Pergamon

0731-7085(95)01315-6

Journal of Pharmaceutical & Biomedical Analysis, Vol. 13, No. 4/5, pp. 635–638, 1995
Elsevier Science Ltd
Printed in Great Britain
0731-7085/95 $9.50 + 0.00

A simple and robust HPLC method for the determination of paroxetine in human plasma*

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Abstract: The objective of this investigation was to establish and validate an HPLC method with UV detection for the determination of paroxetine in human plasma. The method was validated in the concentration range from 6 to 100 ng ml^{-1}. The lower limit of reliable quantification (LLQ) was 6 ng ml^{-1}. The extraction efficiency varied from 67.1 to 85.5% over this range. Accuracies calculated at three concentrations in each of three separate runs were between 99.4 and 109.6%, and precision data were between 1.86 and 9.1%. The means of the between-day precision at all concentrations were between 2.77 and 7.32%. The corresponding means of the accuracy data were in the region of 102.4 to 106.3%.

Keywords: *Paroxetine; HPLC; plasma; assay validation.*

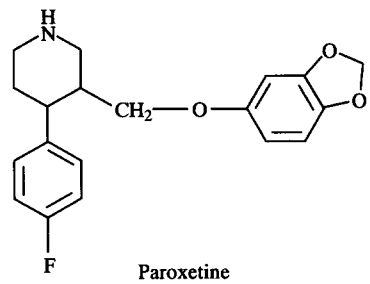
Introduction

Paroxetine, a potent, selective 5-HT uptake inhibitor, is currently in use as an antidepressant drug; it has been extensively studied in man to examine its metabolism and pharmacokinetics [1]. It can be determined in biological fluids by either gas–liquid chromatography [2] or high performance liquid chromatography (HPLC) with fluorescence detection [3]. The objective of this investigation was to establish and validate an HPLC method with UV detection which is more rapid than either of the previously reported methods but with enough sensitivity to support repeat-dose studies.

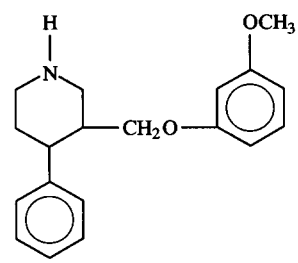
Experimental

Materials

All solvents were of HPLC grade and all reagents were of analytical grade. The following chemicals were used: acetonitrile, ethanol, toluene, heptane (Riedel de Haen, Seelze, FRG) glacial acetic acid, sodium hydroxide, sodium phosphate, sodium lauryl sulphate (Merck, Darmstadt, FRG) tetrabutylammonium hydrogen sulphate (Sigma Chemical Company, Deisenhofen, FRG), paroxetine hydrochloride (assigned potency: 87.4% pure free base) (SmithKline Beecham) and norfemoxetine hydrochloride, which was a kind gift from Novo Nordisk (Malov, Denmark). Paroxetine (Scheme I) is (−)-trans-5-(4-*p*-fluorophenyl-3-piperidylmethoxy)-1,3-benzodioxole (CAS 61869-08-7). The internal standard norfemoxetine (MW 297) is also shown in Scheme I ($C_{19}H_{23}NO_2$).



Paroxetine



Norfemoxetine

Scheme 1

*Presented at the Fifth International Symposium on Pharmaceutical and Biomedical Analysis, Stockholm, Sweden, September 1994.

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Instrumentation

The following instruments and apparatus were used for the HPLC assay: a high-pressure pump (model 655A-12, Merck Hitachi, Darmstadt, FRG), a variable wavelength UV-detector (Kratos 783, Applied Biosystems, Bensheim, FRG) an Autosampler (WISP 712, Waters Eschborn, FRG), a data capture system (Multichrom 2.0, Fisons, Mainz, FRG), an RP-Select B column (Merck, Darmstadt, FG), an automatic shaker (type Automix 2, ASID Bonz & Sohn GmbH, FRG) and a sample concentrator (SC-3, Techne Instruments, FRG).

