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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CITALOPRAM AND FOUR OF ITS METABOLITES IN PLASMA AND URINE SAMPLES FROM PSYCHIATRIC PATIENTS

E. ØYEHAUG* and E.T. ØSTENSEN

Agder College, 4600 Kristiansand S (Norway)

and

B. SALVESEN

Institute of Pharmacy, University of Oslo, Blindern, Oslo 3 (Norway)

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SUMMARY

A high-performance liquid chromatographic method is used for the determination of citalopram [1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phthalancarbonitrile] and four of its metabolites (the methylamino, amino, propionic acid and N-oxide derivatives) in plasma and urine. The plasma samples were extracted with diethyl ether at pH 10 and pH 4. Filtered urine samples could be injected directly on to the column. Steady-state drug and metabolite levels were investigated in fifteen psychiatric patients. In urine, 12 ± 5% (mean ± S.D.) of a given dose of citalopram was excreted in unchanged form. The propionic acid derivative was further conjugated, possibly to glucuronic acid. Mean steady-state plasma levels and metabolites in 24-h urine are given as percentages of the dose.

INTRODUCTION

Citalopram (I, Fig. 1), an antidepressant, is a potent and selective inhibitor of serotonin re-uptake [1]. From previous investigations it appears that the kinetics in man is characterized by approximately complete systemic availability and slow elimination [2]. Compared to the tricyclic antidepressants, which are eliminated mainly by hepatic metabolism [3], citalopram seems to be less extensively metabolized. Thus roughly 13% of a given dose of citalopram has been recovered unchanged in urine, suggesting elimination by renal as well as hepatic processes [2].

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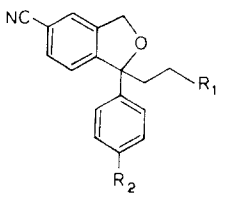

Fig. 1. Formulae of the compounds investigated

	R_1	R_2
I: Citalopram	$-CH_2N(CH_3)_2$	F
II: Methylamine metabolite	$-CH_2NHCH_3$	F
III: Amino metabolite	$-CH_2NH_2$	F
IV: Propionic acid metabolite	$-COOH$	F
V: N-Oxide	$-CH_2\overset{\uparrow}{N}(CH_3)_2$ (O)	F
VI: Internal standard	$-CH_2N(CH_3)_2$	Cl

Citalopram is metabolized by demethylation (II and III, Fig. 1). These metabolites are less potent than citalopram, but retain its specific effect [1]. The N-oxide of citalopram and a deaminated metabolite (IV and V, Fig. 1) have been observed in human urine [4]. We have therefore extended our previously described high-performance liquid chromatographic (HPLC) method [5] to include determination of IV (the propionic acid metabolite) in plasma. This paper also describes a method for the determination of unconjugated and conjugated compounds of I—V (Fig. 1) in urine.

EXPERIMENTAL

Standards and reagents

Lu-10-177 HBr (I), Lu-11-109 HCl (II), Lu-11-161 oxalate (III), Lu-16-073 (IV), Lu-11-305 HCl (V) and the internal standard Lu-10-202 HBr (VI) were supplied by Lundbeck (Copenhagen, Denmark). Methanolic stock solutions (5 mg/ml) of compounds I—VI were stable at room temperature for at least two months.

All reagents were analytical-reagent grade and aqueous solutions were prepared using glass-distilled water; 1 *M* sodium hydroxide was ether-washed; 0.6% (w/v) potassium dihydrogen phosphate was adjusted to pH 3 with concentrated orthophosphoric acid; 0.2 *M* sodium acetate was adjusted to pH 5 with concentrated acetic acid; β-glucuronidase—arylsulphatase from *Helix pomatia* was supplied by Boehringer Mannheim (Mannheim, F.R.G.); acetonitrile (HPLC grade) and diethyl ether were supplied by Rathburn Chemicals (Walkerburn, U.K.).

Chromatography

HPLC analyses were performed as described earlier [5] 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 300 nm, slit

20 nm. The column was Spherisorb ODS 5 μm (25 cm × 3 mm I.D.) with an MPLC™ guard column of RP-18 (3 cm × 4.6 mm I.D.). The mobile phase used for determination of I, II and III was 55% (v/v) acetonitrile in potassium dihydrogen phosphate buffer at a constant flow-rate of 1.3 ml/min. The mobile phase used for determination of IV was 45% (v/v) acetonitrile in potassium dihydrogen phosphate buffer at a flow-rate of 1 ml/min.

