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Short communication

Determination of paroxetine levels in human plasma using gas chromatography with electron-capture detection

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Abstract

A simple, rapid and sensitive procedure using gas chromatography with electron-capture detection to measure paroxetine levels in human plasma has been developed. The analyte was extracted from plasma with ethyl acetate after basification of the plasma and then derivatized with heptafluorobutyric anhydride before gas chromatographic separation. The calibration curves were linear, with typical r^2 values >0.99. The assay was highly reproducible and gave peaks with excellent chromatographic properties.

Keywords: Paroxetine; Heptafluorobutyric anhydride

1. Introduction

Paroxetine is a potent selective serotonin reuptake inhibitor (SSRI) antidepressant which has also been indicated for the treatment of obsessive compulsive disorder, panic disorder, generalized social phobia, premenstrual dysphoric disorder and chronic headache (review see Ref. [1]). For therapeutic drug monitoring purposes, several methods for paroxetine analysis have been reported (summary see Table 1). It is apparent that considerable effort has been focused on utilitizing high-performance liquid chromatography (HPLC) to measure levels of paroxetine in human plasma. However, Eap et al. [2] described a gas chromatographic procedure using mass spectrometry as the detector, but this method involved extensive plasma preparation steps before gas chromatographic separation and mass spectrometry is expensive and hence often not readily accessible in general laboratories. A gas chromatographic method using a nitrogen detector was established to detect paroxetine levels in monkey plasma [11], but this method was also laborious and time consuming. Described in the present report is a simple, rapid and

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Table 1
Reported methods for determination of paroxetine levels in human plasma

Instrumentation	Extraction method	Derivatizing reagent	Internal standard	Remarks	References
GC–MS[a]	Liquid–liquid extraction	*N*-Methyl-bis(trifluoro-acetamide)	Methylmaprotiline	Extensive plasma sample preparation and extraction	[2]
HPLC-FLU	Liquid–liquid extraction	Dansyl chloride	Maprotiline		[3]
HPLC–UV	NA	NA	Trimipramine	Automated method with column switching	[4]
HPLC–FLU	Solid-phase extraction	NA	Dibucaine		[5]
HPLC–UV	Liquid–liquid extraction	NA	Norfemoxetine		[6]
HPLC–UV	Liquid–liquid extraction	NA	Fluoxetine		[7]
HPLC–FLU	Liquid–liquid extraction	NA	Dibucaine		[8]
HPLC–FLU	Solid-phase extraction	NA	Dibucaine		[9]
TLC–DEN	Liquid–liquid extraction	NA	NA		[10]

[a] Abbreviations: GC=gas chromatography, MS=mass spectrometry, HPLC=high-performance liquid chromatography, FLU=fluorimetric detection, UV=ultraviolet detection, TLC=thin layer chromatography, DEN=densitometric scanning after staining, NA=not applicable.

sensitive assay for the extraction of paroxetine from plasma and its subsequent analysis using gas chromatography with electron-capture detection.

2. Experimental

2.1. Materials

Paroxetine hydrochloride and fluoxetine hydrochloride were provided by SmithKline Beecham (Burlington, MA, USA) and Lilly Research Laboratories (Indianapolis, IN, USA), respectively. Heptafluorobutyric anhydride (HFBA) was purchased from Sigma (St. Louis, MO, USA). Sodium phosphate tribasic was obtained from J.T. Baker (Phillipsburg, NJ, USA), and ethyl acetate and toluene (both HPLC grade) were supplied by Fisher Scientific (Fair Lawn, NJ, USA).

Stock standard solutions of paroxetine and fluoxetine were prepared at concentrations of 1 mg/ml (based on free-base weight) in double-distilled water and stored at −70°C. Serial dilutions of working paroxetine solution were prepared freshly for each calibration curve from the stock solution.

