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Note

Fluorimetric determination of fluvoxamine or clovoxamine in human plasma after thin-layer chromatographic or high-performance liquid chromatographic separation*

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Fluvoxamine (Fevarin), 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone (*E*)-O-(2-aminoethyl)oxime, and its chloro analogue clovoxamine, 5-methoxy-1-[4-(chloro)phenyl]-1-pentanone (*E*)-O-(2-aminoethyl)oxime, are new antidepressive agents (Fig. 1). Both drugs are almost completely absorbed from the gastrointestinal tract if administered orally. The fluvoxamine 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 [1]. From urine eleven metabolites were isolated; non-metabolized fluvoxamine did not appear [2].

Fig. 1. Structural formulae of (a) fluvoxamine and (b) clovoxamine.

A direct high-performance liquid chromatographic (HPLC) method for the detection of fluvoxamine and clovoxamine was developed by De Jong in 1979 [3]. It is a rapid procedure but the detection limit (25 ng/ml in plasma) is not sufficient for plasma studies. A gas chromatographic method was developed by De Bree and Kaal in 1981 [4]. Since this method involves a triple extraction,

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an additional re-extraction step and hydrolysis to ketones, the whole procedure is time-consuming. Therefore, it seemed desirable to find a more rapid procedure for the determination of fluvoxamine or clovoxamine that allows the determination of very low concentrations in human samples.

The method described in this paper consists of four steps: (1) extraction from an alkaline sample with *n*-heptane—isopropanol; (2) derivatization with NBD chloride (4-chloro-7-nitrobenzofurazan) [5]; (3) separation of fluvoxamine or clovoxamine from plasma constituents by thin-layer chromatography (TLC) or HPLC; (4) fluorimetric measurement of the product.

EXPERIMENTAL

Chemicals and materials

Fluvoxamine maleate and clovoxamine fumarate were supplied by Duphar (Weesp, The Netherlands). Methanolic solutions of fluvoxamine or clovoxamine (1 mg/ml) were stored at 4°C. Under these conditions they were stable for months.

4-Chloro-7-nitrobenzofurazan (NBD chloride) and solvents (analytical-reagent grade or LiChrosolv) and HPTLC materials were obtained from E. Merck (Darmstadt, F.R.G.). NBD chloride was used as a freshly prepared solution containing 0.1 g in 100 ml of isobutyl methyl ketone for extraction analysis.

The HPTLC plates (20 × 10 cm) used were pre-coated with silica gel 60 (for nano-TLC) without a fluorescence indicator and with a concentrating zone (2.5 × 20 cm). The plates were used without any activation prior to chromatography.

Equipment

A DuPont HPLC column (25 × 0.46 cm I.D.) filled with Zorbax Sil (7 μm) as the stationary phase was used. The pre-column (DuPont) (5 × 0.46 cm I.D.) was filled with LiChrosorb Si 100 (30 μm). For TLC scanning a KM 3 chromatogram spectrophotometer from Carl Zeiss (Oberkochen, F.R.G.) with a Linseis (Selb, F.R.G.) recorder was used. Solutions were applied to the TLC plates using a Linomat III (Camag, Muttenz, Switzerland).

For HPLC a 64 pump from Knauer (Berlin, F.R.G.), a Rheodyne injection valve with a 100-μl loop, an RF-530 fluorescence HPLC monitor from Shimadzu (Düsseldorf, F.R.G.) and an Sb recorder from Servogor (Nürnberg, F.R.G.) were used.

The vacuum centrifuge used was a Speed Vac concentrator (Bachofer Laboratoriumswerke, Reutlingen, F.R.G.).

General procedure for plasma samples

The extraction, derivatization procedures and measuring conditions are identical for fluvoxamine and clovoxamine.

Extraction. In a screw-capped glass centrifuge tube 1 ml of plasma is extracted with 5.6 ml of *n*-heptane—isopropanol (99:1) after addition of 1 ml of 1 *M* sodium hydroxide solution. After shaking on a mechanical shaker

(30 min) and centrifugation (10 min at 3000 *g*), 5 ml of the organic layer are aspirated, transferred to another centrifuge tube and evaporated to dryness in a vacuum centrifuge at 60° C.

Derivatization procedure. A 0.1 *M* sodium hydrogen carbonate solution (0.2 ml) and 0.2 ml of a 0.1% NBD chloride solution are added to the residue in the centrifuge tubes. The tubes are shaken on a vortex mixer for 10 s and the reaction mixture is kept at 80°C for 30 min. After cooling to room temperature, 0.1 g of sodium chloride is added. After stirring on a vortex mixer the contents are centrifuged for 5 min.