The mobile phase used for urine analysis was 50% (v/v) acetonitrile in potassium dihydrogen phosphate buffer at a flow-rate of 1.3 ml/min.

Extraction from plasma

Citalopram and the amino metabolites (I, II and III) were extracted according to the reported method [5] with minor modifications. To 1 ml of plasma (either patient plasma or spiked plasma blank) were added 75 ng of internal standard (VI) followed by 50 μl of 1 *M* sodium hydroxide 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 100 μl of potassium dihydrogen phosphate buffer pH 3. The diethyl ether was evaporated under a stream of nitrogen in a 40°C water-bath. The residual extract was purified by whirlmixing with 0.5 ml of diethyl ether for 10 sec and centrifuged at 625 *g*. The ether layer was then removed and discarded; 15—20 μl of the remaining extract were injected on to the column.

The propionic acid metabolite (IV) was extracted from plasma as follows. To the residual plasma sample after basic extraction were added 150 μl of 1 *M* hydrochloric acid and 1 ml of 0.6% phosphate buffer pH 3, and 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 glass tubes. The diethyl ether was evaporated under a stream of nitrogen in a 40°C water-bath. The residue was dissolved in 100 μl of mobile phase and 20 μl of the extract were injected on to the column.

Four to five standards of blank plasma spiked with I, II, III and IV, and patient plasma samples were run simultaneously. Amounts of I, II and III were determined by peak height ratios of compound to internal standard. The propionic acid metabolite (IV) was determined by peak height measurement.

Pretreatment of urine samples

To 200 μl of urine (either patient urine or spiked blank urine) were added 200 μl of 0.2 *M* sodium acetate pH 5 containing 5 μg/ml internal standard (VI). The solution was sterile filtered through a MF-7 centrifugal Microfilter with an OE 0.2-μm filter (Bioanalytical Systems, West Lafayette, U.S.A.) and 20 μl were injected on to the column.

For determination of conjugated metabolites, the patient urine samples were also incubated with β-glucuronidase. To 1 ml of patient urine were added 1 ml of 0.2 *M* acetate buffer pH 5 containing internal standard 5 μg/ml and 2 μl of β-glucuronidase reagent. The mixture was incubated for 15 h at 37°C and sterile filtered; 20 μl were injected on to the column. Standards and patient

urine samples were run simultaneously. Calibration curves of spiked blank urines were constructed and patient urine levels of compounds I—V were determined using peak height ratios of compound to internal standard.

Plasma samples

Plasma samples were obtained from thirteen psychiatric patients, eight men and five women, aged 22—79 years, who had been treated with citalopram 40—60 mg/day for at least fourteen days. The drug was given as a single morning dose. No other medication, except for nitrazepam, was given.

Citalopram and metabolites were measured in plasma at minimum steady-state levels; i.e. before the morning dose, 10—15 ml of venous blood were drawn into heparinized glass tubes and centrifuged. The plasma samples were stored frozen at —20° C for a maximum of four months before analysis.

Urine samples

Twenty-four-hour urine samples were collected from fifteen psychiatric patients, nine men and six women, aged 29—79 years, who had been treated with citalopram 40—60 mg/day for at least fourteen days. In addition the patients had been given nitrazepam, alimemazine or levomepromazine. The samples were stored frozen at —20° C for a maximum of four months before analysis.

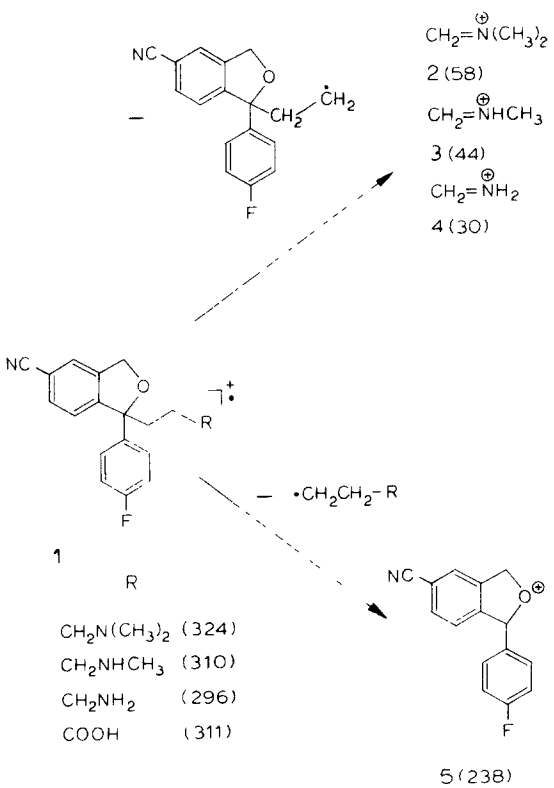

Fig. 2. Fragmentation of citalopram derivatives.

