ELSEVIER

Journal of Chromatography B, 780 (2002) 295–300

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

High-performance liquid chromatography–mass spectrometry method for the determination of paroxetine in human plasma

Zhimeng Zhu*, Len Neirinck

Pharmascience, Suite 100, 6111 Royalmount Ave., Montreal, Quebec, Canada H4P 2T4

Received 23 May 2002; received in revised form 23 July 2002; accepted 2 August 2002

Abstract

A rapid and specific liquid chromatographic mass spectrometric (LC–MS–MS) method has been developed for the determination of paroxetine in human plasma. The procedure involves a liquid–liquid extraction of paroxetine and fluoxetine (internal standard) with cyclohexane–ethyl acetate. The standard curve was linear over a working range of 0.2–50 ng/ml. The lower limit of quantitation was 0.2 ng/ml. No endogenous compounds were found to interfere with the analysis. The absolute recovery was 70.8% for paroxetine and 84.1% for the internal standard. The accuracy of inter-assay and intra-assay accuracy was in the ranges −4.8 to −0.5% and −3.4 to 4.8%, respectively. This method proved to be suitable for bioequivalence studies by being simple, selective and reproducible.

Keywords: Paroxetine

1. Introduction

Paroxetine is a potent and selective serotonin reuptake inhibitor (SSRI), indicated for the treatments of depression, obsessive-compulsive disorder, panic disorder and social phobia [1]. Paroxetine is a phenylpiperidine derivative that is chemically unrelated to the tricyclic or tetracyclic antidepressants [2]. Paroxetine is both a substrate and an inhibitor of cytochrome isoenzyme P450 2D6. This can give rise to drug–drug interactions and wide inter-individual variations [3,4]. Paroxetine is well absorbed orally and undergoes extensive first pass metabolism that is partially saturable [1].

Several chromatographic methods have been developed to measure SSRI's in serum or plasma. Among the methods reported in the literature, are gas chromatography (GC) with nitrogen–phosphorus detector (NPD), electron-capture detector (CED) or mass spectrometry detection [5–8]. These GC methods involved a derivatization procedure. HPLC with UV [2,9,10] or fluorescence detection [4,11–16] have reported detection limits ranging from 5 to 15 ng/ml. The run time ranged from 8 to 30 min.

In this paper, we present a rapid, specific and sensitive method for the determination of paroxetine in human plasma. This validated method requires an LC–MS–MS system and involves liquid–liquid ex-

*Corresponding author. Tel.: +1-514-340-5040; fax: +1-514-342-4118.

E-mail address: zzhu@pharmascience.com (Z. Zhu).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00537-8

traction. It allows a low detection limit (0.2 ng/ml) and short runtime (2 min). It also provides excellent reproducibility which makes it suitable for bioequivalence and pharmacokinetic studies.

2. Experimental

2.1. Materials

Paroxetine HCl and fluoxetine HCl were obtained from Pharmascience (Montreal, Quebec, Canada). The drug-free human plasma was obtained from Biological Specialty (Colmar, PA, USA). The formic acid was obtained from BDH. HPLC grade water was supplied from an in-house Nano-pure water purification system. All other chemicals were purchased from Fisher (Nepean, Ontario, Canada).

2.2. Stock solutions and standards

Stock solutions of paroxetine and fluoxetine (internal standard) were prepared by mixing appropriate amounts of paroxetine or fluoxetine with methanol to a final concentration of 0.1 mg/ml. The stock solutions were stored at -20 ± 5 °C. A set of seven non-zero calibration standards, ranging from 0.2 to 50 ng/ml was prepared by spiking the drug-free human plasma containing EDTA with an appropriate amount of paroxetine. The quality control samples at three concentration levels (0.6, 7.5 and 30 ng/ml) were prepared in a similar manner to the calibration standards. Drug-free human plasma was tested before spiking to ensure that no endogenous interference was found at retention times of paroxetine and fluoxetine.

