High Performance Liquid Chromatographic Determination of Fluvoxamine in Human Plasma

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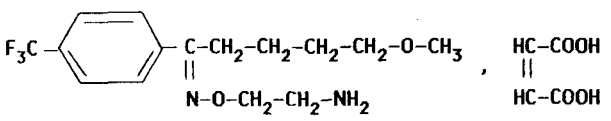
A method has been developed for the separation and measurement of fluvoxamine in human plasma by high performance liquid chromatography. The method uses metapramine as an internal standard and provides a limit of detection of about 1.5 ng/mL for fluvoxamine. At a concentration of 25 ng/mL, fluvoxamine could be measured within a coefficient of variation of ±5.82 of the mean and at 100 ng/mL within a CV of ±2.78 of the mean. The method has been applied to the analysis of plasma from patients undergoing fluvoxamine therapy.

INTRODUCTION

Fluvoxamine maleate (Floxypral) (**1**) is an antidepressant drug belonging to the series of 2-aminoethyl-



Structure 1. Chemical structure of fluvoxamine maleate.

oximethers of aralkylketones. Compounds of these series can inhibit re-uptake of neuronal noradrenaline (NA) and 5-hydroxytryptamine (5-HT). The relative activity with respect to NA re-uptake and 5-HT re-uptake is quite structure-specific. Fluvoxamine was chosen from the series as being a compound with a rather selective inhibition of 5-HT re-uptake and no effect upon NA re-uptake (Claasen *et al.*, 1977).

There is almost no data on the pharmacokinetics of fluvoxamine in humans, mainly because of the lack of a convenient assay. Fluvoxamine is rapidly absorbed after oral administration. The plasma levels reach their maximum between 2 and 8 h after administration, the peak levels ranging from about 30 to 90 ng/mL after a 100 mg dose. The terminal plasma half-life is about 15 h (De Bree *et al.*, 1983) and fluvoxamine is not extensively bound to plasma proteins (77% in man) (Claasen, 1983).

The antidepressant activity of fluvoxamine is due to the activity of the parent compound and the metabolism leads to therapeutically inactive products.

Metabolism of fluvoxamine has been studied in both animals and man. ^{14}C-Labelled fluvoxamine was used to establish the metabolic pattern in urine by thin-layer chromatography and high performance liquid chromatography. A gas chromatographic method was developed by De Bree and Kaal (1981). This last method involves a triple extraction, an additional re-extraction step and hydrolysis to ketones, the whole procedure being time-consuming.

A direct high performance liquid chromatographic method for the detection of fluvoxamine was developed by De Jong (1980). It is a rapid procedure but the detection limit (25 ng/mL) is not sufficient for plasma studies.

Schweitzer *et al.* (1986) have developed a fluorimetric determination of fluvoxamine in human plasma after thin-layer chromatographic or high performance liquid chromatographic separation. This method consisted of four steps: extraction from an alkaline sample with n-heptane+isopropanol, derivatization with NBD chloride (4-chloro-7-nitrobenzofurazan), separation of fluvoxamine from plasma constituents by TLC or HPLC and fluorimetric measurement of the product, but did not use internal standardization.

In this paper, we describe a selective and sensitive method involving derivatization followed by high performance liquid chromatography. This assay was developed to allow further monitoring of drug concentration in patients treated by fluvoxamine and to allow further study of the pharmacokinetics of fluvoxamine in humans.

EXPERIMENTAL

Standard solutions.

Standard stock solutions of fluvoxamine and the internal standard (metapramine) were prepared in methanol at a concentration of 1.0 mg/L in Royal blue, stoppered, Vacutainers (Becton Dickinson, Grenoble, France). They were kept frozen at −20 °C for 4 weeks. 200 μL of the internal standard stock solution was used for internal standardization (200 ng).

Plasma standards (calibration standards) were prepared at concentrations of 10.0; 25.0; 50.0; 100.0; 200.0 and 400 ng/mL of fluvoxamine. The 400 ng/mL standard was prepared by adding 5 mL of drug-free human plasma to the residue obtained by evaporation of 2 mL of fluvoxamine stock solution (1.0 mg/L in methanol). The other standards were then prepared by stepwise dilutions with drug-free human plasma. The calibration standards were stored deep-frozen (−20 °C) in small portions until needed for analyses.