Identification of compounds by fluorescence ratios

Fluorescence ratios of the pure compounds were determined by stop flow and fluorescence measurements at three different wavelengths as described by Yost et al. [6]. When measuring a possible citalopram derivative in a patient urine sample, the fluorescence ratios of the compound were determined by the same technique. Compounds that eluted at the retention times of citalopram (I), the methylamino metabolite (II) and the amino metabolite (III) were identified by this method.

Mass spectrometric identification

Citalopram derivatives eluted from a patient urine by repeated injection of the sample on to the analytical column were collected separately. The acetonitrile was evaporated and the concentrated isolates of the basic and acid compounds were extracted with diethyl ether at pH 10 and 3, respectively. The diethyl ether extracts were evaporated and analysed by electron-impact mass spectrometry (MS) at 20 eV.

A probable fragmentation pattern of citalopram derivatives is shown in Fig. 2. In the mass spectra of isolates with retention times identical to those of citalopram (I), the methylamino metabolite (II), the amino metabolite (III)

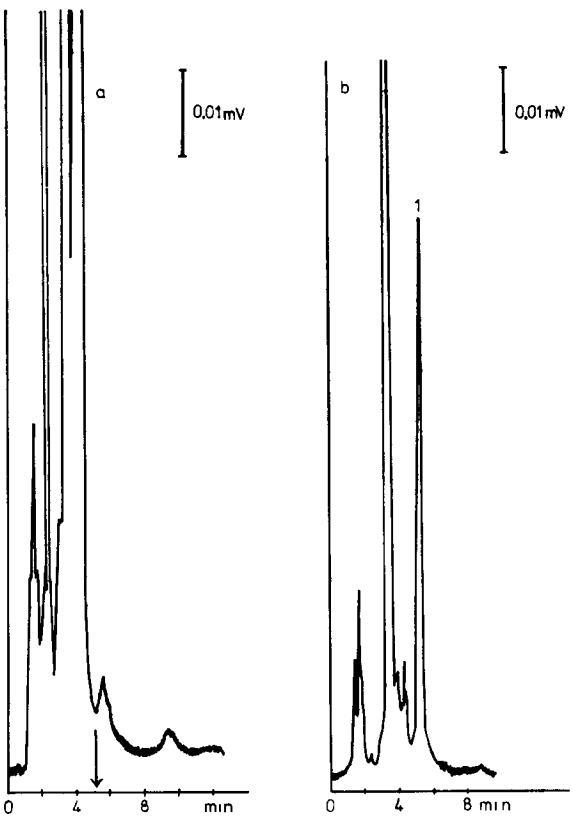

Fig. 3. Chromatograms of extracts from plasma at pH 4. (a) Plasma blank. (b) Patient plasma containing 75 nmol/l propionic acid metabolite (1). Daily dose of citalopram was 40 mg. Mobile phase: acetonitrile—phosphate buffer 0.6%, pH 3 (45:55) at a flow-rate of 1 ml/min.

and the propionic acid metabolite (IV), major ions of *m/e* 238 were found, corresponding to fragment 5 (Fig. 2). In addition, ions (*m/e* 44 and 58) corresponding to fragments 3 and 2 were evident in the mass spectra of citalopram and the methylamino metabolite isolates.

RESULTS

Determination in plasma

Chromatograms of an acidic extract of blank plasma from a healthy person and patient plasma containing 75 nmol/l propionic acid metabolite (IV) are shown in Fig. 3a and b. Inter-individual variations in the amounts of early eluted plasma constituents were observed. Equations for the calibration curves were as follows:

I: $X = 2.73Y - 6.3$; $n = 5$; $r^2 = 1.00$; range 60—375 nmol/l.

II: $X = 1.95Y - 14.0$; $n = 5$; $r^2 = 1.00$; range 60—375 nmol/l.

III: $X = 1.86Y - 3.2$; $n = 5$; $r^2 = 1.00$; range 13—90 nmol/l.