Chromatographic conditions

Each sample was analysed with detection at 295 nm on an RP-Select B column (250 × 4 mm). The mobile phase was a mixture of 65% 0.05 M glacial acetic acid (adjusted to pH 4.5 with 1 N NaOH) containing 2 g tetrabutylammonium hydrogen sulphate and 35% acetonitrile–ethanol (3:2, v/v). The flow rate was 1 ml min^{-1} and norfemoxetine was used as the internal standard.

Calibration samples

Paroxetine stock solutions and subsequent dilutions were made in appropriate volumetric flasks with distilled water. Norfemoxetine was also dissolved and further diluted with distilled water. In order to prepare standard curves for paroxetine within the range of 5–100 ng ml^{-1} plasma, stock solutions of approximately 1 μg ml^{-1} were used.

Quality control samples

Quality control samples were prepared by adding an appropriate volume of stock paroxetine solution (about 100 μg per 100 ml) to 25 ml of drug free human plasma (in volumetric flasks), dividing into 1 ml portions and storing at −20°C until analysis. Stock solutions for quality control samples were prepared independently from stock solutions used for the calibration curves.

Storage of samples

Authentic and spiked plasma samples were stored at −20°C, at which temperature paroxetine has been previously shown to be stable for at least 9 months (internal report).

Sample preparation

75 μl (about 100 ng) of the internal standard solution were added to 1 ml plasma of calibration solution and authentic samples, acidified with 200 μl of 1 N $HClO_4$ and washed using 5 ml of toluene on a tumble mixer for 20 min. This preliminary step removed nonpolar compounds of the plasma matrix which otherwise interfered with the subsequent chromatography. After centrifugation at 1500*g* (10 min) and standing at −20°C for 20 min the organic layer was removed and discarded. 750 μl of 0.025 M phosphate buffer (pH 12) together with 200 μl of a 1% lauryl sulphate solution were then added to the aqueous phase, the samples extracted with 5 ml of heptane–toluene (4:1, v/v) on a tumble mixer for 20 min and centrifuged at 2000*g* for 10 min. Lauryl sulphate solution was added to the aqueous phase to prevent the formation of foam during the extraction step with heptane–toluene.

The upper layer was removed and evaporated to dryness with nitrogen using a sample concentrator at 50°C. The residue was redissolved in 220–250 μl of methanol–acetate buffer (pH 4.5) (30:70, v/v), thoroughly mixed and 180–200 μl injected into the HPLC system.

Results and Discussion

The assay described here provided a specific and rapid method for the determination of paroxetine in human plasma by high performance liquid chromatography using UV-detection. Representative chromatograms of blank plasma and the lowest calibration standard are shown in Fig. 1. The calibration curves were linear and reproducible over the analysed concentration range with correlation coefficients >0.990. The specificity of the method was confirmed by the analysis of a variety of different blank human plasmas none of which yielded any endogenous interference.

Six quality control samples at each of three concentrations were analysed on 3 separate days with freshly prepared calibration lines on each occasion. Within-day and between-day precision were calculated for all concentrations, together with the mean precision. Similarly, the accuracy within each run and the mean accuracy were determined.

The results for paroxetine are given in Table 1. For the 3-day validation, accuracies calculated at all concentrations in each of the three runs were between 99.4 and 109.6%, and