2.3. Extraction procedure for plasma samples

A 0.5-ml aliquot of human plasma was placed into a screw cap glass tube. Then, 0.1 ml of internal standard working solution (160 ng/ml fluoxetine) and 0.5 ml of 0.5 *M* sodium phosphate dibasic buffer were added and the mixture was vortexed for 3 s; 7 ml of extraction solvent was then added. The mixture was shaken and centrifuged at 3000 rpm for 15 min. The organic layer was then evaporated to dryness under a nitrogen evaporator at 40 ± 5 °C, reconstituted in 0.1 ml reconstitution solution consisting of acetonitrile–0.05% formic acid in water (1:1), and then injected (8 μl) into the LC–MS–MS.

2.4. Chromatography and quantitation

The chromatographic system consisted of an Agilent 1100 HPLC system coupled to a PE Sciex API 2000 Mass Spectrometer. The separation was achieved by using a 50×2.1 mm, 4 μm Genesis C_{18} column with a mobile phase consisting of acetonitrile–5 m*M* ammonium formate (4:3, v/v). The mobile phase was delivered into the LC–MS–MS system at a flow-rate of 0.22 ml/min. The total run time was set at 2.2 min.

A Sciex API 2000 mass spectrometer (Concord, Canada) equipped with an ion-spray source was used to obtain the mass spectra. Positive mode was used for the analysis. The data acquisition was carried out by Analysis 1.1 software. A product ion scan was performed on both paroxetine and internal standard. The strongest fragment of each compound was selected and used as Q3 ion to be monitored. MRM was performed on the mass transition ion-pair of $330.1\rightarrow192.2$ for paroxetine and $310.1\rightarrow148.1$ for internal standard. Unit resolution was used for both Q1 and Q3 mass detection. The ion source parameters were set as follows: curtain gas=40 p.s.i., collision gas=6 p.s.i., ion spray voltage=4250 V, temperature=350 °C, ion source gas 1=65 p.s.i. and ion source gas 2=75 p.s.i.

A peak area ratio method was used for quantitation. The paroxetine concentration in human plasma samples was determined by a seven-point standard curve that was analyzed with weighted least squares linear regression. The retention times of paroxetine and fluoxetine were 1.3 and 1.6 min, respectively.

3. Results

3.1. Limit of quantitation, linearity and precision

The limit of quantitation (LOQ) in human plasma for paroxetine was 0.2 ng/ml. Over a concentration range of 0.2–50 ng/ml, a linear response was observed for the peak area ratio versus concentration for paroxetine. The correlation coefficients were

Table 1
Summary of paroxetine calibration standards

Concentration added (ng/ml)	Concentration found (ng/ml)	R.E. (%)	C.V. (%)	*n*
0.2	0.1958±0.00172	−2.1	0.9	5
0.4	0.4090±0.00865	2.3	2.1	5
1.0	1.0436±0.01360	4.4	1.3	5
5.0	5.0690±0.04486	1.4	0.9	5
10.0	10.0990±0.22147	1.0	2.2	5
40.0	38.3334±0.41957	−4.2	1.1	5
50.0	48.6098±0.75276	−2.8	1.5	5

R.E., relative error; C.V., coefficient of variation.

greater than or equal to 0.9981. The coefficient of variation of calibration standards was in the range of 0.9–2.2%. The detailed results for the paroxetine calibration samples are presented in Table 1.

The inter-assay precision and accuracy were determined by analyzing five calibration curves with quality control samples on five different occasions. The inter-assay precision was between 2.6 and 3.5% as shown in Table 2. The intra-assay precision was determined by analyzing six replicates of LOQ and quality control samples extracted on the same day. The intra-day precision for the quality control samples (0.60, 7.50 and 30.0 ng/ml) was between 1.4 and 4.0%, and for the LOQ (0.20 ng/ml) was 0.5%. Detailed results for the intra-assay precision and accuracy are described in Table 3.

Table 2
Inter-assay precision of paroxetine in human plasma

Concentration added (ng/ml)	Concentration found (ng/ml)	R.E. (%)	C.V. (%)	*n*
0.60	0.5969±0.02096	−0.5	3.5	18
7.50	7.2647±0.19020	−3.1	2.6	18
30.0	28.5741±1.01114	−4.8	3.5	18

R.E., relative error; C.V., coefficient of variation.