IV: $X = 1.28Y + 12.6$; $n = 5$; $r^2 = 0.99$; range 37.5—225 nmol/l.

Day-to-day variation in the calibration curve of IV was observed; relative standard deviation was 9% and the detection limit 15 nmol/l. The extraction procedure yielded a recovery of IV better than 80% from plasma (Table I). The N-oxide could not be determined by the given method for plasma analysis.

TABLE I

ANALYSIS OF PLASMA

$n = 10$.

	Within-run analysis of spiked blank plasma		Recovery (%) (mean ± S.D.)
	Mean conc. (nmol/l)	Relative S.D. (%)	
Citalopram	250	2	84 ± 4
Methylamino metabolite	260	3	88 ± 5
Amino metabolite	26	5	87 ± 5
Propionic acid metabolite	60	9	87 ± 7
Internal standard			84 ± 4

Determination in urine

Chromatogams of blank urine and of patient urine are shown in Fig. 4a and b.

Equations for the calibration curves were as follows:

I: $X = 6.7Y - 0.2$; $n = 5$; $r^2 = 1.00$; range 2.5—12.5 μmol/l.

II: $X = 4.4Y - 0.3$; $n = 5$; $r^2 = 1.00$; range 2.5—12.5 μmol/l.

III: $X = 3.8Y - 0.2$; $n = 5$; $r^2 = 1.00$; range 1.0—7.0 μmol/l.

IV: $X = 3.2Y - 0.1$; $n = 5$; $r^2 = 1.00$; range 1.0—7.0 μmol/l.

V: $X = 4.5Y - 0.02$; $n = 5$; $r^2 = 1.00$; range 0.15—0.7 μmol/l.

No day-to-day variation was observed. Relative standard deviations were < 5% (n = 10) in the upper and lower concentration ranges for compounds I—IV. The relative standard deviation of the N-oxide (V) was < 11% in the lower concentration range (Table II). The detection limits were in the range 0.03—0.06 μmol/l.

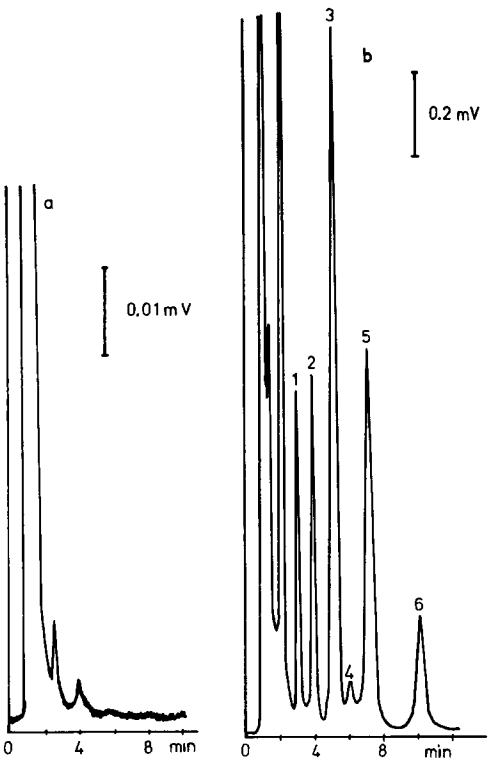

Fig. 4. Chromatograms of urine samples. (a) Urine blank. (b) Patient urine containing propionic acid metabolite 7 μmol/l (1), amino metabolite (10 μmol/l (2), methylamino metabolite 23 μmol/l (3), N-oxide 1.8 μmol/l (4) and citalopram 22 μmol/l (5); internal standard (6). Mobile phase: acetonitrile—phosphate buffer 0.6%, pH 3.2 (50:50), at a flow-rate of 1.3 ml/min.

Plasma samples

Minimum steady-state plasma levels of citalopram and metabolites are shown in Table III. Citalopram (I) plasma levels were in the range 108—334 nmol/l. The methylamino metabolite (II) levels were on average 50% of the citalopram levels and were in the range 70—167 nmol/l. The amino metabolite (III) plasma levels were approximately 10% of the parent drug (I) levels and were in the range 7—37 nmol/l.

In one patient, the propionic acid metabolite (IV) could not be detected. In twelve patients, the plasma levels of IV ranged from 33 to 117 nmol/l and were approximately 30% of the citalopram plasma levels (Table III).

The N-oxide could not be detected in plasma.

TABLE II

ANALYSIS OF URINE

n = 10.

