

Sensitive one-step extraction procedure for column liquid chromatographic determination of fluvoxamine in human and rat plasma

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ABSTRACT

A normal-phase column liquid chromatographic assay for fluvoxamine, a recently introduced atypical antidepressant, has been developed. Prior to analysis, aliquots of alkalinized plasma were extracted with *n*-hexane. These extracts were then injected into a 5- μ m Resolve spherical silica column. Fluvoxamine and the internal standard, clovoxamine, were detected at a wavelength of 254 nm. Standard curves were linear over the concentration range 2–400 ng/ml. Acceptable coefficients of variation were obtained for both within-run and day-to-day studies. The estimated limit of detection is 0.5 ng/ml, and selected drugs checked for coadministration show no analytical interference. This assay was used to evaluate the pharmacokinetics of fluvoxamine in rats.

INTRODUCTION

Fluvoxamine, 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone (*E*)-*O*-(2-aminoethyl)oxime, is one of several recently developed drugs (Fig. 1) that specifically inhibit the neuronal uptake of serotonin (5-hydroxy-tryptamine) [1]. These agents have exhibited antidepressant activity but they are structurally different from the tricyclics, such as clomipramine.

There is little information available on the pharmacokinetics of fluvoxamine in humans and almost none on pharmacokinetics in animals, mainly because of the lack of a convenient assay. Fluvoxamine is rapidly absorbed after oral administration. The plasma level reaches a maximum between 2 and 8 h after adminis-

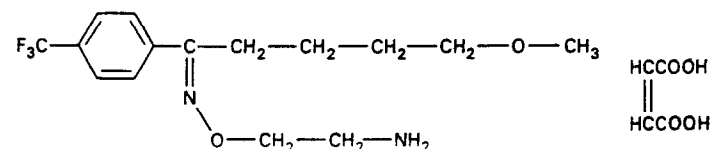


Fig. 1. Structure of fluvoxamine maleate.

tration. The terminal plasma half-life is *ca.* 15 h, and fluvoxamine is not extensively bound to plasma protein (77% in humans) [2]. The metabolism of fluvoxamine has been studied in both animals and humans [3]. Radiolabelled fluvoxamine was used to establish the metabolic pattern in urine by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

A gas chromatographic method was developed by De Bree and Kaal [4]. This method involves triple extraction, additional re-extraction, and hydrolysis to ketones, and the whole procedure is time-consuming.

A direct HPLC method for the detection of fluvoxamine was developed by De Jong [5]. It is a rapid procedure, but the detection limit (25 ng/ml) is not sufficient for pharmacokinetics studies. It is evident that this procedure, *i.e.* the use of a pre-column, is more time-consuming than an isocratic method, mainly as a consequence of the column equilibration delay.

Schweitzer *et al.* [6] have developed a fluorimetric determination of fluvoxamine in human plasma after TLC or liquid chromatographic separation. This method involves the formation of derivatives, and the retention times for fluvoxamine (34.5 min) and its chloro analogue clovoxamine (31.5 min) are too long for routine analysis. HPLC methods using fluorescence or UV detection [7,8] require two extraction steps and a large volume of plasma (2 ml), and do not yet reach a suitable level of sensitivity (detection limit 25 ng/ml).

We have developed a rapid HPLC procedure without complex purification, which allows the determination of fluvoxamine in plasma after a single extraction step, using clovoxamine as an internal standard (I.S.). The method is adequate for routine drug monitoring and for studies on the pharmacokinetics of fluvoxamine in humans and animals: an example from a recent pharmacokinetics study is provided.

EXPERIMENTAL

Reagents and standard

Fluvoxamine maleate and clovoxamine fumarate were kindly supplied by Duphar (Villeurbanne, France). Methanolic solutions of fluvoxamine and clovoxamine (1 mg/ml) were stored at 4°C. Under these conditions, they were stable for months. Methanol and acetonitrile (both HPLC grade) were purchased from Merck (Darmstadt, Germany). Tetrahydrofuran and sodium hydrogencarbonate were supplied by Sigma (St. Louis, MO, U.S.A.). Hexane (HPLC grade) and diethylamine (Rectapur) were obtained from Prolabo (Paris, France).

Apparatus and chromatographic conditions

The chromatographic system consisted of a Beckman pump, Model 114 M (Gagny, France), connected to a Kontron 660 T autosampler (Montigny-Le-Bretonneux, France), a Beckman Model 160 spectrophotometric detector set at a wavelength of 254 nm, where absorbance is maximum (Fig. 2) (0.002 a.u.f.s.) and a Shimadzu C-R6 A integrator (Touzart et Matignon, Vitry, France).