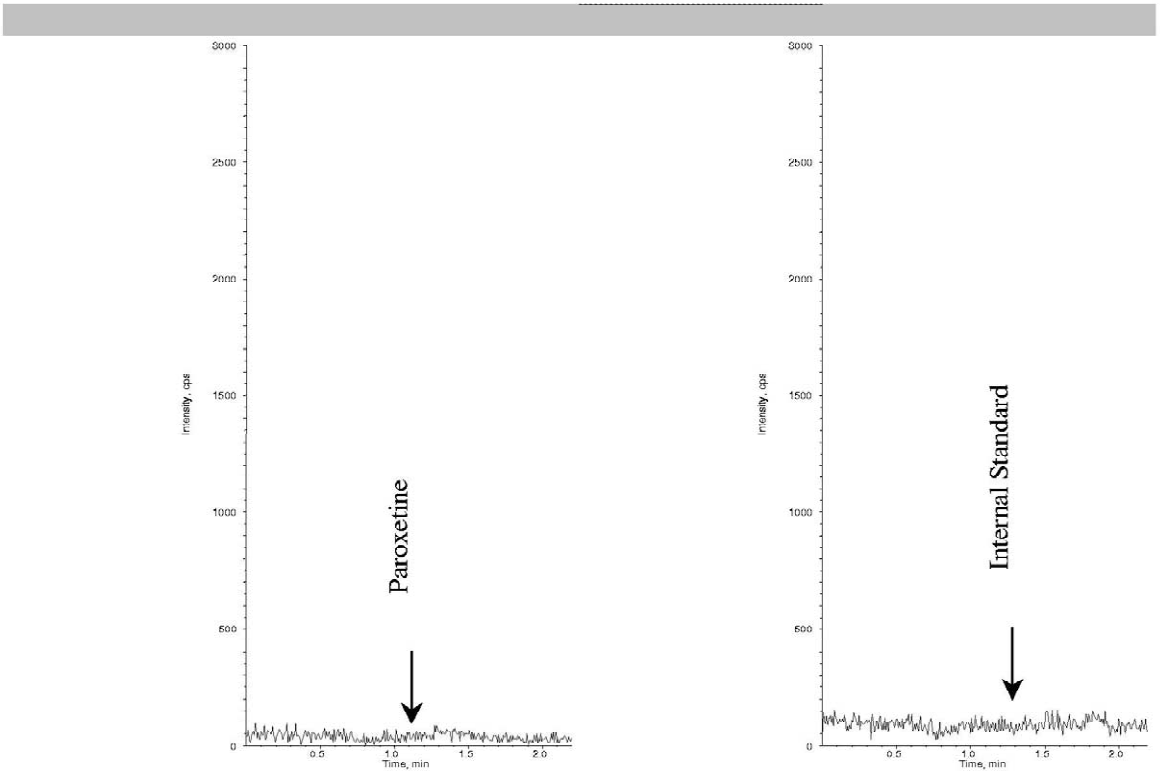

Fig. 1. Representative chromatogram for extracted blank plasma sample.

Table 3
Intra-assay precision of paroxetine in human plasma

Concentration added (ng/ml)	Concentration found (ng/ml)	R.E. (%)	C.V. (%)	*n*
0.20	0.2095±0.00096	4.8	0.5	6
0.60	0.5833±0.02321	−2.8	4.0	6
7.50	7.3193±0.10387	−2.4	1.4	6
30.0	28.9902±0.41138	−3.4	1.4	6

R.E., relative error; C.V., coefficient of variation.

Table 4
Extraction yield of paroxetine and internal standard

Description	Extraction yield (%)	C.V. (%)	*n*
Low QC (0.60 ng/ml)	65.9	5.7	6
Mid QC (7.50 ng/ml)	69.9	2.0	6
High QC (30.0 ng/ml)	76.6	3.4	6
Internal standard (fluoxetine)	84.1	4.0	18

3.2. Recovery

Paroxetine recovery was assessed by comparing the peak area of six replicates of extracted QC samples (at low, mid and high range) to reference QC samples prepared in solutions at the same concentration levels. Concentrations of the six replicates were 0.60, 7.50 and 30.0 ng/ml. The overall recovery of paroxetine was 70.8%, while the recovery of the internal standard (fluoxetine) was 84.1%. Results are shown in Table 4.

