response of the analyte toward ionization, and after the MRM channels tuned, we changed the mobile-phase from organic phase to more aqueous phase with acid dopant to get a fast run LC method to enhance the throughput capability in detriment of the chromatographic separation, and the better signal was obtained for formic acid 0.1% in acetonitrile: water (6:4; v/v).

Validation Selectivity

To test the selectivity of the method, four regular, one hyperlipemic, and one haemolysed blank samples of human plasma were obtained from six individuals and then analysed using the proposed extraction procedure and chromatographic conditions to compare those obtained with an aqueous solution of the analyte at a concentration near to the limit of quantification.

To test the possibility of interference, blank samples were tested versus aqueous analyte solution

using the proposed extraction procedure and chromatographic conditions at a concentration near to the limit of quantification. No significant interference with the drug, metabolites or internal standard was found (Figure 2).

Figure 3 shows MRM chromatogram channels of non-zero 0.6 ng/mL paroxetine standard and IS obtained from a regular analytical run.

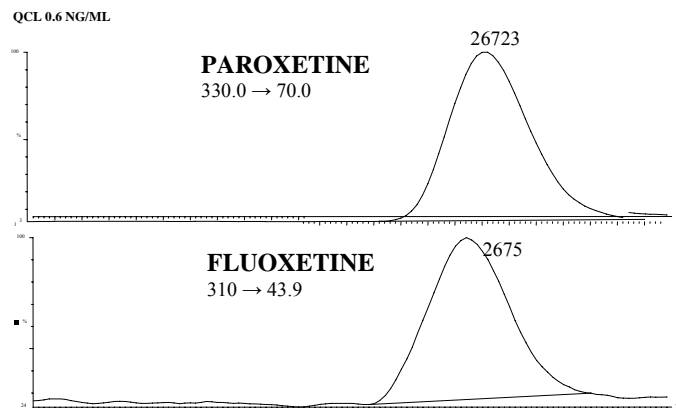


Figure 3: Representative MRM chromatograms of QCL: (a) channel for paroxetine spiked human plasma containing 0.6 ng/mL (b) channel for fluoxetine (IS).

Recovery

The extraction efficiency of paroxetine from human plasma was determined by analyzing the quality controls samples. The recovery in three concentrations were determined by comparing peak areas obtained from plasma sample and those found by direct injection of a methanolic standard solution at the same concentration using the same conditions (Table 1).

The recovery of paroxetine in three concentrations were determined by comparing peak areas obtained from plasma sample and those found by direct injection of a methanolic standard solution at the same concentration, using the same conditions, the mean recovery of paroxetine was 78.7% (Table 1). The recovery of IS fluoxetine tested using the method described for paroxetine was 87.34%. The observed close recoveries for the drug and IS illustrate the suitability of the extraction procedure.

Table 1: Recovery validation data

Sample	Paroxetine			Paroxetine 500.0 ng/mL
	QCL	QCM	QCH	
1	0.42	5.23	17.33	433.29
2	0.31	6.63	14.18	431.24
3	0.38	6.42	13.39	498.95
4	0.37	7.37	13.95	414.22
5	0.41	7.71	12.99	405.68
MEAN (NG/ML)	0.38	6.67	14.37	436.68
CV (%)	12.06	14.44	11.97	8.40
RECOVERY (%)	62.90	83.39	89.79	87.34

Nominal Concentration: QCL= 0.6 ng/mL, QCM= 8.0 ng/mL, QCH= 16.0 ng/mL

Analytical Curve and Detectability

The analytical calibration curves were constructed with 6 non-zero standards ranging from 0.2 to 20 ng/mL. The quantification limit (LQ) was calculated using signal to ratio of 9 obtaining 0.1 ng/mL. Otherwise the lowest point in the analytical curve was defined as 0.2 ng/mL due to the better precision and accuracy.

The linear regression analysis of paroxetine was made by plotting the peak area ratio (y) versus the inverse of analyte concentration (1/x) in ng/mL. The linearity of the relationship between peak area and concentration was demonstrated by the determination coefficients (r^2) obtained for the regression lines of paroxetine. Precision and accuracy of the analytical curve were < 15% relative standard deviation (RSD). Figure 4 shows an analytical curve for paroxetine using paroxetine as IS.

Determination of the Quality Control Concentrations

Quality control samples concentrations were defined using some rules. The low QC concentration was 3 times the lowest point in the analytical curve (0.6 ng/mL), the average QC was calculated as just about the intermediate between low QC and high QC samples (8 ng/mL), and the high QC 80% of the highest analytical curve point (16 ng/mL).

Intra- and Inter-Batch Validation Parameters

The precision and accuracy of the method were evaluated by quintuplicate analyses of three quality control samples and the lowest point in the analytical curve. Calibration standards, the quality controls and the LQ were analyzed on three different batches in order to determine intra- and inter-batch precision and accuracy. The acceptance criteria for each quality control was that the coefficient of variation (CV) and accuracy must not exceed 15% and for the LQ tolerance of 20%.