2.2. Plasma samples

Plasma samples were obtained from healthy non-medicated volunteers and from depressed patients who had been treated with paroxetine with daily doses of 30 or 60 mg. Venous blood was collected into EDTA-containing tubes. These tubes were centrifuged (1200 g for 10 min; Sorval T6000B centrifuge, Du Pont Canada, Mississauga, Canada) after collection and the resultant plasma separated and stored at −80°C until analysis.

2.3. Sample extraction and derivatization

An aliquot (1 ml) of each human plasma sample was pipetted into a screw-cap glass culture tube (150 mm×16 mm) and the internal standard fluoxetine (1000 ng) was added. The samples were basified by adding 1 ml of 10% (w/v) sodium phosphate tribasic solution and mixing briefly on a vortex-mixer. Ethyl acetate (4 ml) was added, and the tubes were capped and shaken vigorously for 5 min in an IkaVibrex-VXR vortex-mixer (Janke and Kunkel, Staufen, Germany) and centrifuged at 1000 g for 10 min in a benchtop centrifuge (Sorvall GLC-2B general laboratory centrifuge, Du Pont, Canada). The upper ethyl acetate layers were transferred to screw-cap glass culture tubes (100 mm×13 mm) and taken to dryness in a SpeedVac® SC110 evaporator (Savant Instruments, Farmingdale, NY, USA). The residue in each tube was then reacted with HFBA (50 μl, derivatizing reagent) in the presence of 25 μl ethyl acetate at 60°C for 30 min. After cooling the tubes at room temperature (10 min), the excess reagent was evaporated using a SpeedVac® SC110 evaporator. The residues were reconstituted in 150 μl of toluene. Of each of these solutions, 2 μl was used for gas chromatographic analysis.

A calibration curve consisting of six concentration points over the range of 0, 40, 80, 160, 320 and 640 ng of paroxetine per 1 ml of human plasma was run in parallel with each assay. These calibration curves were prepared by adding internal standard (1000 ng) and varying concentrations of authentic paroxetine to human plasma (1 ml) obtained from drug-free volunteers. The ratios of the peak area of varying concentrations of authentic paroxetine to that of internal standard were calculated and plotted against the varying concentrations of paroxetine. The levels of paroxetine in plasma of patients treated with this drug were estimated using this calibration curve in each assay run.

2.4. Equipment

Analyses were performed on a Hewlett–Packard (HP) 5890 gas chromatograph equipped with an electron-capture detector and linked to a HP 3396 printer/integrator (Hewlett–Packard Canada, Mississauga, Canada). A fused-silica capillary column (15 m×0.25 mm I.D.) coated with a 0.25 μm film thickness of 5% phenylmethyl silicone (J&W Scientific, Folsom, CA, USA) was employed. To confirm the structure of the final derivative of paroxetine, gas chromatography–mass spectrometry was conducted by using an HP 5890 gas chromatograph linked to a HP MSD mass selective detector.

2.5. Method development

The conditions for gas chromatographic separation were as follows. An initial oven temperature of 105°C was maintained for 0.5 min, then was increased to 295°C at a rate of 12°C/min. The final oven temperature 295°C was maintained for 15 min. Temperatures at the injection port and detector were 270°C and 325°C, respectively. All injections of samples (injection volume: 2 μl of each sample) were carried out using an HP 6890 injector with purge off time of 0.5 min. Ultra-pure helium (Praxair, Mississauga, Canada) was used as carrier gas and argon/methane (95:5) was used as make-up gas, with flow-rates of 2 ml/min and 30 ml/min, respectively. Mass spectrometer operating conditions were as follows: ion source temperature, 200°C; interface temperature, 295°C; column pressure, 34.5 kPa; accelerating voltage, 2200 eV; and ionization voltage, 70 eV.

3. Results and discussion

3.1. Chromatography and specificity

Representative chromatograms of extracts of plasma obtained from a drug-free volunteer and a patient treated with 30 mg of paroxetine are shown in Fig. 1. Extracts obtained from plasma of drug-free volunteers showed no chromatographic peaks that interfered with paroxetine or internal standard peaks. Gas chromatography combined with electron-impact mass spectrometry gave a molecular ion of m/z 525 for derivatized paroxetine, consistent with the structure shown in Fig. 2.