3.3. Specificity

Screening of six different sources of drug-free human plasma showed no endogenous interference at the retention times of paroxetine and the internal standard. A chromatogram of extracted blank human plasma sample as well as representative chromatograms of extracted calibration sample at the lower limit of quantitation (LOQ) and high QC samples are provided in Figs. 1–3.

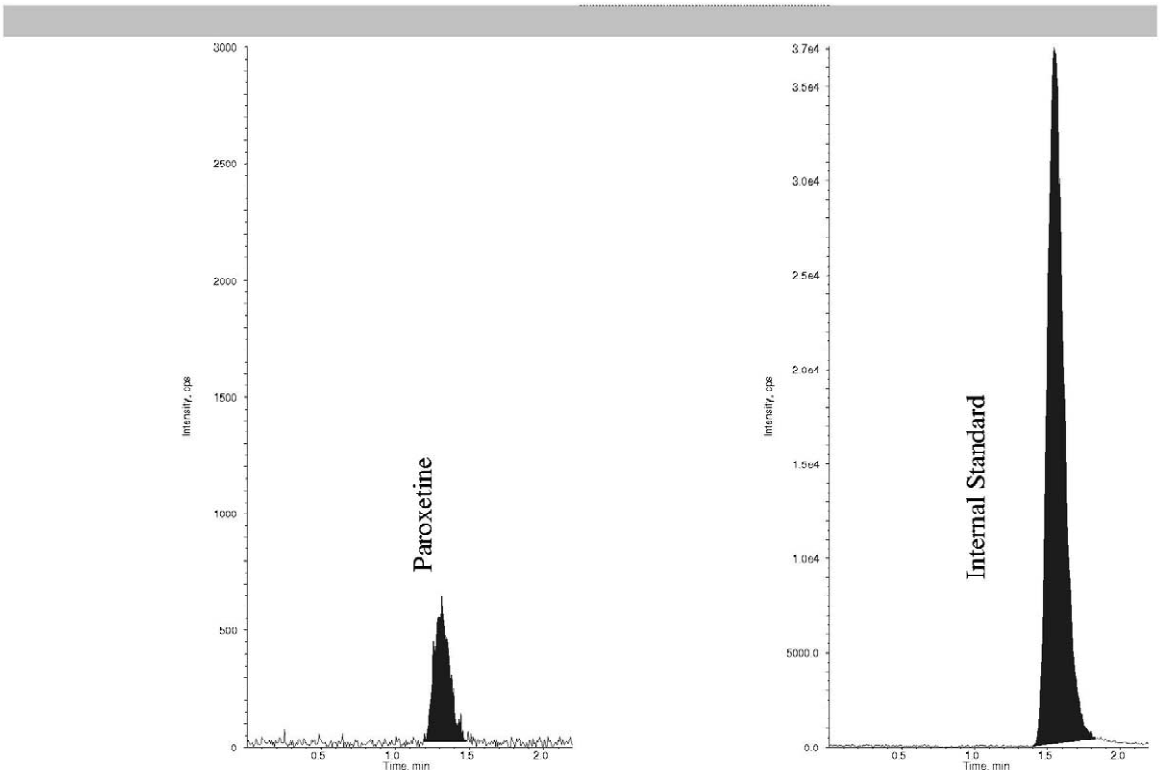

Fig. 2. Representative chromatogram for extracted LOQ sample.

Table 5
Minimum stability of paroxetine under various storage conditions

Conditions	Stability
Short term (bench top)	At least 2 h at room temperature
In-process	At least 2 h at room temperature
Autosampler	At least 76 h at 4±2 °C
Freeze–thaw	Three freeze/thaw cycles
Long term (frozen sample)	At least 69 days at −20±5 °C

3.4. Stability

The stability of paroxetine was evaluated under the conditions described in Table 5. Both bench top stability tests and in-process stability tests showed that paroxetine in human plasma is stable for at least 2 h at room temperature.