Instrumentation and chromatographic conditions

A model 5000 liquid chromatograph (Varian, Orsay, France) equipped with a Fluorichrom detector (excitation passed by 7.54; 7.60 filter combination and emission collected by 3.71;

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4.76 filter combination) was used throughout. Analyses were performed on a 5 μm Hypersil ODS column (125 × 4.6 mm ID) (Chroma-Sciences, France) operating at 30 °C. Elution solvents were water (solvent A) and acetonitrile (solvent B).

The mobile phase was a linear gradient of 45–65% solvent B in solvent A, run for 10 min, then water + acetonitrile (35 : 65 v/v) was held for 20 min, at a flow rate of 1.5 mL/min.

Extraction procedure

Calibration curve. Plasma standards (2.0 mL) were pipetted into a 125 × 16 mm culture tube, containing 200 ng of internal standard. The plasma was made alkaline with 2.0 mL of 1 M phosphate buffer pH 10.0; 6 mL of a mixture of diethylether + hexane (1 : 1 v/v) were added. The tube was then shaken for 15 min and centrifuged at 4000 × **g** for 5 min. The organic layer was transferred to another tube and acidified with sulfuric acid (0.125 N, 2 mL). The tube was vortexed for 5 min and centrifuged at 4000 × **g** for 5 min. The aqueous phase was then made alkaline with sodium hydroxide (0.5 N, 1.0 mL). The solution was vortexed and a 6.0 mL volume of a mixture of diethylether + hexane (1 : 1, v/v) was added. The tube was shaken for 10 min and centrifuged at 4000 × **g** for 5 min. The organic phase was transferred to another tube and evaporated under a stream of nitrogen at 50 °C. The residue was redissolved in 0.1 M sodium carbonate and 10 μL of dansyl chloride (10 mg/mL in acetone) was added. The tube was vortexed for 1 min and placed in a water bath at 45 °C for 30 min. Finally, the solution was evaporated under a stream of nitrogen at 50 °C. The residue was then dissolved in 200 μL of mobile phase (acetonitrile + water, 45 : 55 v/v) and 100 μL were injected into the liquid chromatograph.

Samples. A suitable volume of plasma (up to 2 mL) was combined with 200 μL of internal standard (200 ng). This mixture was further treated as described for the calibration curve.

Quantitation. Calibration standards covering the anticipated concentration range (10.0–400.0 ng/mL) in methanol and plasma were processed. Peak-area ratios of fluvoxamine to the internal standard were measured and the calibration was obtained from linear regression of the peak-area ratios against concentrations. This line was then used to calculate the concentration of the drug in the unknown samples.

Recovery

Extracts from plasma, prepared as described above were compared with a direct assay of standards in methanolic solutions. These relative recoveries were determined for two different concentrations.

Interferences

Interference from endogeneous material and from other antidepressants was researched. Drugs were tested at concentrations of 400 ng/mL (Table 1).

Patient samples. The procedure was used to analyse plasma samples from patients undergoing therapy with fluvoxamine. Patient blood samples were collected in Royal blue, stoppered, Vacutainers (Becton-Dickinson, Grenoble, France) and centrifuged within 2 h of sampling to obtain plasma. The plasma was then stored at −20 °C in a clean Royal blue Vacutainer tube until analysed.

RESULTS AND DISCUSSION

Figure 1 shows the separation and quantitation of fluvoxamine in human plasma using metapramine as internal standard. In the chromatograms which were obtained after extraction of 2.0 mL blank plasma, no additional peaks that could interfere with the determination of fluvoxamine and internal standard are present. Figure 1(a) represents a chromatogram of a blank plasma. Figure 1(b) is a chromatogram obtained after extraction of 2.0 mL plasma containing 100 ng/mL fluvoxamine. Fluvoxamine and internal standard are well separated with retention times of 19 and 28 min, respectively. Baseline separation of each compound was easily achieved. The peak shapes were symmetrical with no evidence of tailing.