3.2. Linearity

Six-point calibration curves were prepared over the concentration range of 0 to 640 ng/ml of paroxetine in plasma of drug-free volunteers in parallel with the samples in each assay run. Regression analysis of the correlation between the chromatographic peak area ratios of paroxetine/internal standard and varying concentrations of paroxetine yielded a linear correlation over the concentration range analyzed, with typical r^2 values >0.99 (ranging from 0.9907 to 0.9981, $n=7$).

3.3. Recovery, precision and accuracy

The recovery of paroxetine in the extraction procedure was determined by comparison of the peak heights obtained after the complete extraction and derivatization procedure of plasmas containing 250 ng/ml of paroxetine with those obtained after direct derivatization of the same quantity of the pure standard. The recovery of paroxetine from plasma was 76.9%.

The intra-day precision of the assay was assessed by calculating the coefficient of variation (C.V.) for replicated samples ($n=6$) at concentration of 100 ng/ml paroxetine in plasma. The inter-day precision of the assay was assessed by analyzing the C.V. of five plasma samples spiked with 100 ng/ml of

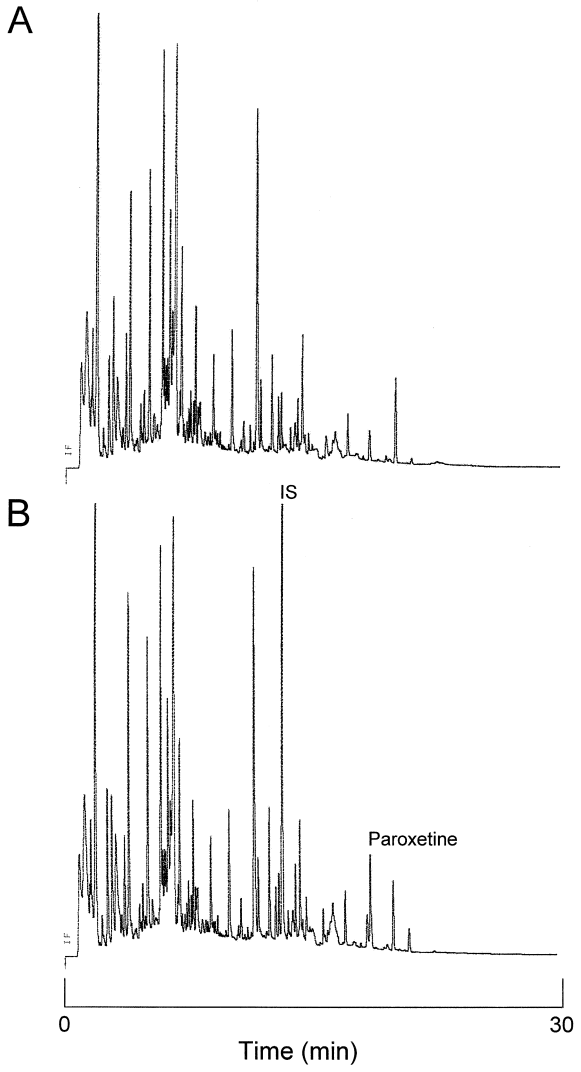

Fig. 1. Representative chromatograms of derivatized extracts of plasma obtained from: (A) a drug-free healthy volunteer and (B) a depressed patient treated with 30 mg of paroxetine and spiked with and the internal standard (IS) fluoxetine. The retention times for the fluoxetine and paroxetine were approximately 13.1 and 18.5 min, respectively.

paroxetine on separate days. Accuracy (expressed as % bias) was calculated as the percent difference between the amount of paroxetine added (100 ng) and detected. Table 2 summarizes the precision and accuracy of the gas chromatographic assay for the analysis of paroxetine in human plasma. The observed values indicate that the assay was reproducible and accurate.