The stability of extracted paroxetine and internal standard in reconstitution solution (processed sample stability) showed that processed samples are stable at 4±2 °C for at least 76 h. The processed sample stability was evaluated by comparing the extracted plasma samples that were injected immediately (time 0) with the samples that were re-injected 76 h after sitting in the autosampler at 4±2 °C. Evaluation was based on back-calculated concentrations.

The freeze–thaw stability test was also evaluated and showed that paroxetine in human plasma is stable for at least three freeze–thaw cycles. The test involved a comparison of replicate stability samples which had been frozen and thawed three times with a fresh plasma sample that had been thawed only once.

Long-term (frozen plasma sample) stability evaluation involved an analysis of the low, mid and high quality control samples that were stored at −20±5 °C for 69 days, together with freshly spiked calibration standard and quality control samples. The analysis was carried out on the same day.

3.5. Application

The method described in this paper was applied to a bioequivalence study that generated over 1400

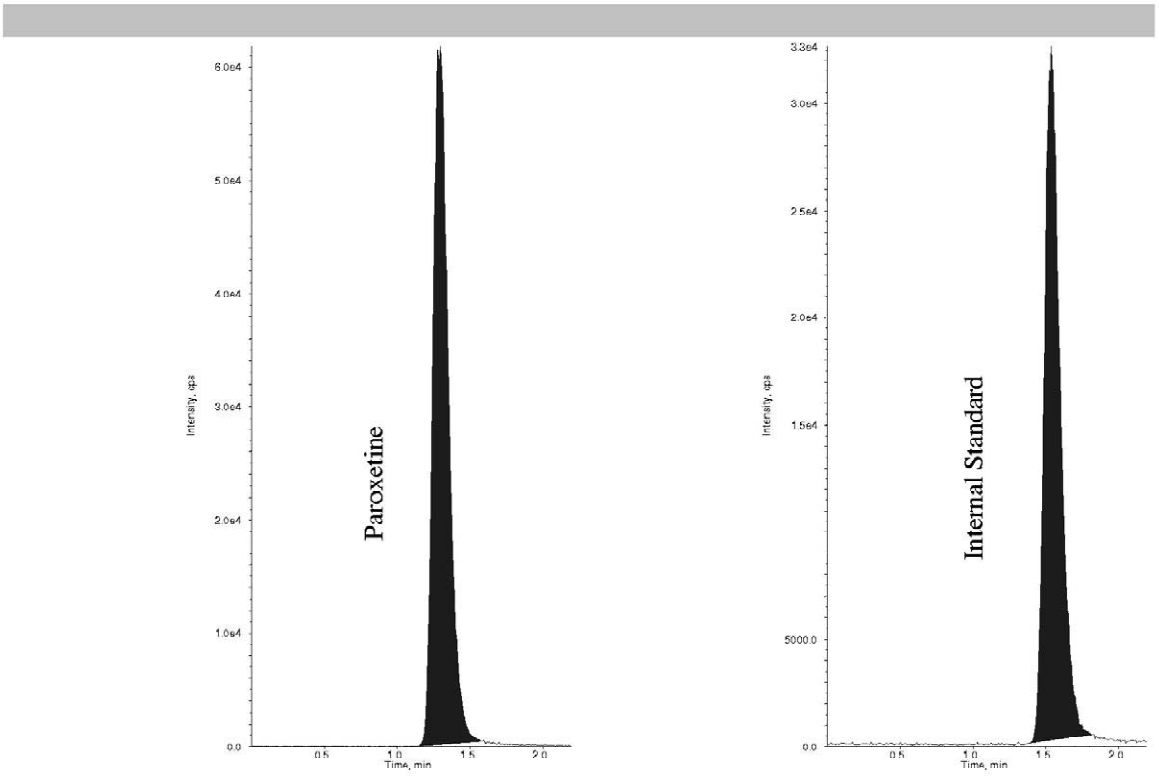

Fig. 3. Representative chromatogram for extracted highest QC sample.

