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DETERMINATION OF THE ANTIDEPRESSANT AGENT CITALOPRAM AND METABOLITES IN PLASMA BY LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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SUMMARY

A high-performance liquid chromatographic method is described for the determination of citalopram [1-(3-(dimethylaminopropyl)-1-(4-fluorophenyl)-5-phthalancarbonitrile] and its two main metabolites (the methylamino and amino derivatives). The compounds were extracted from alkaline plasma with diethyl ether. The combined ether layers were evaporated after addition of 50 μl of 0.1 *N* HCl. The residual extracts were purified with diethyl ether and 20 μl were injected into a Spherisorb ODS 5-μm column with acetonitrile—0.6% phosphate buffer pH 3 (55:45, v/v) as the mobile phase. Using a fluorescence detector the detection limits are 1 ng/ml of plasma for citalopram and the methylamino metabolite and 0.5 ng/ml for the amino metabolite.

INTRODUCTION

Citalopram (I, Fig. 1), a new bicyclic antidepressant agent, is a potent and selective serotonin re-uptake inhibitor [1, 2]. It is demethylated in vivo to its methylamino (II) and amino (III) metabolites (Fig. 1). In urine, another metabolite, the N-oxide of citalopram (V) (Fig. 1) has been observed [3, 4].

A drug/methylamino metabolite ratio of 1:2 in steady-state plasma samples was found. The pharmacokinetics of citalopram in man have been reported using an analytical procedure that includes extraction from alkaline plasma with hexane and separation of the drug from its metabolites through thin-layer

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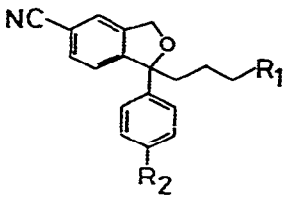

Fig. 1. Formulae of the compounds investigated.

	R_1	R_2
I: LU 10-171 (citalopram)	$-N(CH_3)_2$	F
II: LU 11-109 (methylamino metabolite)	$-NHCH_3$	F
III: LU 11-161 (amino metabolite)	$-NH_2$	F
IV: LU 10-202 (internal standard)	$-N(CH_3)_2$	Cl
V: LU 11-305 (N-oxide)	$-\overset{\uparrow}{N}(CH_3)_2$ (O)	F

chromatography of the evaporated hexane extracts. The drug and the methylamino metabolite were then determined fluorimetrically after ion-pair formation with 9,10-dimethoxyanthracene-2-sulphonate. The detection limits were 20 ng of I and 50 ng of II in 2-ml plasma samples [3].

This paper describes a simple, selective and sensitive high-performance liquid chromatographic (HPLC) method for the determination of I and II, and in addition the amino metabolite (III), from steady-state plasma samples using a reversed-phase column and fluorescence detection.

EXPERIMENTAL

Standards and reagents

Citalopram (Lu 10-171 (I) hydrobromide, Lu 11-109 (II) oxalate, Lu 11-161 (III) oxalate, Lu 11-305 (V) hydrochloride and the internal standard Lu 10-202 (IV) hydrobromide were supplied from Lundbeck & Co. (Copenhagen, Denmark).

Methanolic stock solutions (0.5%, w/v) of the hydrobromides (I and IV) and the oxalates (II and III) were stable at room temperature for at least two months. Working standard solutions A and B were prepared in water. Solution A: I (1 μg/ml), II (1 μg/ml), III (0.2 μg/ml). Solution B: IV (1 μg/ml). The solutions were stable for at least 14 days when stored at 4°C. All reagents were analytical-reagent grade and aqueous solutions were prepared using glass-distilled water. Acetonitrile (HPLC grade) and diethyl ether were supplied by Rathburns Chemicals (Walkerburn, Great Britain); 1 *N* NaOH was ether-washed; 0.6% (w/v) KH_2PO_4 was adjusted to pH 3 with H_3PO_4.

Glassware

Conical glass-stoppered centrifuge tubes (10 ml) were silanized with 5% dimethyldichlorosilane in toluene for 24 h, then washed with methanol and acetone. Evaporating tubes were rinsed with methanol in an ultrasonic bath and dried just before use.