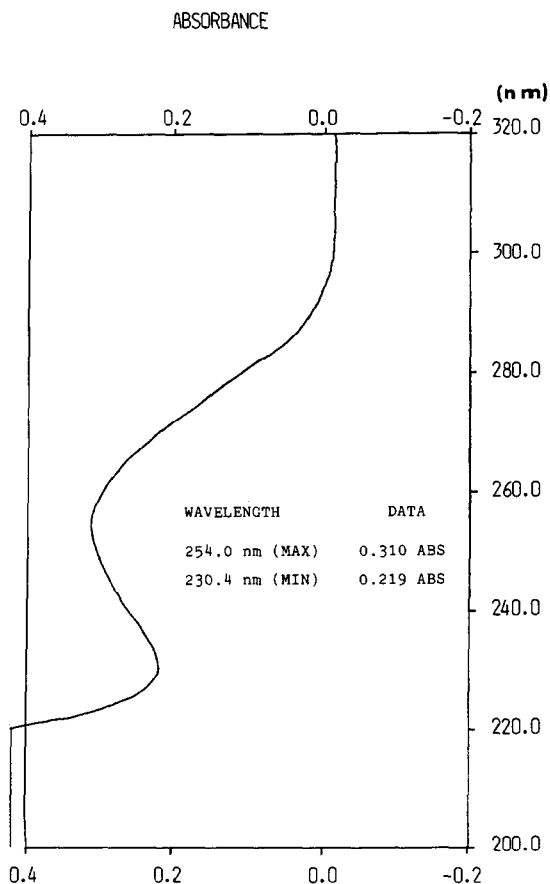


Fig. 2. Absorption spectrum of fluvoxamine.

Separation was achieved at room temperature, using a Resolve spherical silica column (15 cm \times 3.9 mm I.D., particle size 5 μ m) from Waters Millipore (Milford, MA, U.S.A.). The eluent was degassed methanol–acetonitrile–tetrahydrofuran–water–diethylamine (98.59:1:0.2:0.2:0.01, v/v). The flow-rate was 1.0 ml/min.

Extraction procedure

In a 15-ml centrifuge tube, 1 ml of plasma (from heparinized blood) alkalinized with 500 μ l of 2 M sodium hydrogencarbonate was extracted by hexane (10 ml) after the addition of the I.S. The tube was capped, shaken horizontally for 20 min, then centrifuged at 2000 g for 10 min. The upper organic layer was removed and transferred to 12-ml centrifuge tubes, dried under a gentle stream of nitrogen in a water-bath set at 35°C. After reconstitution with 200 μ l of the mobile phase, a 50- μ l aliquot of the final preparation was injected into the column.

Preparation of the calibration curve

A standard curve was constructed for each assay by adding known amounts of fluvoxamine maleate to 1 ml of drug-free plasma. A single calibration curve was used for pharmacokinetic analysis (range 0–400 ng/ml).

Concentrations of the drug equivalent to 2, 5, 10, 15, 20, 30, 50, 100, 200, 300 and 400 ng/ml (as base) were measured. An aliquot (100 μ l) of the I.S. in methanolic solution (0.1 or 1 μ g/ml) was added to each spiked plasma sample.

RESULTS

Quantification, separation and plasma interference

The equation describing the standard curve determined by linear least squares regression analysis was y (peak-height ratio) = $0.0859x$ (amount of fluvoxamine injected, ng) + 0.0115 when 10 ng of the I.S. were added, and $y = 0.00841x + 0.0098$ for 100 ng of the I.S. The corresponding correlation coefficients (r) were 0.9998 and 0.9997, respectively.

Chromatograms obtained from blank plasma and from plasma spiked with fluvoxamine maleate and the I.S. are shown in Fig. 3. The retention times were 4.7 and 5.2 min for fluvoxamine and clovoxamine, respectively. These two peaks were well resolved without any interference from endogenous compounds.

