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Note

Simultaneous determination of citalopram, monodesmethylcitalopram and didesmethylcitalopram in plasma by high-performance liquid chromatography after column extraction

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Citalopram is a potent and specific serotonin reuptake inhibitor with antidepressant properties [1,2]. It is metabolized by N-demethylation and N-oxidation [3]. In human plasma monodesmethylcitalopram and didesmethylcitalopram are found in addition to citalopram itself; the metabolites are pharmacologically active, but less potent than the parent drug [3]. The N-oxide derivative has also been observed in urine [4,5].

We previously published a high-performance liquid chromatographic (HPLC) procedure for determining citalopram and its monodesmethylated metabolite in steady-state plasma samples of treated patients [6,7]. Prior HPLC techniques were also published by Øyehaug et al. [8,9]. The present study was undertaken to develop a procedure for routine therapeutic monitoring of citalopram and both of its desmethylated metabolites.

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EXPERIMENTAL

Reagents and glassware[a]

All reagents were of analytical grade. Potassium dihydrogenphosphate (Normapur) and sodium hydroxide (Normapur) were from Prolabo (Paris, France). Phosphoric acid (RPE), methanol (RS), diethyl ether (RS) and acetonitrile (RS per HPLC) were from Carlo Erba (Milan, Italy). The prepacked 3-ml Extrelut® columns were from Merck (Nogent-sur-Marne, France).

All glassware was first washed with a 3% 'RBS 25 biodegradable' alkaline solution from Biolyon (Dardilly, France), which contains a mixture of anionic and non-ionic detergents, then rinsed with distilled water and dried before use.

Standards

The standard compounds, citalopram·HBr (Lu 10-171 PB), monodesmethylcitalopram·HCl (Lu 11-109 PC) and didesmethylcitalopram oxalate (Lu 11-161 PO) were supplied by H. Lundbeck (Copenhagen, Denmark). Desipramine·HCl (internal standard) was supplied by Ciba-Geigy (Rueil-Malmaison, France). Stock solutions of citalopram, mono- and didesmethylcitalopram and desipramine were made in methanol at a concentration of 1 $\mu g\,\mu l^{-1}$ and stored at +4°C. Quality remained good for at least one month. Stock solutions were diluted with methanol for preparation of calibration standards.

Procedure

A 1–2 ml volume of the plasma to be analysed was pipetted into a 5-ml glass tube, and 50 μl of desipramine (internal standard) solution in methanol (10 ng μl^{-1}) were added. The volume was made up to 3 ml with 0.1 *M* NaOH. After vortex-mixing for 1 min, the mixture was passed onto a 3-ml Extrelut cartridge. Elution was then carried out with 15 ml of diethyl ether. The eluate was collected in a 20-ml evaporation glass tube containing 50 μl of 0.05 *M* H_3PO_4. The diethyl ether was evaporated under a stream of air in a 40°C water-bath. The residual acid solution was washed by vortex-mixing with 1 ml of diethyl ether for 20 s. After centrifugation at 2800 *g* for 5 min, the ether layer was removed and discarded. Then 20 μl of the acid extract were injected into the chromatograph.

Apparatus and chromatographic parameters

Chromatographic analysis was performed on a Waters system consisting of an M 510 pump, a μBondapak C_{18} column (30 cm × 3.9 mm I.D.; particle size 10 μm, ambient temperature) connected to a μBondapak C_{18} T.M. guard-pak column (5 mm × 6 mm I.D.; two filters, one at either end, hold the packing in place and provide a 2 μm filtering capability), an M 481 multi-wavelength

[a]RPE = Reagente Puro Erba; RS = Reagente Speciale.

detector and an M 380 control station. A 20-μl high-pressure loop injector (M 7125 Rheodyne) was used to introduce the sample.

The mobile phase was acetonitrile–0.025 *M* potassium dihydrogenphosphate–water (45:55:10, v/v, or 41:50:9 %) at a flow-rate of 0.8 ml min^{-1}; the detection wavelength was 239 nm.