Chromatography

HPLC analyses were performed on a Perkin-Elmer Series 2/2 liquid chromatograph with a Perkin-Elmer 3000 fluorescence spectrometer (Perkin-Elmer, Norwalk, CT, U.S.A.) operating at an excitation wavelength of 240 nm, slit 15 nm, and an emission wavelength of 296 nm, slit 20 nm. Expansion was in the range X5 to X100. Using UV detection at 240 nm a Perkin-Elmer spectrophotometer Model LC 55 was used. The column was Spherisorb ODS 5 μm (25 cm X 3 mm I.D.) with a MPLC™ guard column of RP-18 (3 cm X 4.6 mm I.D.). The chromatographic system was operated at room temperature.

The mobile phase used was 55% (v/v) acetonitrile in potassium dihydrogen phosphate (0.6%, w/v) adjusted to pH 3 with phosphoric acid, at a constant flow-rate of 1.3 ml/min.

Extraction procedure

To 1 ml of plasma (either patient plasma or spiked blind plasma) were added 75 μl of working standard solution B followed by 50 μl of 1 *N* NaOH to bring the solution to a pH of about 10. The mixture was extracted twice with 3-ml portions of diethyl ether by mechanical shaking for 15 min. After centrifuging for 10 min at 3200 *g*, the combined ether layers were transferred to 10-ml evaporation tubes containing 50 μl of 0.1 *N* HCl. The diethyl ether was evaporated under a stream of nitrogen in a 40°C water-bath.

The residual extracts were purified by whirlmixing with 0.5 ml of diethyl ether for 10 sec and centrifuged at 625 *g*. The ether layers were then removed and discarded; 15—20 μl of the residual extracts were injected onto the column.

Calibration curves

Preliminary steady-state plasma level determinations of I, II and III from patients given citalopram showed that the concentration ratio of I/III was about 7:1 and that of I/II about 1:1. The concentration ranges to be covered appeared to be from the detection limits upwards to about 200 ng/ml for I and II and to about 50 ng/ml for III. Spiked plasma samples were therefore prepared by adding 25, 50, 75, 100, 150 and 200 μl of working standard solution A to 1 ml of blank plasma; these were worked-up as described under extraction procedure. The calibration curves were calculated using regression analysis.

Recovery studies

A 1-ml volume of plasma spiked with 50 μl of working standard solution A was extracted as described above. The internal standard was added after extraction by substituting the 0.1 *N* HCl solution in the evaporating tubes with 50 μl of a 1 μg/ml solution of IV in 0.1 *N* HCl. Peak height ratios of these extracts were compared to peak height ratios of a standard aqueous solution of I, II and IV at 1 μg/ml and III at 0.2 μg/ml injected directly.

The procedure was repeated using a single extraction with 5 ml of diethyl ether.

Preliminary studies

A chromatogram of a mixture of citalopram, the methylamino and amino

metabolites, the N-oxide of citalopram, the internal standard, caffeine, nitrazepam, levomepromazine and alimemazine is shown in Fig. 2a. A UV detector operating at 240 nm was used. The order of elution is given in Table I. The hypnotics nitrazepam, levomepromazine and alimemazine, together with caffeine, represent possible interfering substances from patient plasma. Caffeine and nitrazepam, however, both eluted ahead of citalopram and its metabolites, while levomepromazine and alimemazine, not separating on the column, were eluted with a longer retention time.

The sample which gave the chromatogram in Fig. 2a was further detected with the fluorescence detector coupled in series with the UV detector, resulting in the chromatogram shown in Fig. 2b. The only peaks which appeared were due to citalopram, the internal standard, and metabolites II, III and V.

The N-oxide (V) could not be detected either in extracts of plasma spiked with 200 ng/ml (Fig. 2c), or in extracts of plasma samples from steady-state patients (Fig. 3), and it is hardly extracted from alkaline solution with ether.

A chromatogram of an extract from 2 ml of blank plasma revealed very few absorbing endogenous plasma constituents using the UV detector (Fig. 4a). Thus, due to the absence of interfering peaks, the method might well be used for analysis with this detector. However, on comparing the peak heights in Fig. 2a and b, when the UV detector was operating at maximum sensitivity (0.02 a.u.f.s.) while the fluorescence signal could still be expanded ten times, it could