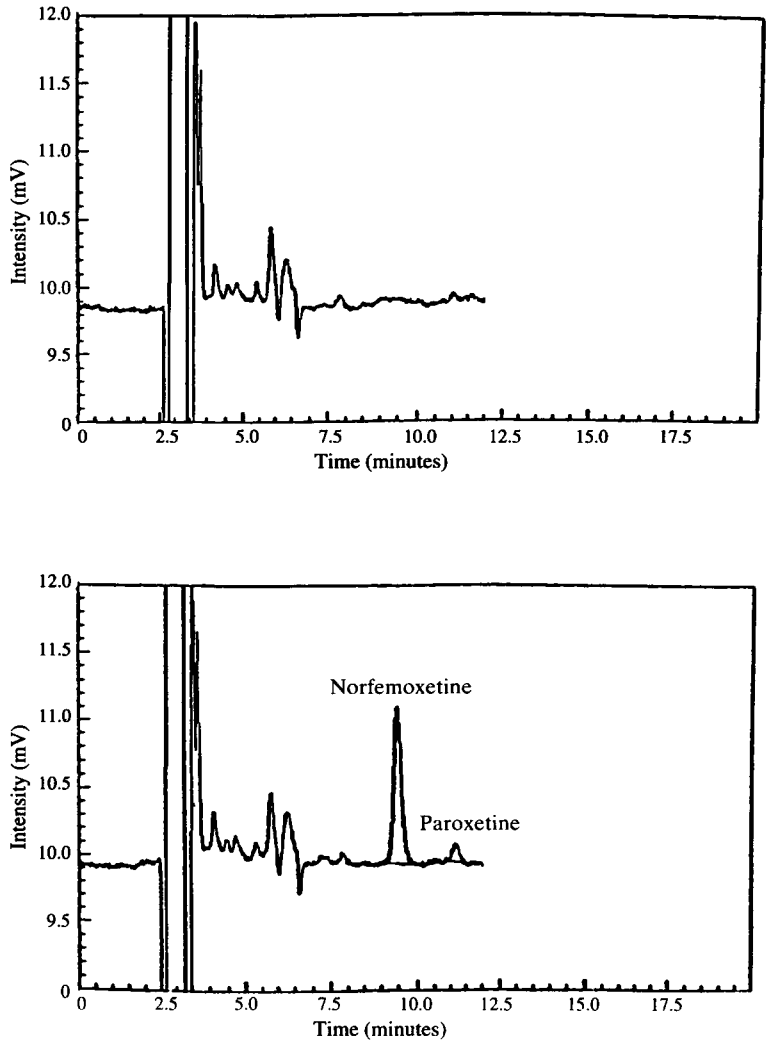

Figure 1
(A) Blank plasma; (B) lowest calibration standard (5.8 ng ml^{-1}).

Table 1
3-day validation of paroxetine in human plasma

Nominal concentration (ng ml^{-1})		Day 1	Day 2	Day 3	Average within-day precision	Between-day precision	Average accuracy
	Mean	6.51	6.19	6.48			
	SD	0.59	0.44	0.37			
6.23	RSD	9.10	7.13	5.73	7.32	2.76	
	ACC	104.50	99.40	103.30			102.4
	n	6.00	6.00	6.00			
	Mean	44.27	40.49	42.73			
	SD	0.82	1.73	0.93			
40.54	RSD	1.86	4.28	2.18	2.77	4.47	
	ACC	109.20	99.90	105.40			104.8
	n	6.00	6.00	6.00			
	Mean	95.38	87.70	94.39			
	SD	4.17	3.38	3.92			
87.03	RSD	4.37	3.86	4.15	4.13	4.52	
	ACC	109.60	100.80	108.50			106.3
	n	6.00	6.00	6.00			

precision data were between 1.86 and 9.1%. The means of the within-day precision data for the three days at all concentrations were between 2.77 and 7.32%. The corresponding means of the accuracy data were in the region of 102.4–106.3%. The between-day precision varied from 2.76 to 4.52%. The extraction efficiency of paroxetine in human plasma was found to be 81.3, 72, 85.5 and 67.1% determined at concentrations of 5, 15, 50 and 100 ng ml^{-1}, respectively ($n = 6$).

Conclusion

This HPLC method provides a rapid and robust assay for the determination of paroxetine in plasma samples derived from clinical studies after multiple-dose administration. Good correlation was obtained between this method and the previously reported HPLC method [3] using *in vivo* samples in the 6–90 ng ml^{-1} concentration range. The lower limit of quantification (LLQ) was assessed to be 6 ng ml^{-1}, corresponding to an RSD value of ca 7%.

For supporting pharmacokinetic studies with single dose administration of paroxetine, however, the more sensitive fluorescence method [3] following derivatization with dansylchloride, is still required on sensitivity grounds.

The robustness of the assay was shown with plasma samples from a variety of multiple dose studies which were analysed using the HPLC method with UV detection: no interference of endogenous plasma components with the quantification of paroxetine was observed.

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[*Received for review 23 September 1994; revised manuscript received 24 November 1994*]