The selectivity of resolve spherical silica column enables other antidepressants or compounds used as concomitant therapy, or present in cases of intoxication, to

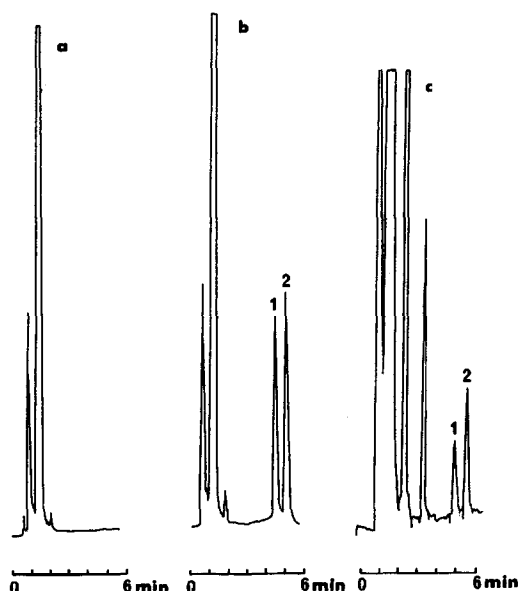


Fig. 3. Chromatograms obtained from (a) a blank human plasma sample, (b) a spiked human plasma (50 ng/ml) and (c) a sample from a patient. Peaks: 1 = fluvoxamine; 2 = internal standard, clovoxamine.

be resolved. Various other antidepressants, benzodiazepines and neuroleptics were assayed by the proposed method; retention data are given in Table I. Clearly, only imipramine can interfere with the determination of fluvoxamine.

The three main metabolites (fluvoxamino acid, fluvox ethanol and acetylated fluvoxamino acid) are not eluted within 30 min.

Accuracy, recovery and limit of detection

Accuracy and precision, and day-to-day and within-day reproducibility data are shown in Tables II and III.

The assay recovery with four different concentrations (5, 20, 100 and 400 ng/ml, $n = 6$) had an average (\pm S.D.) for fluvoxamine of $88 \pm 5.3\%$. The I.S. recovery was $83 \pm 8\%$.

TABLE I

RETENTION TIMES OF DRUGS TESTED FOR INTERFERENCE WITH FLUVOXAMINE

Drug	Retention time (min)
Propericiazine	1.83
Haloperidol	2.25
Viloxazine	2.45
Trimipramine	2.55
Levomepromazine	2.65
Alimemazine	2.70
Amitryptiline	3.06
Chlorpromazine	3.21
Clomipramine	3.26
Imipramine	4.08
Fluvoxamine	4.7
Clovoxamine I.S.	5.2
Fluoxetine	9.91
Norfluoxetine	10.61
Nortryptiline	12.21
Desmethyldomipramine	14.10
Desipramine	18.55
Buspirone	N.E. ^a
Amineptine	N.E.
Diazepam	N.E.
Desmethyl diazepam	N.E.
Lorazepam	N.E.
Clorazepate, dipotassium salt	N.E.
Clobazam	N.E.
Clonazepam	N.E.
Flunitrazepam	N.E.
Nitrazepam	N.E.
Oxazepam	N.E.

^a N.E. = not eluted within 30 min.

TABLE II

INTER-DAY ACCURACY AND PRECISION

Day-to-day assays were performed over six months using spiked human plasma samples ($n = 10$).

Fluvoxamine added (ng/ml)	Fluvoxamine found (ng/ml)	Coefficient of variation (%)
2	1.99	13.4
5	5.07	5.1
10	9.99	2.9
15	15.31	2.8
20	19.79	4.9
30	30.13	2.2
50	49.17	1.8
100	100.33	2.2
200	197.90	3.2
300	301.50	3.6
400	398.30	2.7

The choice of the detection wavelength (254 nm) was suggested by the remarkable increase of sensitivity compared with that obtained at other wavelengths, as previously reported. The lower limit of detection (at a signal-to-noise ratio of 3) was 0.5 ng/ml. The limit of quantitation with good accuracy was 2 ng/ml during routine analysis. This limit is adequate for pharmacokinetic studies in animals, and the levels found in patients during therapeutic drug monitoring were greater than 50 ng/ml.

TABLE III

INTRA-DAY ACCURACY AND PRECISION

Spiked human plasma samples were used ($n = 6$).