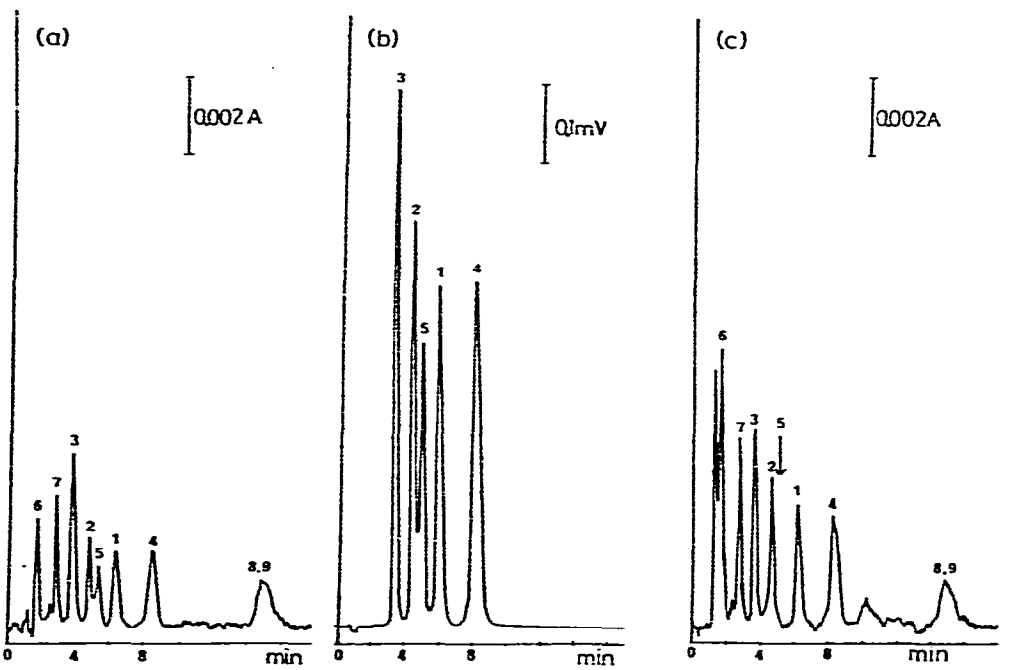

Fig. 2. Chromatograms of about 40 ng of caffeine (6), nitrazepam (7), the amino metabolite of citalopram (3), the methylamino metabolite (2), the N-oxide (5), citalopram (1), internal standard (4), alimemazine (8) and levomepromazine (9) injected on the column and detected with (a) the UV detector and (b) the fluorescence detector, (c) chromatogram of an extract of 1 ml of plasma spiked with 200 ng of the same compounds as in (a) and (b) and detected with the UV detector.

TABLE I

ELUTION OF CITALOPRAM, METABOLITES AND SOME POSSIBLE INTERFERING SUBSTANCES

Conditions: Spherisorb ODS 5 μm, acetonitrile—0.6% phosphate buffer pH 3 (55:45, v/v), 1.3 ml/min

Drug	Chromatographic peak number*	Retention time (min)	Retention time relative to I.S.
Caffeine	6	1.6	0.19
Nitrazepam	7	2.8	0.33
Amino metabolite	3	3.7	0.44
Methylamino metabolite	2	4.6	0.55
N-Oxide	5	5.2	0.62
Citalopram	1	6.3	0.75
Internal standard (I.S.)	4	8.4	1.00
Levomepromazine	9	15.0	1.79
Alimemazine	8	15.0	1.79

*Figs. 2—4.

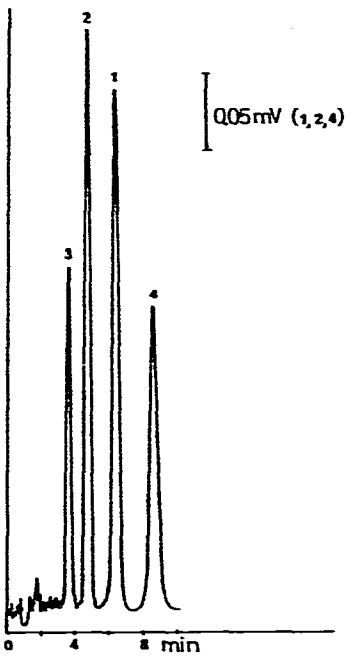

Fig. 3. Chromatogram of an extract of a steady-state patient plasma containing 340 nmol/l citalopram (1), 293 nmol/l methylamino metabolite (2) and 26 nmol/l amino metabolite (3). The amino metabolite was detected with a sensitivity range five times the sensitivity range used for the detection of 1, 2 and 4 (internal standard). The patient had been given a dose of 70 mg citalopram daily.

be concluded that the fluorescence detector gave a much better response with a detection limit of 0.2—0.5 ng while the detection limit of the UV detector could be estimated to be about 10—15 ng. The method was therefore developed using the fluorescence detector.