	Within-run analysis of spiked blank urine	
	Mean conc. (μmol/l)	Relative S.D. (%)
Citalopram	1.25	3
	12.50	5
Methylamino metabolite	1.30	3
	13.00	4
Amino metabolite	0.65	4
	6.50	4
N-Oxide	0 67	6
	0 13	11
Propionic acid metabolite	0 75	5
	7 50	5

TABLE III

MINIMUM STEADY-STATE PLASMA LEVELS

n = 13. Citalopram dose was 40 mg/day.

	Plasma concentration (nmol/l)	
	Range	Mean ± S.D
Citalopram	108—334	206 ± 69
Methylamino metabolite	70—167	105 ± 27
Amino metabolite	7—37	23 ± 9
Propionic acid metabolite*	<15—117	66 ± 27

*n = 12.

TABLE IV

PERCENTAGES OF A GIVEN DOSE IN 24-h URINE AT STEADY-STATE

n = 15.

	Range (%)	Mean ± S.D (%)
Citalopram	6.3—21.0	12 2 ± 5.1
Methylamino metabolite	6.4—22.2	12.3 ± 4.0
Amino metabolite	0 3— 3.5	1 5 ± 1 2
Amino metabolite, conjugated	0.0— 1.9	
Propionic acid metabolite	0.1— 1 4	0.6 ± 0 4
Propionic acid metabolite, conjugated	2 1— 7.1	4.3 ± 1 5
N-Oxide	0.0— 1.0	
Total of drug and known metabolites	18 7—44.8	31.8 ± 5 1

Urine samples

Table IV shows amounts of citalopram and metabolites in 24-h urine samples as percentage of a given dose. Volume and pH of excreted urine were in the range 0.65—2.15 l per 24 h and 5.4—6.4, respectively. Ranges of excreted unchanged drug (I) and the methylamino metabolite (II) were 6.3—21.0% and 6.4—22.2%, respectively, with averages of 12% of a given dose.

The amino and propionic acid metabolites (III and IV) appeared to be conjugated in urine. The ranges of unconjugated and conjugated amino metabolite were 0.3—3.5% and 0—1.9%, respectively, of a given dose. Excreted unconjugated and conjugated propionic acid metabolite were in the range 0.06—1.44% and 2.1—7.1%, respectively.

The N-oxide (V) could not be detected in seven patients. The range of excreted amounts of V was 0—1.0% of a given dose.

DISCUSSION

In our clinical study, either nitrazepam, alimemazine or levomepromazine was given in addition to citalopram. However, because of the selectivity of the fluorescence detection these compounds do not give interfering peaks [5]. The 9% standard deviation in the determination of the propionic acid metabolite (IV) in plasma reflects the absence of internal standard and the presence of co-extracted endogenous plasma constituents (Fig. 3a). The presence of the propionic acid metabolite (IV) in plasma and urine seems to verify that citalopram is deaminated in the human body, possibly by α-oxidation as described by Beckett et al. [7].

This side-chain degradation is identical to the degradation of the corresponding phthalanes Lu-3-010 [8] and Lu-5-003 [9] and is different from degradation of the tricyclic antidepressants [3].

The propionic acid metabolite (IV) appears further to be conjugated to glucuronic acid. Conjugation of the amino metabolite (III) was also observed in some of the patient urine samples, while citalopram and the methylamino metabolite (I and II) did not seem to be excreted in conjugated form.

Observed amounts of citalopram N-oxide in urine were close to the detection limit of the analytical method and it seems likely that N-oxidation only plays a minor role in citalopram metabolism.

The average amount of citalopram excreted unmetabolized was 12% of a given dose. This result is in agreement with observations in an earlier investigation [2] and confirms that citalopram is eliminated by renal as well as hepatic clearance. The mean amount of excreted drug and metabolites in 24-h urine was 31.8% of the dose (Table IV). This result may in addition indicate faecal elimination or degradation pathways of citalopram other than demethylation and deamination.

In this study the steady-state citalopram and methylamino metabolite plasma levels were in the same range as reported earlier [1]. The corresponding amino metabolite and propionic acid metabolite levels were about one-tenth and one-third, respectively, of the citalopram levels.

Determination of plasma and urine levels of drug and metabolites may be useful in investigating drug interactions caused by induction or inhibition of

hepatic metabolism. We have been investigating the effect of phenothiazines on steady-state plasma levels of citalopram and metabolites. The results will be published elsewhere [10].

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