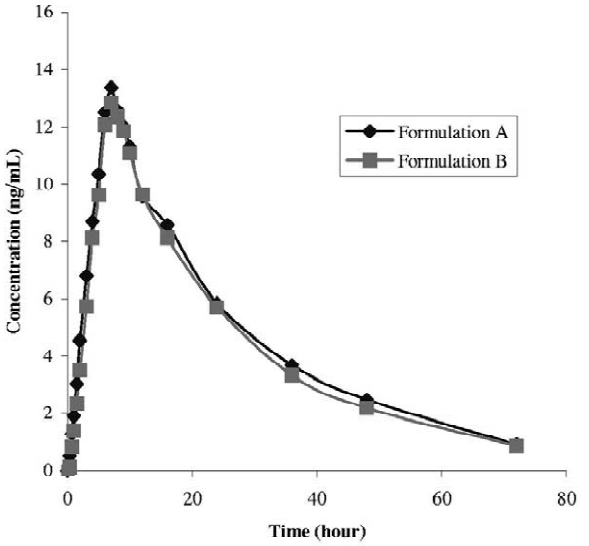

Fig. 4. Concentration–time profile for 40 subjects after 30 mg dosing.

human plasma samples. Twenty blood samples were drawn over a period of 72 h after administration of a single oral dose of 30 mg paroxetine to healthy male volunteers. Samples were stored at −20 °C until analyzed. Comparison of peak area ratios from the unknown samples with those from the calibration curve allowed quantitation of the assayed samples. An example of concentration versus time profile is presented in Fig. 4.

4. Conclusions

The method consists of a simple liquid–liquid extraction procedure and a simple isocratic chromatography condition. Mass spectrometric detection allows the method to go to a very low detection limit and results in a very short run time. This method has excellent sensitivity, reproducibility and it is simple and rapid. It allows the analysis of over 100 samples within 4 h. This method is suitable for bio-equivalence/bio-availability studies with controlled subject selection. It is important to make sure the subjects are not on the anti-depressant fluoxetine to avoid possible interference.

Acknowledgements

The authors wish to thank C. Dong and L. Wang for providing validation and plasma sample analysis results. We also wish to thank A.M. Donetti and R. Francis in helping prepare this manuscript.

References

[1] M. Bourin, P. Chue, Y. Guillon, CNS Drug Rev. 7 (1) (2001) 25.
[2] J.P. Foglia, D. Sorisio, M. Kirshner, B.G. Pollock, J. Chromatogr. B 693 (1997) 147.
[3] C. Hiemke, S. Hartter, Pharmacol. Ther. 85 (1) (2000) 11.
[4] J.G. Shin, K.A. Kim, Y.R. Yoon, I.J. Cha, Y.H. Kim, S.G. Shin, J. Chromatogr. B 713 (1998) 452.
[5] C.T. Lai, E.S. Gordon, S.H. Kennedy, A.N. Bateson, R.T. Coutts, G.B. Baker, J. Chromatogr. B 749 (2) (2000) 275.
[6] N.H. Jourdil, P.D. Fontanille, G.M. Bessard, Clin. Chem. 43 (1997) 2209.
[7] E. Lacassie, J.M. Gautier, P. Marquet, J.F. Rabatel, G. Lâchatre, J. Chromatogr. B 742 (2000) 229.
[8] H.J. Leis, W. Windischhofer, G. Raspotnig, G. Fauler, J. Mass Spectrom. 36 (2001) 923.
[9] G. Tournel, N. Houdret, V. Hedouin, M. Deveau, D. Gosset, M. Lhermitte, J. Chromatogr. B 761 (2001) 147.
[10] J. Knoeller, R. Vogt-Schenkel, M.A. Brett, J. Pharm. Biomed. Anal. 22 (1995) 635.
[11] A. Lucca, G. Gentilini, S. Lopez-Silva, A. Soldarini, Ther. Drug Monit. 22 (2000) 271.
[12] C. López-Calull, N. Dominguez, J. Chromatogr. B 724 (1999) 393.
[13] M.A. Brett, H.D. Dierdorf, J. Chromatogr. 419 (1987) 438.
[14] D.S. Schatz, A. Saria, Pharmacology 60 (2000) 51.
[15] J. Lambropoulos, G.A. Spanos, N.V. Lazaridis, J. Pharm. Biomed. Anal. 19 (1999) 793.
[16] R.N. Gupta, J. Chromatogr. B 661 (1994) 362.