Thin-layer chromatography. A 10-μl aliquot of the upper organic layer is carefully aspirated from the surface with a Hamilton syringe and applied to an HPTLC plate in a 5-mm strip with a Linomat III. Three plasma standards of fluvoxamine or clovoxamine are also spotted. For the analysis of samples obtained after oral administration of a single dose of 100 mg, spiked plasma standards with a concentration of 20 ng/ml were used. The plate is developed at room temperature in an unlined glass tank (Desaga) containing chloroform—ethyl acetate (100:4) (solvent mixture 1) as mobile phase for fluvoxamine. For clovoxamine, the ratio of the components of the mixture is 100:1 (solvent mixture 2).

After developing the plate for 30 min, it is air-dried. To enhance the fluorescence of the derivatization product the plate is dipped into paraffine liquid—cyclohexane (30:60) and again air-dried. Under the described conditions the derivatization products move 3.0 cm (fluvoxamine, solvent mixture 1) and 2.4 cm (clovoxamine, solvent mixture 2). Finally the plate is scanned with the spectrophotometer.

Fluorimetric detection. The following conditions were used: excitation, 434-nm line (ST 41 medium-pressure mercury lamp); emission, 546 nm (M 546 monochromatic filter); slit 1 × 6 mm.

The concentrations of plasma samples are calculated from spiked plasma standards (peak-area method). For each plate three plasma standards of the same concentration are used.

High-performance liquid chromatography. The following conditions were used: injection volume, 100 μl of the organic layer (after derivatization with NBD chloride), loop filling; stationary phase, see *Equipment*; mobile phase, chloroform—isopropanol (250:2); flow-rate, 1 ml/min; pressure, 7 MPa; temperature, ambient; detection wavelengths, excitation 455 nm, emission 513 nm (xenon lamp as light source, two monochromators).

The retention time of fluvoxamine is 34.5 min and that of clovoxamine is 31.5 min. The concentrations are calculated as described for HPTLC.

Recovery and linearity studies

Recovery studies were performed at three different concentrations by analysing spiked plasma samples and comparing the peak areas with those of non-extracted standards. The linearity of the calibration graph was tested between 0 and 200 ng/ml with samples containing 0, 5, 10, 20, 50, 100 and 200 ng/ml fluvoxamine or clovoxamine.

Reproducibility

Reproducibility experiments were carried out by investigating eight samples per concentration and three different concentrations of fluvoxamine and clovoxamine in plasma.

Applicability

With fluvoxamine, but not clovoxamine, clinical studies in man have already been performed. Hence the applicability of the fluvoxamine method could be tested by analysing plasma samples from two patients having received a single oral dose of 100 mg of fluvoxamine. Plasma samples were taken over a period of 36 h. The plasma was stored at −22°C until analysed.

RESULTS

Fluorescence properties and chromatography

The derivatization products can be detected by measuring the fluorescence. Excitation and emission maxima of the derivatization products of both compounds are 455 and 513 nm, respectively. There are no differences between the relative fluorescence properties on the TLC plates and in the organic solvents used as mobile phases. Representative chromatograms of HPTLC and HPLC separations are given in Figs. 2—4.

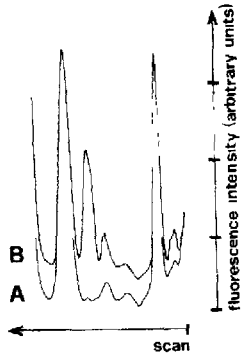

Fig. 2. Representative HPTLC traces of (A) blank plasma sample and (B) fluvoxamine in a patient's plasma (32 ng/ml).

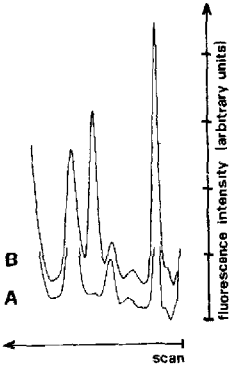

Fig. 3. Representative HPTLC traces of (A) blank plasma sample and (B) clovoxamine in spiked plasma (50 ng/ml).

Recovery, linearity and reproducibility

Mean recoveries from plasma of 99.3% for fluvoxamine and 96.7% for clovoxamine were obtained (means of three different concentrations) (Table I).