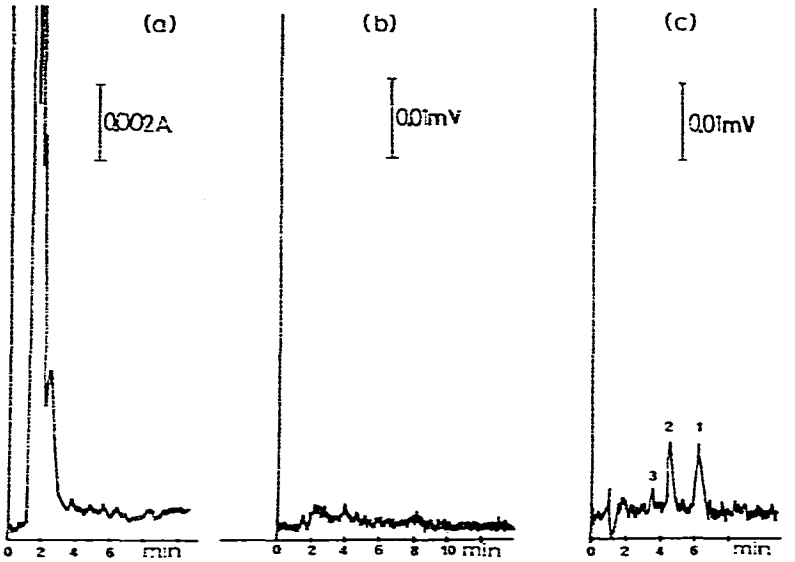

Fig. 4. Chromatograms of extracts of blank plasma: (a) 2 ml of plasma detected with the UV detector; and (b) 1 ml of plasma detected with the fluorescence detector. (c) Chromatogram of an extract of plasma containing 2.5 ng/ml citalopram (1) and methylamino metabolite (2), and 0.5 ng/ml amino metabolite (3), detected with the fluorescence detector.

RESULTS AND DISCUSSION

From the chromatogram of a plasma blank extract (Fig. 4b) it can be concluded that very few fluorescent endogenous plasma constituents are extracted. A chromatogram of an extract of plasma containing I and II at 2.5 ng/ml and III at 0.5 ng/ml is shown in Fig. 4c. The detection limits were 1—2 ng/ml for citalopram and the methylamino metabolite and 0.5—1 ng/ml for the primary amino metabolite.

The equations of the calibration curves were: citalopram, $x = 1.85y + 1.25$, $r^2 = 1.00$; methylamino metabolite, $x = 1.60y + 1.38$, $r^2 = 1.00$; amino metabolite, $x = 1.14y + 1.56$, $r^2 = 0.99$; x = nmol/l, y = percentage peak height relative to internal standard, number of observations = 49.

Relative standard deviations ($n = 9$ for each point) were $< 4\%$ for I, $< 3\%$ for II and $< 5\%$ for III. Eight aliquots of a plasma pool from several patients were analysed. The results showed a somewhat lower standard deviation than in the calibration graphs where the day-to-day variation is incorporated.

The mean recovery from plasma was better than 83% for the two metabolites and better than 79% for citalopram. A single extraction step with 5 ml of diethyl ether gave a lower recovery (Table II).

A series of plasma samples from patients who had received citalopram for several weeks was analysed in duplicate together with the standards. It was possible to analyse 8—10 patient samples during the day.

The ratio of I/II was found to be between 0.4 and 2.5 with a mean of 1.6, and confirmed earlier results [4]. The ratio I/III was between 2.0 and 10.7 with a mean of 7.3. The results revealed that the mean steady-state concentration of III was below the detection limit of the UV detector. Thus, in studying metab-

TABLE II

ANALYSIS OF PLASMA

Compound	Within-run analyses of 8 aliquots of a plasma pool		Recovery [mean (%) ± S.D., n = 10]	
	Mean (nmol/l) ± S.D.	Relative S.D. (%)	Extraction A*	Extraction B**
Citalopram	185 ± 4	2.1	84 ± 5	62 ± 5
Methylamino metabolite	122 ± 3	2.3	88 ± 5	79 ± 5
Amino metabolite	21 ± 0.5	2.6	87 ± 5	78 ± 7

*Extraction A: 2 × 3 ml diethyl ether.
**Extraction B: 1 × 5 ml diethyl ether.

olites of citalopram, fluorescence detection gives a very sensitive and selective method. The method may also be used in studying the pharmacokinetics of the drug.

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