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Simplified high-performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites

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Abstract

A simplified method for the determination of racemic citalopram and its main metabolite desmethylcitalopram in serum using HPLC was developed. The compounds were extracted with heptane–isoamyl alcohol (98:2) and subsequently transferred into phosphate buffer pH 2.5 for direct injection into the HPLC apparatus. The analytes were separated with an acetonitrile–phosphate buffer, pH 2.5–tetraethylamine mobile phase on a C_{18} column and measured by UV detection at 240 nm. Within the typical range of serum concentrations (30–200 ng/ml) the inter-day variation was <6% for both compounds. Possible analytical interference from a number of commonly coadministered psychoactive drugs and their metabolites was studied by extracting sera from patients receiving these drugs. Interference was not a problem for the developed method.

Keywords: Citalopram; Desmethylcitalopram

1. Introduction

Citalopram is a drug which belongs to the group of specific serotonin reuptake inhibitors (SSRIs) used in the treatment of depression [1,2]. The major advantage of the SSRIs compared to the tricyclic antidepressants (TCAs) is that the SSRIs are less cardiotoxic and exert less anticholinergic side-effects [2,3]. Whether the clinical effect of the SSRI drugs against major depression is comparable to that of the TCAs is still under investigation [3]. Citalopram is marketed as a racemic compound and the *S*-(+)-citalopram enantiomer is considered the main responsible for the inhibition of serotonin reuptake [4]. A method for the separate determination of the two enantiomers of citalopram in serum using HPLC and a chiral analytical column has recently been described [5]. A high positive correlation be-

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tween the serum levels of the *S* and *R* enantiomers was found indicating that for therapeutic drug monitoring it may still be sufficient to measure the serum concentration of racemic citalopram [6].

A large variation of the serum citalopram concentrations is obtained when equal 24-h doses are given to different people [3,7], but a therapeutic range for serum citalopram has not yet been established. It has been suggested that the minimal effective serum concentration for clinical response is about 30 ng/ml, and the majority of patients given standard doses have serum levels up to about 200 ng/ml [6]. Thus, in order to control for non-compliance and to disclose extremely high or low serum concentrations, measurement of citalopram concentration in serum may be valuable.

Several methods for determination of racemic citalopram in serum have been published using TLC, GC–MS or HPLC techniques [8–15]. Concerning the HPLC methods, we noticed that the extraction steps were somewhat laborious. Although the procedure for extracting citalopram from serum described by Rop et al. [15] is less time consuming than their previous method [12], it still includes both solid-phase clean up and an evaporation step. Furthermore, in routine therapeutic drug monitoring (TDM) at a psychiatric hospital a major problem is the analytical interference from other drugs, because only a small percentage of the patients are in monotherapy. Neuroleptics, sedatives and even tricyclic antidepressants are given simultaneously, and the presence of these drugs and their metabolites in serum may disturb the quantitation of citalopram. Insufficient information is provided about a possible interference from these compounds in the previously published methods.

The aim of the present study was to simplify the extraction of citalopram and desmethylcitalopram from serum and use a HPLC method, which allows a quantitative determination of the two compounds in the presence of commonly used psychoactive drugs and their metabolites.

2. Experimental

2.1. Chemicals

Citalopram, 1-(3-(dimethylamino)propyl)-1-(*p*-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile, N-desmethylcitalopram, N-didesmethylcitalopram and LU 10-202, which is citalopram where chlorine substitutes fluor in the phenyl ring, were all donated by Lundbeck (Copenhagen, Denmark). HPLC-grade heptane, isoamyl alcohol, methanol and acetonitrile were from Fisons Scientific Equipment (Loughborough, UK). Triethylamine, potassium dihydrogenphosphate, phosphoric acid, sodium carbonate and sodium bicarbonate, all analytical grade, were obtained from Merck (Darmstadt, Germany). Water was deionized and purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

All stock solutions were prepared by dissolving pure substances in ethanol: citalopram, desmethylcitalopram or LU 10-202 in a concentration of 5 mmol/l (1.621, 1.552 or 1.704 mg/ml). For further dilution of stock solutions ethanol–water (50:50, v/v) was used. Stock solutions were kept at −20°C and found to be stable for at least three months, and aqueous dilutions were stable for at least fourteen days when stored at 4°C [10].