The calibration curves were obtained using methanolic solution of standards, human plasma spiked with 10–400 ng/mL fluvoxamine and 100 ng/mL internal standard (metapramine). There was a good correlation between the amount of fluvoxamine added to human plasma and the amount detected in the samples of both 2.0 mL of plasma. The linear regression equations of data are shown in Table 2 with correlation coefficients >0.99. Calibration curves in plasma showed good linearity between peak-area ratios and concentrations from 10 to 400 ng/mL and the present method is able to detect 1.5 ng/mL fluvoxamine (0.75 ng per peak). Schweitzer *et al.* (1986) determined fluvoxamine at levels down to 5 ng/mL using HPTLC and 1–2 ng/mL using HPLC. De Jong (1980) found a detection limit of 3 ng/mL. The accuracy calculated as 100 × (target con-

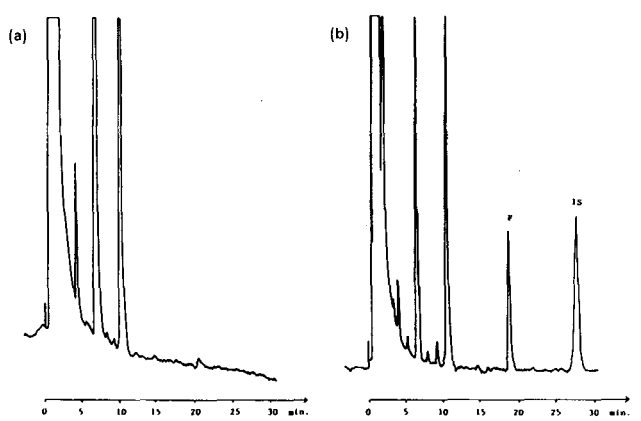

Figure 1. HPLC profiles of (a) extracted blank plasma, (b) human plasma (2 mL) spiked with 100 ng/mL fluvoxamine and 100 ng/mL internal standard. Peaks: F = fluvoxamine; IS = internal standard.

Table 1. Drugs tested for possible interference in the HPLC assay of fluvoxamine

Alimemazine	Levomepromazine
Alprazolam	Loprazolam
Amineptine	Lorazepam
Amitriptyline	Meprobamate
Caffeine	Nitrazepam
Clobazam	Oxazepam
Clomipramine	Cyamemazine
Diazepam	Clorazepate
Demethyldiazepam	Triazolam
Flunitrazepam	Viloxazine

Table 2. Linear regression equations for fluvoxamine

Methanolic solution	$y=0.00585x-0.013$ ($r=0.999$)
Plasma extraction	$y=0.00477x-0.022$ ($r=0.998$)

y = peak area ratio fluvoxamine to internal standard.
x = fluvoxamine concentration.

Table 3. Intra- and interassay coefficient of variation for fluvoxamine

Concentration (ng/mL)	Within day CV (%) ($n=5$)	Day to day CV (%) ($n=5$)
25	5.00	5.82
100	2.01	2.78

n = Number of determinations.

centration − calculated concentration) was approximately 4.8% for 100 ng/mL fluvoxamine. The intra- and inter-assay precision data for fluvoxamine in plasma are summarized in Table 3. There was little variation in fluvoxamine determination with coefficient of variation below 6%.

Analytical relative recoveries of fluvoxamine in plasma were determined at concentrations of 50 and 200 ng/mL and were 62% and 77% respectively. The values are not in agreement with those reported by Schweitzer *et al.* (1986). These authors reported a recovery of about 100% and compared only the peak areas without adding internal standard before the extraction step. De Jong (1980) found 60% of recovery without internal standardization. The method described here used an internal standard.

The assay was shown to be selective, without interference from endogeneous material and from other antidepressant drugs commonly used in antidepressant treatment or often found in poisoned patients.

The HPLC procedure described here has been used for the assay of human plasma samples obtained from patients treated by fluvoxamine. Results show a level of 169 ng/mL for patients receiving 200 mg/day for 4 days and 258 ng/mL for patients receiving 200 mg/day for 20 days.

In summary, this HPLC assay shows good reproducibility, sensitivity, accuracy and selectivity. It has the advantage of being a relatively convenient and simple method. This method was developed in response to a clinical problem and could be applied to pharmacokinetic studies in humans.

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