The accuracy and intra- inter-batches of the method are shown on Table 2.

Long-Term Stability

To evaluate long-term stability, the time between the date of the first sampling and the date of last sample analysis was used to define the long-term period. Aliquots of each sample type were initially frozen at -70°C and then thawed to be extracted and tested. Then the performed tests indicate that the analyte on human plasma can be stored at -70 °C for at least 73 days without showing any degradation.

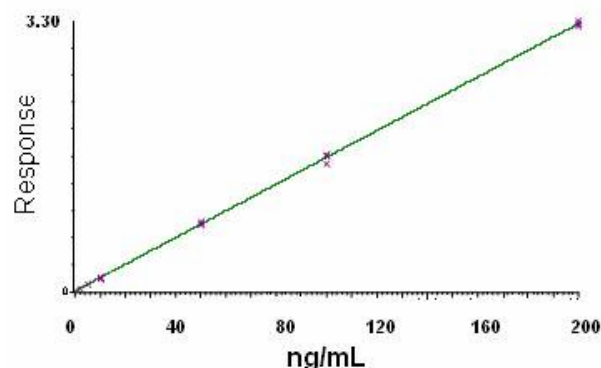


Figure 4. MRM abundances of characteristic fragment ion of paroxetine (330→70) as a function of paroxetine in plasma concentration (fluoxetine as IS).

Table 2: Accuracy and intra- inter-batches variability

Concentration n (ng/mL)	First batch (n=5)		Second batch (n=5)		Third batch (n=5)		Pooled (n=15)	
	Accuracy (%)	C.V. (%)	Accuracy (%)	C.V. (%)	Accuracy (%)	C.V. (%)	Accuracy (%)	C.V. (%)
0.20	107.00	7.09	111.00	2.01	111.00	3.77	109.67	4.71
0.60	100.67	7.37	103.00	3.86	104.00	2.91	102.56	4.87
8.0	100.83	4.76	107.48	3.61	105.73	0.94	104.68	4.23
16.0	102.14	5.24	113.01	0.92	112.40	1.56	109.18	5.50

Application to Biological Samples

The proposed method was applied to the determination of paroxetine in plasma samples for the purpose of establishing the bioequivalence of a 20 mg formulation capsule in 28 healthy volunteers. Typical plasma concentration vs time profiles are shown in Figure 5. Plasma concentrations of paroxetine were in the standard curve range and remained above the 0.2 ng/mL quantitation limit for the entire sampling period. The pharmacokinetic parameters, for the standard (reference drug) and test (generic drug), obtained were described as follows. The value of area under the plasma concentration vs time curve from time 0 to the last sampling time (AUC_{0-t}) was 209.46 ± 289.86 for the standard and 225.04 ± 291.91 for the test (ng.h/mL), and area under the plasma concentration vs time curve from time 0 to time infinite (AUC_{∞}) was 238.19 ± 335.97 for the standard and 246.11 ± 316.02 (ng.h/mL) for the test. The observed maximum plasma concentration (C_{max}) that is collect time independent was 8.23 ± 8.08 for the standard and 9.02 ± 8.82 for the test (ng/mL), time to observed maximum plasma concentration (T_{max}) was 4.97 ± 1.97 for the standard and 5.03 ± 1.91 for the test (h), and elimination half-life was 21.31 ± 17.26 for the standard and 17.37 ± 12.24 for the test (h). In addition, the mean ratio of the plasma concentration vs time for bromopride profile of AUC_{0-t} divided by AUC_{∞} was above 84% that is higher than the US Food and Drug Administration. These results demonstrate that this method is simple, sensitive, reproducible and accurate and meets the requirement of the report of the conference on Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic studies (11).

The last sampling time concentration (t=120h) was predicted using a one compartment

open pharmacokinetic model. Therefore, in light of the present and previously reported (2,3,5) data, it is conclude that validated pharmacokinetic parameters can be generated using the analytical method described herein.

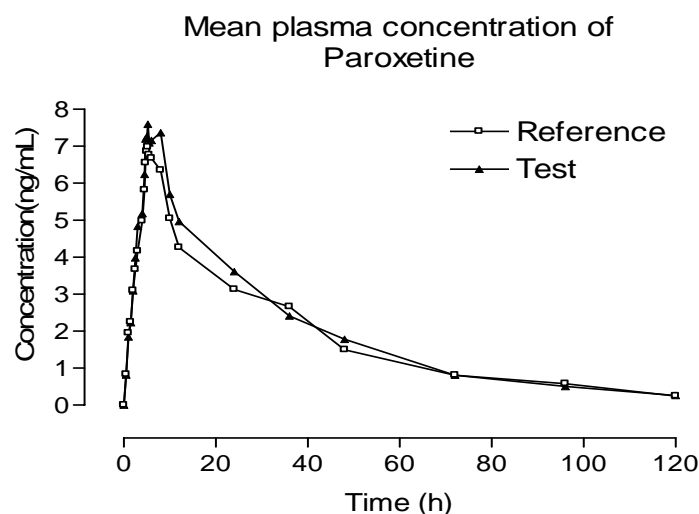


Figure 5. Mean plasma concentrations of test vs reference after a 20 mg single oral dose (28 healthy volunteers).