Serum standards and controls containing known amounts of citalopram and desmethylcitalopram were prepared by spiking serum from healthy drug-free donors.

2.3. Extraction

In 12-ml centrifuge tubes, 1.0 ml of serum was mixed with 0.5 ml 0.75 *M* sodiumcarbonate/bicarbonate buffer, pH 10, and 50 μl LU 10-202 solution as internal standard. An aliquot of 8 ml heptane–isoamyl alcohol (98:2, v/v) was added and the mixture was shaken for 5 min at 250 shakings/min on a HS 500 (Janke & Kunkel, Staufen, Germany) shaking apparatus. After

centrifugation at 1500 *g* for 10 min, the aqueous layer was frozen by immersing the tubes into a cooling bath consisting of dry ice and ethanol. The heptane layer was decanted into centrifuge tubes and 150 μl 22 m*M* potassium dihydrogenphosphate/phosphoric acid buffer pH 2.5 was added. The mixture was shaken, centrifuged and frozen as described above, and the organic layer discarded by suction and 65 μl of the aqueous phase injected into the chromatograph.

2.4. Chromatography and calculations

The chromatographic analysis was performed on a Perkin-Elmer (Norwalk, CT, USA) system consisting of a LC Model 250 pump, ISS-200 autosampler, LC 90 UV photometer set at 240 nm and chromatograms were recorded by PE-Nelson Omega software. The analytical column was a 250 × 4.6 mm I.D. Supelco C_{18} (Supelco, Bellefonte, PA, USA). The mobile phase was a 44 m*M* KH_2PO_4 buffer containing 1.5 ml triethylamine per 1000 ml and adjusted to pH 2.5 with H_3PO_4–acetonitrile (55:45, v/v). The flow-rate was 1.5 ml/min.

From the recorded peak heights, the ratios of drug to internal standard were calculated. The results obtained from serum standards spiked with different known amounts of citalopram and desmethylcitalopram were used to calculate the factor for multiplying the ratios between heights of unknown and internal standard peaks.