Calculation

The ratios between the peak heights of the compounds analysed and that of the internal standard were calculated and plotted against the concentrations of the compounds after analysis of blank plasma spiked with increasing concentrations (20, 50, 100 and 200 ng ml^{-1}) of citalopram, monodesmethylcitalopram and didesmethylcitalopram, and a constant amount (500 ng) of the internal standard. Within these concentration ranges the relations were linear for the three compounds and allowed the determination of their steady-state plasma levels. For the lower concentrations (0.8, 2, 10 and 15 ng ml^{-1}), the added amount of the internal standard must be less, e.g. 50 ng; under these conditions the relations were also linear.

The equations of the standard curves and their correlation coefficients were as follows. Range 20–200 ng ml^{-1} (internal standard amount 500 ng): citalopram: $y=0.010x-0.008$, $r=0.999$; monodesmethylcitalopram: $y=0.012x-0.012$, $r=0.999$; didesmethylcitalopram: $y=0.013x-0.013$, $r=0.999$; range 0.8–15 ng ml^{-1} (internal standard amount 50 ng): citalopram: $y=0.011x-0.0029$, $r=0.999$; monodesmethylcitalopram: $y=0.012x-0.0070$, $r=0.998$; didesmethylcitalopram $y=0.017x-0.0012$, $r=0.999$; where y is the ratio of analysed compound to internal standard and x is the amount of spiked compound.

RESULTS

The retention times and the relative retention times (related to desipramine, the internal standard) of the analysed compounds are shown in Table I.

Fig. 1 shows the chromatograms of (A) a blank plasma extract after spiking with citalopram, monodesmethylcitalopram, didesmethylcitalopram and desipramine as internal standard, (B) a plasma extract from a patient before receiving citalopram and (C) a plasma extract from the same patient at the 20th day of a 60-mg daily treatment with citalopram.

Recovery

A 1-ml volume of a blank plasma (spiked with 5, 20 and 100 ng of each compound) was extracted as described above. The internal standard was added to the diethyl ether eluate just before evaporating in the 50 μl of 0.05 *M* H_3PO_4. Peak-height ratios of the extracts were compared with those obtained from direct injection of the residue of the methanolic standard solutions (mixture of 5, 20 and 100 ng of each compound and 500 ng of internal standard) after

TABLE I

RETENTION TIMES OF THE ANALYSED COMPOUNDS AND OF SOME DRUGS TESTED FOR POSSIBLE INTERFERENCE

Drug	Retention time (min)	Retention time relative to desipramine (internal standard)
Solvent front	4	0.27
Heptaminol	–	–
Meprobamate	–	–
Caffeine	–	–
Metoclopramide	6.80	0.47
Didesmethylcitalopram[a]	8.50	0.58
Oxazepam	8.70	0.60
Dihydroergotamine	9.20	0.63
Lorazepam	9.70	0.67
Monodesmethylcitalopram[a]	10.10	0.70
Citalopram[a]	11.50	0.79
Bromazepam	12.40	0.86
Cisapride	12.40	0.86
Levomepromazine	12.50	0.86
Nordiazepam	13	0.89
Norclobazam	13	0.89
Desipramine[a]	14.50	1
Clobazam	15.60	1.07
Diazepam	16	1.10
Cyamemazine	16	1.10
Alimemazine	17.85	1.23

[a]Analysed compounds.

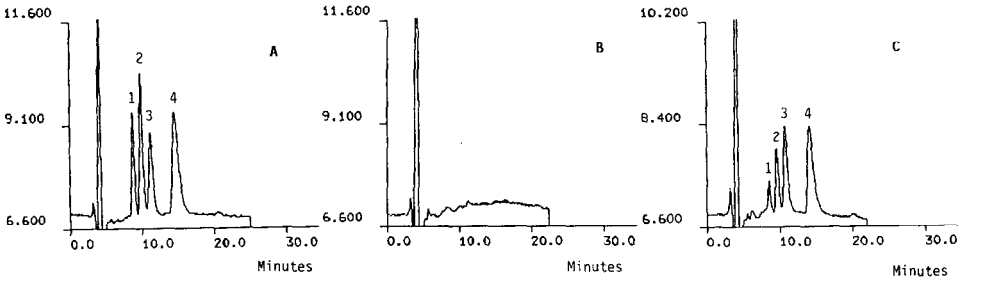

Fig. 1. Chromatograms of (A) a 1-ml blank plasma spiked with 76 ng didesmethylcitalopram (1), 110 ng of monodesmethylcitalopram (2), 80 ng of citalopram (3) and 500 ng of desipramine as internal standard (4), (B) a 1-ml plasma extract from a patient before receiving citalopram and (C) a 1-ml plasma extract from the same patient at the 20th day of a 60-mg daily treatment with citalopram; amounts found were 17 ng of didesmethylcitalopram (1), 55 ng of monodesmethylcitalopram (2) and 92 ng of citalopram (3) with desipramine as internal standard (4).