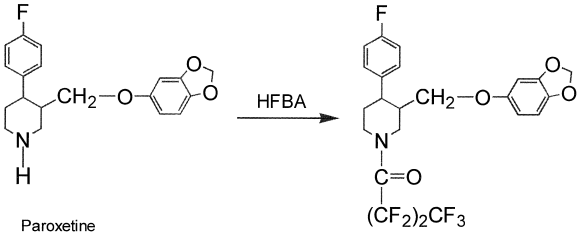

Fig. 2. Scheme showing derivatization of paroxetine with heptafluorobutyric anhydride (HFBA).

3.4. Application to patient plasma samples

The limit of detection (LOD) is calculated as $LOD=3s_b/S$, where 3 is a factor for a 99.9% level of confidence, s_b and S represent the standard deviation of the blank measurement and the slope of the calibration curve, respectively [12]. The limit of quantitation (LOQ) is estimated to be $LOQ=Ks_b/S$, where K is a factor indicating the desired precision at the lower limit (for example, 10 for 10% relative standard deviation was used for this report) [12]. The LOD of paroxetine was 8.5 ng/ml, whereas the LOQ was 28.4 ng/ml. This assay has now been used to measure paroxetine levels in the plasma of patients treated with this drug, and the results are presented in Table 3.

In summary, described here is an assay using gas chromatography with electron-capture detection to measure the levels of paroxetine in plasma from paroxetine-treated depressed patients. It is simple,

Table 2
Precision and accuracy of the gas chromatographic assay for the analysis of paroxetine in human plasma[a]

Amount added (ng)	Intra-day studies		Inter-day studies	
	C.V.	% bias[b]	C.V.	% bias[b]
100	3.2%	3.5%	7.2%	2.7%

[a] Abbreviation: C.V.=coefficient of variation.

[b] Accuracy is represented by % of bias, which is defined as the percent difference between the amount of paroxetine added and detected.

Table 3
Steady-state levels of paroxetine in plasma of two depressed patients who were treated with paroxetine[a].

Sample No.	Dose (mg/day)	Plasma level (ng/ml plasma)
1A	30	106.7
1B	30	95.1
1C	30	115.1
1D	30	82.4
1E	30	108.6
2A	60	257.2
2B	60	215.9
2C	60	275.6
2D	60	278.1
2E	60	225.3

[a] Measurements were made at five consecutive 2-week intervals.

rapid, sensitive and readily applicable to routine analysis of paroxetine.

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References

[1] N.S. Gunasekara, S. Noble, P. Benfield, Drugs 55 (1998) 85.
[2] C.B. Eap, G. Bouchoux, M. Amey, N. Cochard, L. Savary, P. Baumann, J. Chromatogr. Sci. 36 (1998) 365.
[3] M.A. Brett, H.-D. Dierdorf, B.D. Zussman, P.E. Coates, J. Chromatogr. 419 (1987) 438.
[4] S. Härtter, B. Hermes, A. Szegedi, C. Hiemke, Ther. Drug Monit. 16 (1994) 400.
[5] R.N. Gupta, J. Chromatogr. B 661 (1994) 362.
[6] J. Knoeller, R. Vogt-Schenkel, M.A. Brett, J. Pharm. Biol. Anal. 13 (1995) 635.
[7] J.P. Foglia, D. Sorisio, M. Kirshner, B.G. Pollock, J. Chromatogr. B 693 (1997) 147.
[8] J.-G. Shin, K.-A. Kim, Y.-R. Yoon, I.-J. Cha, Y.-H. Kim, S.-G. Shin, J. Chromatogr. B 713 (1998) 452.
[9] C. López-Calull, N. Dominguez, J. Chromatogr. B 724 (1999) 393.
[10] Danish University Antidepressant Group, J. Affect. Disord. 18 (1990) 289.
[11] E.N. Petersen, E. Bechgaard, R.J. Sortwell, L. Wetterberg, Eur. J. Pharmacol. 52 (1978) 115.
[12] H.T. Karnes, G. Shiu, V.P. Shah, Pharm. Res. 8 (1991) 421.