CONCLUSIONS

A sensitive, accurate, precise, and robust method based on LC-MS/MS has been developed for determination of paroxetine at subnanogram level in human plasma. The method was validated to meet the requirements of the pharmacokinetic investigation.

REFERENCES

- 1) Boyer, W. F.; Feighner, J. P., an overview of Paroxetine. *J Clin Psychiatry*. 53: 3-6, 1992.
- 2) Dechant, K. L.; Clissold, S. P., paroxetine. a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in depressive illness. *Drugs*. 41(2):225-53, 1991.
- 3) Catterson, M.L.; Preskorn, S.H., pharmacokinetics of selective serotonin reuptake inhibitors: clinical relevance. *Pharmacol Toxicol*. 78(4):203-8, 1996.
- 4) Nemeroff, C. B., paroxetine: an overview of the efficacy and safety of a new selective serotonin reuptake inhibitor in the treatment of depression. *J Clin Psychopharmacol*. 13:10S-17S, 1993.
- 5) Hiemke, C., paroxetine: pharmacokinetics and pharmacodynamics *Fortschr Neurol Psychiatr*. 62 1:2-8, 1994.
- 6) (a) Eap, C.B.; Bouchoux, G.; Amey, M.; Cochard, N.; Savary, L.; Baumann, P., simultaneous determination of human plasma levels of citalopram, paroxetine, sertraline, and their metabolites by gas chromatography-mass spectrometry. *J Chromatogr Sci*. Jul;36(7):365-71, 1998. Maurer, H.H.; Bickeboeller-Friedrich, J., screening procedure for detection of antidepressants of the selective serotonin reuptake inhibitor type and their metabolites in urine as part of a modified systematic toxicological analysis procedure using gas chromatography-mass spectrometry. *J Anal Toxicol*. 24(5):340-7, 2000.
- 7) (a) Erk, N.; Biryol, I., voltammetric and HPLC techniques for the determination of paroxetine hydrochloride. *J Pharmazie*. 58(10):699-704, 2003 (b) Duverneul, C.; de la Grandmaison, G.L.; de Mazancourt, P.; Alvarez, J.C., a high-performance liquid chromatography method with photodiode-array UV detection for therapeutic drug monitoring of the nontricyclic antidepressant drugs. *Ther Drug Monit*. 25(5):565-73, 2003, (b) Lopez-Calull, C.; Dominguez, N.; determination of paroxetine in plasma by high-performance liquid chromatography for bioequivalence studies, *J Chromatogr B Biomed Sci Appl*. 724(2):393-8, 1999.
- 8) (a) Zhu, Z.; Neirinck, L., high-performance liquid chromatography-mass spectrometry method for the determination of paroxetine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 780(2):295-300, 2002.
- 9) Juan, H.; Zhiling, Z.; Huande, L. simultaneous determination of fluoxetine, citalopram, paroxetine, venlafaxine in plasma by high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI). *J Chromatogr B Analyt Technol Biomed Life Sci*. 820(1):33-9, 2005.
- 10) Naidong, W.; Eerkes, A. development and validation of a hydrophilic interaction liquid chromatography-tandem mass spectrometric method for the analysis of paroxetine in human plasma. *Biomed Chromatogr*. 18(1):28-36, 2004.
- 11) (a) Federal Register Part. 320: Bioavailability and Bioequivalence Requirements. Food and Drug Administration: Washington, DC, 1985; 154, (b) Food and Drug Administration. Pharmacopeial Fórum 1993; 19: 6501.
- 12) a) Chapman, J.R. *Practical Organic Mass Spectrometry*, 2^a ed., 1993. b) Hoffmann, E.; Charette, J.; Stroobant, V. *Mass Spectrometry Principles and Applications*. c) Watson, J. T. *Introduction to Mass Spectrometry*, 3^a ed., 1997.
- 13) (a) Ho, E.N.; Yiu, K.C.; Wan, T.S.; Stewart, B.D.; Watkins, K.L. detection of anti-diabetics in equine plasma and urine by liquid chromatography-tandem mass spectrometry. *J Chromatogr B* Nov 5;811(1):65-73, 2004. (b) Maralikova, B.; Weinmann, W. confirmatory analysis for drugs of abuse in plasma and urine by high-performance liquid chromatography-tandem mass spectrometry with respect to criteria for compound identification. *J Chromatogr B* 5;811(1):21-30. 2004. (c) Taylor, P.J.; Forrest, K.K.; Landsberg, P.G.; Mitchell, C.; Pillans, P.I. The measurement of nicotine in human plasma by high-performance liquid chromatography-electrospray-tandem mass spectrometry *Ther Drug Monit*. Oct; 26(5):563-8, 2004.