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Note

Determination of fluvoxamine in human plasma by high-performance liquid chromatography with ultraviolet detection

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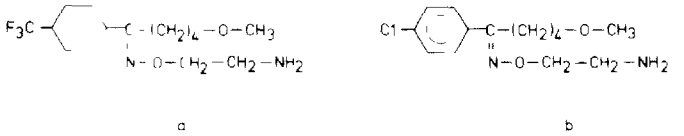
A new generation of agents for the treatment of various depressive states was introduced with the release of fluvoxamine [1,2] Fluvoxamine, 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone (*E*)-O-(2 aminoethyl)-oxime, a new antidepressant (Fig 1), has significant serotonergic activity through the selective blockade of reuptake of serotonin, the metabolites of fluvoxamine show no significant psychotropic activity [3–7]

Until recently, preliminary studies had been unable to demonstrate correlations between fluvoxamine plasma levels and clinical outcome, although in eleven patients the concentration range was found to be 282–515 ng/ml during the second and third treatment week, using a flexible dose regime of 50–300 mg per day [12] A newly completed study with 35 patients, which compared fluvoxamine with desipramine, has demonstrated that the relationship between fluvoxamine plasma level and response is linear with a suggestion that the steady state range of 160–220 ng/ml is optimal for therapeutic response [13] Thus, it appears that simpler methodology with expedient sample prep-



a b

Fig 1 Structures of fluvoxamine (a) and clovoxamine (b)

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aration would be appropriate for steady-state plasma levels with a limit of detection of ca 25 ng/ml and with acceptable between-day reproducibility

Available methods include gas chromatography (GC) using electron-capture detection [8], high-performance liquid chromatography (HPLC) with fluorescence detection, and thin-layer chromatography (TLC) [9–11] The GC method involves a triple extraction, an additional re-extraction step and hydrolysis to ketones with an overall recovery of ca. 60% — a rather lengthy procedure that has adequate specificity. The two fluorescent HPLC methods involve the formation of derivatives such as the ones obtained by reacting fluvoxamine and the internal standard clovoxamine, with 4-chloro-7-nitrobenzofurazan (NBD chloride) or fluorescamine, followed by separation with HPLC or TLC and fluorometry of the products The detection limits are 1–5 ng/ml coefficients of variation (C V) in the range 2 2 -5 9% However, as with GC these methods are time-consuming and, although useful for single-dose pharmacokinetics, are not readily applicable for therapeutic monitoring

The present method involves an initial extraction from an alkaline solution, which is followed by a back-extraction into 0 1 *M* hydrochloric acid The top organic layer is discarded with the acid layer remaining for analysis Both substances are completely resolved in one HPLC run

EXPERIMENTAL

Materials

2-Propanol, *n*-heptane, and methanol were obtained from Burdick and Jackson (Obetz, OH, U S A) Potassium phosphate, HPLC grade, was obtained from Fisher Scientific (Pittsburgh, PA, U S A) and *n*-octylamine was purchased from ICN (Cleveland, OH, U S A) All remaining chemicals used were analytical grade The fluvoxamine maleate and clovoxamine fumarate were kindly supplied by Duphar (Weesp, The Netherlands) All other drugs were obtained from Sigma (St Louis, MO, U S A)

Apparatus

The analysis was performed on a Beckman gradient liquid chromatograph, Model 110A, with Hitachi Model 40 variable-wavelength UV detector at a wavelength of 215 nm (Beckman Instruments, Fullerton, CA, U S A) A straight stainless-steel column (12 cm×4 6 mm I D) packed with 5-μm Nucleosil C_8 was used (Unimetrics, Shorewood, IL, U S A) The chromatograph was also fitted with a 7125 Rheodyne injector and 20-μl loop (Rainin Instruments, Woburn, MA, U S A)

Extraction procedure for plasma samples

The analytical method for HPLC determination of fluvoxamine was as follows 1 ml of plasma was added to a 10-ml screw-capped polypropylene vial

(Fred Morrow Scientific, New Milford, NJ, U S A) with 20 μl of 10 μg/ml solution of clovoxamine added as the internal standard To each of these samples, 120 μl of 2 *M* sodium hydroxide were added to adjust the pH to 12 Then 4 ml of heptane–2-propanol (98 2, v/v) were pipetted into each tube, and the tubes were shaken for 30 min and centrifuged at 3000 *g* for 10 min The organic layer was transferred to another 10-ml vial containing 100 μl of 0 1 *M* hydrochloric acid, which was then shaken for 20 min This solution was transferred to 4 5-ml polypropylene conical tubes and centrifuged at 3000 *g* for 10 min The top organic layer was discarded, and 20 μl of the remaining hydrochloric acid layer were injected into the chromatograph