For both compounds the calibration graphs obtained by HPTLC and HPLC are linear over the range investigated (between 0 and 200 ng/ml) with correlation coefficients > 0.99 [HPTLC: for fluvoxamine 0.9999 ($y = 0.1002x + 0.355$), for clovoxamine 0.9999 ($y = 0.0348x - 0.1100$); HPLC: for fluvox-

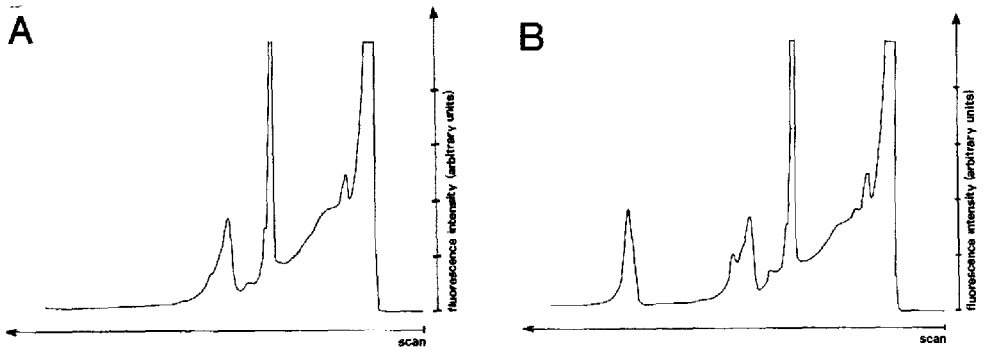

Fig. 4. Representative HPLC traces of (A) blank plasma sample and (B) fluvoxamine in a patient's plasma (7 ng/ml).

TABLE I

RECOVERIES OF FLUVOXAMINE AND CLOVOXAMINE FROM PLASMA (n = 3)

Compound	Amount added (ng)	Mean recovery (%)
Fluvoxamine	20	98.9
	50	99.4
	100	99.6
Clovoxamine	20	96.2
	50	96.6
	100	97.3

TABLE II

RESULTS OF REPRODUCIBILITY STUDIES

Compound	Method	Concentration in plasma (ng/ml)	Number of samples	Coefficient of variation (%)
Fluvoxamine	HPLC	5	7	5.9
		10	7	4 7
		20	8	2.7
		50	8	2.3
		100	8	2.4
	TLC	20	8	2.9
		50	9	2.9
		100	8	2.2
Clovoxamine	HPLC	5	7	5.2
		10	7	3.4
		20	8	2.7
		50	8	3.2
		100	8	2.6
	TLC	20	8	3.9
		50	9	2.9
		100	9	2.9

amine 0.9984 ($y = 0.1671x + 0.0847$), for clovoxamine 0.9985 ($y = 0.2047x - 0.0693$)]. The detection limits of fluvoxamine and clovoxamine are < 2 ng/ml (0.09 ng per peak) using HPTLC and < 0.5 ng/ml (0.22 ng per peak) using HPLC. The compounds can be determined at levels down to 5 ng/ml using HPTLC and 1—2 ng/ml using HPLC. The results from the reproducibility studies are given in Table II.

Interferences

There are no interferences from normal constituents of plasma. Using HPLC the difference between the retention times is small and fluvoxamine and clovoxamine may therefore interfere with each other. Hence neither TLC nor HPLC can be used for the simultaneous determination of fluvoxamine and clovoxamine.

Applicability

After oral administration of 100 mg of fluvoxamine to two patients suffering from liver cirrhosis the highest concentrations found were 31.8 and 18.3 ng/ml. The corresponding t_{max} values were 9 and 3 h and the terminal half-lives were 12 and 15 h.

DISCUSSION

The described procedure is the first method available for the simple and rapid determination of fluvoxamine or clovoxamine in biological materials. However, for the detection of low drug concentrations the excess of derivatization reagent must be high, i.e., the concentration of the reagent solution must be at least 0.1%. As NBD chloride easily crystallizes even from these lower concentrated solutions, frequent column washing is necessary in HPLC; the pre-column has to be washed carefully after about 40—50 samples and the analytical column must also be washed from time to time. Nevertheless, the method is suitable for routine analyses of biological materials, as especially the extraction and derivatization procedures are short.

Further, the intensity of the fluorescence of the derivatization products is sufficient for the determination of therapeutic concentrations after oral administration. If samples with very low concentrations are to be measured, HPLC is preferable. The more rapid TLC method can be applied to samples with fluvoxamine or clovoxamine concentrations above 5 ng/ml. TLC was not as sensitive as HPLC because neither the excitation nor the emission wavelengths totally matched the excitation and emission maxima of the derivatization products, owing to the spectrophotometer used.

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REFERENCES

1 H. de Bree, J.B. van der Shoot and L.C. Post, Eur. J. Drug Metab. Pharmacol., 8 (1983) 175.
2 H. Overmars, P.M. Scherpenisse and L.C. Post, Eur. J. Drug Metab. Pharmacol., 8 (1983) 269.
3 G.J. de Jong, J. Chromatogr., 183 (1980) 203.
4 H. de Bree and J.H.M.A. Kaal, Report No. 56630/81, Analytical Department, Duphar, Weesp, 1981.
5 N. Seiler and L. Demisch, in K. Blau and G.S. King (Editors), Handbook of Derivatives for Chromatography, Heyden, London, 1978, pp. 367—370.