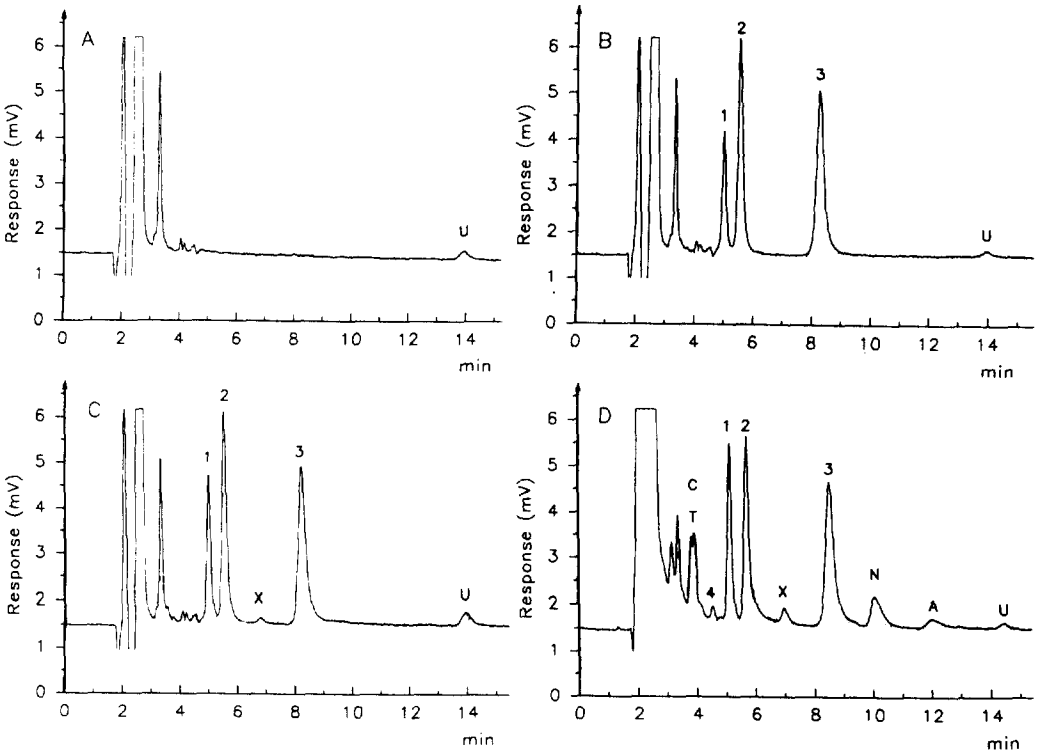

Fig. 1. (A) Chromatogram of a 1-ml blank serum; U indicates an unknown peak originating from serum. (B) Chromatogram of serum blank spiked with 18.6 ng/ml desmethylcitalopram (1), 64.9 ng/ml citalopram (2), and internal standard LU 10-202 (3). (C) Chromatogram of serum from a patient treated with citalopram 40 mg/day; X indicates an unidentified peak found in serum from citalopram-treated patients. (D) Chromatogram of serum from a patient treated with 40 mg/day of citalopram and 100 mg/day of amitriptyline; C = *cis*- and T = *trans*-10-OH-nortriptyline, 4 = didesmethylcitalopram, N = nortriptyline, and A = amitriptyline.

3. Results

3.1. Chromatography, recovery and linearity

A chromatogram of a serum blank from a healthy drug-free blood donor is shown in Fig. 1A. Only one peak (U), which did not interfere, was found after the solvent peaks, and Fig. 1B shows that citalopram, desmethylcitalopram and LU 10-202, the internal standard, were eluted within 9 min. The chromatogram in Fig. 1C originates from a patient receiving 40 mg citalopram per day. It is observed that in addition to citalopram and desmethylcitalopram an unidentified peak (X) was found in sera from citalopram-treated patients. Fig. 1D shows that amitriptyline and its main metabolites did not disturb the quantitation of citalopram, and it further shows that a small amount of the clinically inactive metabolite didesmethylcitalopram is also observed.

The recovery was calculated by comparing the peak heights obtained when citalopram (64.9 ng/ml) and desmethylcitalopram (18.6 ng/ml) were added to blank serum and analyzed according to Section 2 with the peak heights obtained when the corresponding amount of the compounds dissolved in mobile phase was injected. In the extraction procedure the drug content of 1 ml serum is redissolved in 125 μl buffer. After correction for the change in volume the recovery of citalopram was (mean ± C.V., $n = 3$) 81 ± 3.4% and that of desmethylcitalopram 99 ± 4.4%.

Blank serum was spiked with citalopram in the range 4.9–648.4 ng/ml and with desmethylcitalopram in the range 2.9–186.3 ng/ml and analyzed according to the method described. The equations of the regression lines and the correlation coefficients were $y = 0.022x + 0.067$, $r = 1.000$, $n = 16$ for citalopram and $y = 0.042x + 0.074$, $r = 1.000$, $n = 16$ for desmethylcitalopram.

3.2. Precision and accuracy

The within-day and day-to-day precision and accuracy were evaluated by analyzing blank serum spiked with different amounts of citalopram and desmethylcitalopram. The results are given in Table 1 which shows that at typical serum levels for patients on standard doses the day-to-day variations for citalopram and desmethylcitalopram were less than 5% and 6%,

Table 1
Precision and accuracy of the determination of citalopram and desmethylcitalopram in spiked serum

Concentration (ng/ml)	n	Intra-day		Inter-day	
		C.V. (%)	Accuracy (%)	C.V. (%)	Accuracy (%)
Citalopram					
10.8	10	6.79	107		
32.5	10	2.73	100		
32.5	8			3.41	100
64.8	5			4.96	97.0
259.7	10	2.90	108		
259.7	8			3.08	101
Desmethylcitalopram					
3.1	10	6.04	117		
9.3	10	3.17	104		
18.6	5			5.08	105
32.5	8			5.91	100
74.5	10	4.28	109		
74.5	8			3.41	100

respectively. The lower limit of quantitation (signal-to-noise ratio >3) was about 2 ng/ml for citalopram and 3 ng/ml for the metabolite.