Chromatography conditions

The mobile phase for the separation of clovoxamine, the internal standard, and fluvoxamine was a 0 016 *M* phosphate buffer (pH 2 5), which was prepared with 0 016 *M* potassium phosphate (KH_2PO_4) adjusted to pH 2 5 with concentrated phosphoric acid (HPLC grade), and acetonitrile (64 36, v/v) The flow-rate was 1 ml/min For separations, the column was maintained at room temperature

Human plasma samples

Human plasma samples were obtained from a clinical protocol, which involved the random assignment of patients to either a noradrenergic (desipramine) or serotonergic (fluvoxamine) agent The study was performed over a 31-day trial to determine the effectiveness of fluvoxamine in comparison with desipramine in depressed patients

Recovery and linearity

Recoveries were performed with spiked and patient samples at four different concentrations of fluvoxamine with 20 μl of a 10 mg/ml solution of clovoxamine Recovery of clovoxamine was also performed at four different concentrations The linearity of the assay was determined between 25 and 400 ng/ml, with samples containing 25, 50, 100, 150, 200, 250, 300, 350, and 400 ng/ml fluvoxamine and 200 ng/ml clovoxamine

RESULTS

UV detection of fluvoxamine and clovoxamine

UV detection at 215 nm of both fluvoxamine and clovoxamine was sufficiently sensitive for therapeutic monitoring of plasma samples The samples obtained for the 31-day study had steady-state values adequately detected by this technique

The limit of detection of the assay for fluvoxamine was 25 ng/ml with a signal-to-noise ratio of 5 1 The assay recovery (Table I) with four different

TABLE I

ASSAY RECOVERY STUDY FOR FLUVOXAMINE AND CLOVOXAMINE FROM SPIKED PLASMA SAMPLES ($n=6$)

Drug	Amount present (ng/ml)	Mean recovery (%)
Fluvoxamine	25	92
	100	94
	200	93
	400	94
Clovoxamine (internal standard)	100	85
	150	80
	200	83
	250	84

TABLE II

ACCURACY OF FLUVOXAMINE DETERMINATION FROM SPIKED PLASMA SAMPLES ($n=6$)

Concentration spiked (ng/ml)	Average concentration determined (ng/ml)	Coefficient of variation (%)
25	23	5 2
100	98	4 1
200	197	3 5

concentrations had an average ($\pm$ S D) for fluvoxamine of 93 $\pm$ 0 96% and the internal standards recovery was 83 $\pm$ 2 2% The accuracy of the fluvoxamine assay was determined with spiked plasma at three different concentrations (Table II) Recovery studies were also done to establish the correct solvent system (Table III) The linearity of the assay for fluvoxamine over the range 25–400 ng/ml was demonstrated, with a correlation coefficient of 0 9989 (fluvoxamine, $y=0\ 0038x-0\ 0186$) A limit of detection of 25 ng/ml was more than adequate for these analyses because the levels found in patients were greater than 50 ng/ml The reproducibility of spiked plasma and patient samples is reported in Tables IV and V. The C V range was 3 2–9 7% for the spiked samples, and 0 4–3 4% for patient plasma samples.

Dosage adjustments were done on the 17th and 27th day of the protocol on the basis of the plasma levels, in order to maintain therapeutic plasma level uniformity and minimize the side-effects by fluvoxamine In Table VI, the data

TABLE III

RECOVERY RATES OF FLUVOXAMINE AND CLOVOXAMINE WITH SOLVENTS IN HEPTANE

Percentages recoveries of both compounds were determined in triplicate and the mean value calculated

Compound	Solvent	Percentage solvent	Recovery (%)
Fluvoxamine	2-Propanol	2	90
		5	91
	n-Propanol	2	82
		5	81
	n-Butanol	2	83
		5	82
	Isobutanol	2	85
		5	85
Clovoxamine	2-Propanol	2	82
		5	83
	n-Propanol	2	65
		5	68
	n-Butanol	2	53
		5	62
	Isobutanol	2	48
		5	44

TABLE IV

DAY-TO-DAY REPRODUCIBILITY OF FLUVOXAMINE STANDARD CURVES FROM SPIKED PLASMA SAMPLES

Concentration (ng/ml)	*n*	Mean peak-height ratio	Coefficient of variation (%)
50	20	0 210	4 8
100	20	0 389	6 5
150	20	0 586	9 7
200	20	0 805	8 6
250	20	1 019	8 8
300	20	1 146	3 2