Fluvoxamine added (ng/ml)	Fluvoxamine found (ng/ml)	Coefficient of variation (%)
5	4.83	5.6
20	20.75	1.9
100	97.71	1.9
400	387.80	1.8

DISCUSSION

The assay described here provides a method that allows fluvoxamine concentrations to be determined by normal-phase HPLC. This method offers significant advantages over earlier methods. Sample preparation was relatively simple and required less time per sample than procedures requiring derivatization of fluvoxamine and clovoxamine with the fluorogenic reagent fluorescamine [5] or with NBD chloride (4-chloro-7-nitrobenzofurazan) [6]. Furthermore, retention times were shorter than those reported previously [6,7]. The extraction procedure is no more time-consuming than that reported by Foglia *et al.* [8], who performed an initial extraction from an alkaline solution followed by a back-extraction into 0.1 M hydrochloric acid. We found that the single liquid-liquid hexane extraction step was sufficient to remove substantial interfering endogenous peaks found in the blank serum, as described by Diquet *et al.* [9] for antidepressant determination.

Unlike Foglia *et al.* [8], we used an internal standard, clovoxamine. The extraction efficiency did not appear to be concentration-dependent and was determined to be of the same order of magnitude as seen with similar liquid-liquid extractions of this compound.

The accuracy and precision estimates of the data were found to be acceptable for both pharmacokinetic studies and therapeutic monitoring of fluvoxamine in patients.

The detection limit of this assay was determined to be 0.5 ng/ml. The lower limit of reliable estimate may be between this value and the lowest concentration of the calibration curve (2 ng/ml), given the reasonable estimates of precision at that concentration (Table II). The maximum coefficient of variation (Table III) was to be less than 10%. The assay was linear over the concentration range used. The coefficient of correlation exceeded 0.9997 for all calibration curves.

Several substances were evaluated for potential interference with the quantification of fluvoxamine. Table I summarizes the retention times of these compounds. No interference with any of the peaks of interest was observed with any of the listed drugs. Overall, the system was relatively stable and performed well under the conditions of this assay. Of note is a substantial variation in retention times with different compositions of the mobile phase. The α values for fluvoxamine and the I.S. were 1.18, approaching 1.00 when the proportion of diethylamine was increased.

Each column may be used for at least 1000 injections, in contrast to the relatively less stable LiChrosorb RP-8 column used by De Jong [5]. Furthermore, the use of the LiChrosorb RP-8 column means that the whole system must be flushed regularly with methanol in order to ensure the elution of relatively lipophilic substances each time after the analytical step. It is obvious that this procedure is time-consuming, especially because of the column equilibration time. The method described here does not need a long equilibration time. Moreover flushing of the column after analysis is not required.

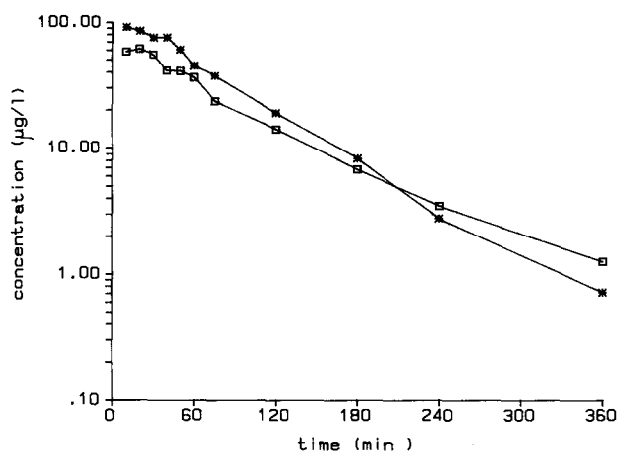


Fig. 4. Mean plasma fluvoxamine concentrations after administration of a 4 mg/kg dose of fluvoxamine (□) or fluvoxamine plus diazepam (1 mg/kg) (*).

This assay was used to evaluate the pharmacokinetics of fluvoxamine in rats. The logarithm of concentration *versus* time plots are illustrated in Fig. 4 for rats after intraperitoneal administration of fluvoxamine (F), or combined administration of fluvoxamine and diazepam (FD). The area under the concentration-time curve (AUC) was determined to be 5279 and 7381 ng min/ml for F and FD administration, respectively.

We found that concomitant administration of diazepam does affect the pharmacokinetics of fluvoxamine, and other investigators [10–12] reported that this may modify the pharmacokinetics and/or pharmacodynamics of each treatment. The AUC values and other pharmacokinetics parameters evaluated by this procedure support these results.

CONCLUSION

The proposed HPLC procedure for the quantitative determination of fluvoxamine in human or animal plasma offers rapidity, good accuracy and precision. The method is sensitive enough for clinical pharmacological studies and for drug monitoring. This procedure also allows the monitoring of numerous tri- or tetracyclic antidepressants and their active metabolites, following minor modifications of the mobile phase and the detection wavelength.

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