3.3. Analysis for interference, serum levels in patients

Sera from patients receiving antipsychotic or antidepressant drugs, which may be used in combination with citalopram, were analyzed in order to study possible interference with regard to citalopram and desmethylcitalopram determination. The retention times of the compounds relative to citalopram are given in Table 2. It appears that only 8-OH-clomipramine may interfere with the determination of citalopram.

In seventeen patients treated with 20 mg citalopram/day the median and range of the steady-state serum concentrations (24-h values) of citalopram and desmethylcitalopram were 49.7 ng/ml (27.0–76.9) and 10.6 ng/ml (6.2–10.9), respectively. In patients given 40 mg/day the corresponding figures were 79.6 ng/ml (46.4–116.9) for citalopram and 21.4 ng/ml (6.2–36.0) for the metabolite. The serum levels measured in patients using the present method are in the range reported by other investigators [3,6].

Table 2
Retention time and relative retention time (in min) of some commonly used psychotropic drugs and their metabolites

Compound	Retention time	Relative retention time
Citalopram	5.56	1.0
Desmethylcitalopram	5.06	0.91
Didesmethylcitalopram	4.51	0.81
LU 10-202 (I.S.)	8.33	1.50
Clozapine	3.54	0.64
Desmethylclozapine	5.06	0.52
Clozapine N-oxid	4.65	0.84
Haloperidol	6.70	1.21
Reduced haloperidol	n.d.	
Perphenazine	7.43	1.34
Dealkylated perphenazine	6.86	1.23
Zuclopenthixol	9.24	1.66
Dealkylated zuclopenthixol	6.88	1.24
Levomepromazine	11.3	2.03
Desmethyllevomepromazine	9.80	1.76
Levomepromazine sulfoxide	3.62	0.65
Flupenthixol	13.8	2.48
Chlorprothixene	16.8	3.02
Chlorprothixene metabolite	14.0	2.52
Nortriptyline	9.73	1.75
cis-10-OH-nortriptyline	3.80	0.68
trans-10-OH-nortriptyline	3.00	0.54
Amitriptyline	11.5	2.07
cis-10-OH-amitriptyline	4.17	0.75
trans-10-OH-amitriptyline	3.00	0.54
Clomipramine	16.9	3.04
8-OH-clomipramine	5.82	1.05
Desmethylclomipramine	14.2	2.55
8-OH-desmethylclomipramine	5.28	0.95
Didesmethylclomipramine	11.5	2.08
Benzodiazepines	n.e.	

n.d. = Not detected; n.e. = not extracted.

4. Discussion

In the extraction step of citalopram and desmethylcitalopram from serum we used a low concentration of acid phosphate buffer with a pH identical to the buffer used in the mobile phase. Thereby it is possible rather selectively to extract basic compounds from the organic phase and inject the aqueous phase directly into the HPLC apparatus without disturbing the chromatogram with respect to retention times or broadening of peaks. Evaporation of organic fluid is avoided and cleaning steps, e.g. back extraction from the aqueous phase into a new organic phase was not necessary. The peak-height ratio between the analytes and the internal standard was linearly correlated to the serum concentration of the two compounds within the range investigated. No attempts were done to extend this range into toxic levels. The reproducibility of the method is sufficient for clinical use.

The mean concentration of desmethylcitalopram in serum from citalopram-treated patients was only 28% of the citalopram concentration and the metabolite is further considered to have a potency equal to a quarter of the parent compound [1]. Therefore, in routine TDM only the concentration of citalopram should be reported. Metabolite concentrations may be of interest in special cases, e.g. to distinguish between ultrarapid metabolism and non-compliance and in suspected cases of drug interaction.

In the development of a routine HPLC method, it is of course not possible to take into account all possible drugs which may interfere with the determination of citalopram. In the present paper we have focused on commonly used psychoactive drugs. For clinical reasons it may not be necessary to determine desmethylcitalopram and in some cases it may be necessary to use another internal standard and in special cases adjustment of the mobile-phase composition must be considered. When considering analytical interference, the importance of metabolites should not be underestimated. Commonly, interference is only evaluated by spiking with parent compounds, which for many neuroleptics and antidepressants are clearly insufficient. By extracting sera from patients receiving the drugs, the real picture emerges.

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