TABLE V

DAY-TO-DAY REPRODUCIBILITY OF FLUVOXAMINE WITH PATIENT PLASMA SAMPLES

Samples were run in triplicate

Patient	Day of study	Mean value (ng/ml)	Coefficient of variation (%)
LA 1	10	139	0 8
KRAY 2	10	123	3 4
	20	282	0 7
BIC 3	10	164	2 6
MUL 4	10	360	0 4

TABLE VI

MEAN DOSE AND PLASMA LEVELS OF FLUVOXAMINE IN SEVENTEEN PATIENTS

Study day	Mean of dose (mg per day)	Plasma level (mean + S D) (ng/ml)	Concentration-to-dose ratio
13	211 8 ± 45 2	256 ± 128	1 2
27	202 9 ± 69 5	269 ± 140	1 3

TABLE VII

POTENTIAL DRUG INTERFERENCES

Drugs were injected neat and also extracted by the described method with detection at 215 nm

Drug	Retention time (min)
Amitriptyline	5 5
Chlorimipramine	7 0
Clovoxamine	3 3
Desipramine	4 5
Desmethylchlorimipramine	6 1
Doxepin	3 8
Fluvoxamine	4 3
Imipramine	4 9
Nortriptyline	4 9
Trimipramine	5 8

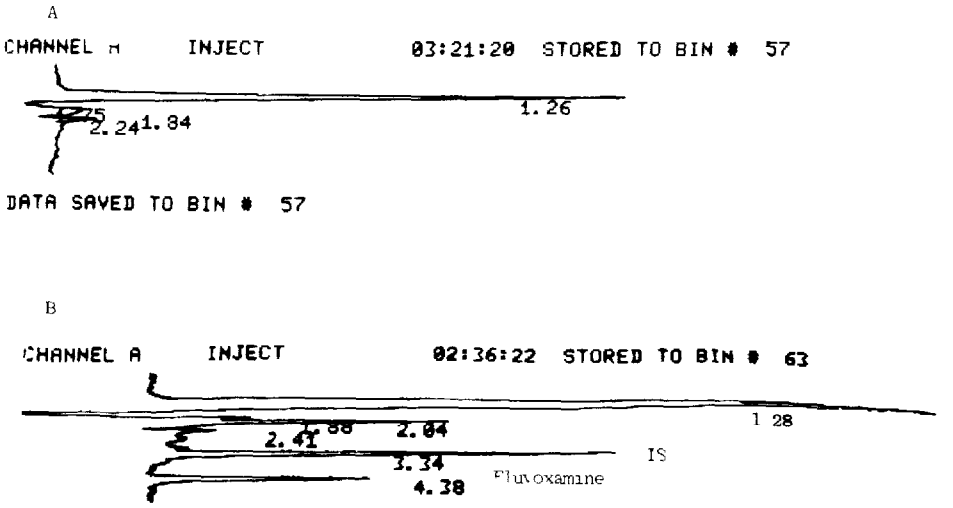

Fig 2 Chromatograms of (A) a plasma extract of a blank sample and (B) a plasma extract containing fluvoxamine (150 ng/ml) and clovoxamine (200 ng/ml)

from the clinical study are summarized, indicating the observed therapeutic plasma levels with concentration/dosage ratios between 1 2 and 1 3

Potential drug interferences were also ruled out at a wavelength of 215 nm as shown in Table VII, which lists the different retention times of the various antidepressant agents Fig 2 shows chromatograms of a blank and a sample, to demonstrate the resolution of the compounds of interest

DISCUSSION

The monitoring of fluvoxamine in depressed patients is directed to ensure the therapeutic steady-state plasma levels and compliance, toxicity is not a major concern because of the high therapeutic index already demonstrated with this agent Another important issue is that fluvoxamine, by virtue of its selective serotonergic effects, produces a higher incidence of untoward effects related to this neuropharmacology, such as headaches, gastrointestinal distress, and sleep disturbances Clinicians have often added a second agent, such as a tricyclic antidepressant, to ameliorate these adverse symptoms This may lead to analytical specificity problems in the reported procedures for fluvoxamine

This assay was developed to insure reproducibility and accuracy for the therapeutic monitoring of fluvoxamine in patients while retaining sufficient simplicity to allow rapid turn-around of the results Although existing procedures are adequate and are also able to measure low levels for pharmacokinetic studies, the present assay procedure had adequate sensitivity without time-con-

suming and laborious analytical steps of previous methods and thus can readily evaluate routine plasma samples Equally important, as shown in Table VII, this procedure is specific for fluvoxamine in the presence of tricyclic antidepressants and their metabolites, and may also be utilized for concurrent measurements of both antidepressants for monitoring as well as ascertainment of potential drug interactions

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