TABLE II

REPRODUCIBILITY AND RECOVERY

Added (ng ml^{-1})	Within-day		Day-to-day						Recovery (mean ± S.D., $n=7$) (%)
	Found (mean ± S.D., $n=7$) (ng ml^{-1})	C.V. (%)	Found (ng ml^{-1})					C.V. (%)	
			Day 1	Day 7	Day 15	Day 30	Mean ± S.D., $n=10$		
Citalopram									
5	5.50 ± 0.60	10.90							73 ± 3
10			8.80	9.65	7.70	8.65	8.70 ± 0.70	8	
20	18.70 ± 1.80	9.70							79 ± 4
40	38.20 ± 2.90	7.65	42.00	38.75	41.45	39.60	40.45 ± 1.36	3.25	
100	96.25 ± 6.25	6.50							82 ± 3
Monodesmethylcitalopram									
5	4.60 ± 5.30	11.50							75 ± 5
10			10.25	7.70	7.85	8.25	8.50 ± 1.02	12	
20	18.30 ± 1.90	10.25							81 ± 2
40	39.75 ± 3.45	8.70	43.45	38.30	42.25	39.30	40.80 ± 2.10	5.10	
100	101.00 ± 5.75	5.70							82 ± 2
Didesmethylcitalopram									
5	4.85 ± 0.50	10.30							72 ± 2
10			9.75	8.25	11.35	10.35	9.90 ± 1.12	11.30	
20	18.70 ± 1.95	10.50							80 ± 4
40	40.45 ± 4.10	10.10	42.00	39.25	36.20	38.80	39.10 ± 2.05	5.25	
100	98.50 ± 9.35	9.50							75 ± 2

evaporating and dissolving in 50 μl of 0.05 M H_3PO_4. The recovery was better than 70% for the three compounds (Table II).

Reproducibility

Reproducibility was tested on a pool of blank plasmas spiked with 5, 20, 40 and 100 ng ml^{-1} of each compound. Coefficients of variation (C.V.) obtained on the same day for citalopram, monodesmethylcitalopram and didesmethylcitalopram were less than 11.50% (Table II). Day-to-day C.V. for plasma concentrations of 10 and 40 ng ml^{-1} were less than 12% for the three compounds (four determinations) over a period of one month (Table II).

Limit of determination

The limits of determination in plasma were 0.8 ± 0.12 ng ml^{-1} ($n=7$) for citalopram and didesmethylcitalopram and 0.7 ± 0.1 ng ml^{-1} ($n=7$) for monodesmethylcitalopram, using 2 ml of sample.

Selectivity

Fig. 1B, the chromatogram of the plasma from a patient before the citalopram treatment, shows no background interference from endogenous constituents. The procedure also proved selectivity towards other drugs often associated with antidepressant therapeutics (Table I). Oxazepam and lorazepam were respectively close to didesmethylcitalopram and monodesmethylcitalopram. However, under the extraction conditions described, only very small amounts of these two compounds remained in the acid extract after washing with diethyl ether.

DISCUSSION

The liquid–liquid extraction of our previous technique for determining citalopram and monodesmethylcitalopram was time-consuming. The solid–liquid extraction in the present method allows sample preparation in 10 min and HPLC analysis within 30 min. The slight change to the acetonitrile–0.025 *M* potassium dihydrogenphosphate–water mobile phase (41:50:9 instead of 45:50:5) provides a better separation of the two metabolites of citalopram. The previous procedure had a better precision, expressed as overall within-day and day-to-day C.V., than the present, but was significantly less rapid and sensitive.

CONCLUSION

The procedure proposed for the simultaneous determination of citalopram, monodesmethylcitalopram and didesmethylcitalopram in plasma is rapid, reproducible, selective and sensitive. It can be applied to pharmacokinetic studies, for therapeutic monitoring, as well as for the diagnosis of cases of possible overdose.

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