ELSEVIER

Journal of Chromatography B, 702 (1997) 234–239

JOURNAL OF CHROMATOGRAPHY B

Short communication

Solid-phase extraction with end-capped C_2 columns for the routine measurement of racemic citalopram and metabolites in plasma by high-performance liquid chromatography

Björn Carlsson*, Björn Norlander

Department of Clinical Pharmacology, Faculty of Health Sciences, Linköping University, S-581 83 Linköping, Sweden

Received 14 March 1997; received in revised form 27 June 1997; accepted 7 July 1997

Abstract

An assay based on solid-phase extraction followed by high-performance liquid chromatography (HPLC) was developed for the measurement of citalopram and its main metabolites desmethylcitalopram and didesmethylcitalopram. The best extraction procedure was performed with end-capped C_2 column utilising secondary silanol interactions to obtain clean extract. The HPLC analysis was done on a phenyl column with a mobile phase without any amine additives. Fluorescence detection gave a limit of detection of 0.8 nmol/l plasma for the compounds analysed. © 1997 Elsevier Science B.V.

Keywords: Citalopram; Desmethylcitalopram; Didesmethylcitalopram

1. Introduction

Citalopram belongs to a new group of antidepressants, selective serotonin reuptake inhibitors (SSRIs), which are today prescribed more than tricyclic antidepressants [1,2]. Citalopram inhibits the transport of the neurotransmitter serotonin into nerve terminals and other cells that accumulate it.

In vitro studies in rat brain synaptosomes have shown that the inhibitory effect resides in the (*S*)-(+)-enantiomer, and that the two metabolites formed by N-demethylation of citalopram, desmethylcitalopram and didesmethylcitalopram, are less potent than the parent compound [3]. In vivo the metabolites do not contribute to the pharmacological effect. No concentration–effect relation for citalopram has yet been established and one explanation might be that citalopram is a racemic compound.

Recently three methods have been published that deal with the separation of the enantiomers of citalopram and its metabolites [4–6]. It is hoped that these will initiate studies about concentration–effect relations for the enantiomers of citalopram.

Until these relations are finally established, monitoring of the concentration of racemic citalopram and its main metabolites may help the physician during treatment by providing valuable information about non-compliance or extremely high or low plasma concentrations.

Several methods using high-performance liquid chromatography (HPLC) [7–13] for achiral assay of citalopram have been published as has one using gas chromatography–mass spectrometry (GC–MS) [14]. Different procedures of extraction have been used,

*Corresponding author.

0378-4347/97/$17.00
PII S0378-4347(97)00366-6

the most common of which are liquid–liquid extraction [7–9,12,14], column switching [11] and extraction with Extrelute columns [10]. To our knowledge no one has used solid-phase extraction (SPE), utilizing the possibility of secondary silanol interactions for extraction of citalopram and its metabolites, a method that has been successfully used for other antidepressants [15].

The aim of the investigation was to investigate the use of SPE for citalopram and demethylated metabolites, and to ascertain what type of column material C_{18}, C_8 or C_2 end-capped and C_2 non-end-capped would give the best recovery and cleanest extract.

2. Experimental

2.1. Standards and reagents

Acetonitrile (far-UV) and methanol of HPLC-grade were purchased from Lab Scan (Ireland) and all other chemicals were of analytical grade: potassium dihydrogenphosphate (Fluka), disodium hydrogenphosphate dihydrate (Merck), potassium hydroxide (Merck), sodium hydroxide (Merck), acetic acid 100% (Merck) and phosphoric acid 85% (Merck).

Ultra-pure water was taken from a Milli-Q station (Millipore).

The SPE columns Isolute (IST, International Sorbent Technology) were C_2, C_8 and C_{18} all end-capped and C_2 non-end-capped containing 100 mg of sorbent and with 10 ml reservoir size.

Eluents were collected in 10×100 mm glass tubes.

The standard compounds, citalopram HBr (Lu 10-171 B) (CT), desmethylcitalopram HCl (Lu 11-109 C) (DCT), didesmethylcitalopram tartrate (Lu 11-161-X) (DDCT) and the internal standard (Lu 10-202-O) were supplied by H. Lundbeck (Copenhagen, Denmark). Stock solutions (1 mg/ml) of CT, DCT and the internal standard were made in methanol and of DDCT in water. The stock solutions were diluted in methanol to 100 μg/ml for the preparation of calibration standards. All standard solutions were stored at 4°C. Serum standards were prepared and frozen in 3 ml aliquots at −20°C and were found to be stable for 6 months.

2.2. Apparatus

A WacMaster-20 (IST) sample processing station, fitted with stainless-steel needles, was used for the extraction procedure.

The HPLC system consisted of a Varian 2510 pump connected to a Nova Pak phenyl column RCM (10 cm×8 mm I.D., particle size 4 μm, Waters) with a Nova Pak phenyl guard-column (4 μm, Waters) at ambient temperature. The samples were detected with a fluorescence detector LS-40 from Perkin-Elmer. The samples were injected automatically with a Waters 717 autosampler.

Chromatograms were recorded with EZ Chrom chromatography data system Version 6.5 (Scientific Software).

2.3. Chromatographic conditions

The mobile phase was acetonitrile–70 mmol/l phosphate buffer (40:60) filtered through a 0.22 μm filter and recycled in the chromatographic system at a flow-rate of 3 ml/min.

An excitation wavelength of 235 nm and an emission wavelength of 290 nm were used.

2.4. Extraction procedure

Patient blood samples were centrifuged and the plasma was separated and stored at −20°C until analysis.

The extraction columns were placed on the WacMaster.

Conditioning of the columns was done with 1 ml acetonitrile followed by 0.5 ml 25 mmol/l phosphate buffer, pH 11.5. Thereafter 1 ml of acetonitrile–25 mmol/l phosphate buffer, pH 11.5 (5:95) was added to the column reservoir followed by 1 ml plasma (sample, standard or control), 100 μl of internal standard 0.5 μg/ml and finally another 1 ml acetonitrile–25 mmol/l phosphate buffer, pH 11.5 (5:95). A gentle underpressure of 15–35 kPa was used to suck the solution through the column.

The columns were washed with 1 ml of ultrapure water, dried for 5 min (15–35 kPa), followed by a second washing step with 1 ml acetonitrile and

finally dried. The tips of the WacMaster were cleaned with a paper napkin and a rack with glass tubes was placed in the WacMaster.

2 ml of 1% acetic acid in methanol was added for elution and left for 5 min, after which the solvent still on the column was drawn through at 15–35 kPa.

The elution solvent was evaporated from the tubes with nitrogen at 70°C. The samples were reconstituted in 100 μl of the mobile phase and 20 μl injected.

3. Results

3.1. Chromatography and calibration

Fig. 1a and Fig. 1b show chromatograms of a blank plasma sample from a healthy drug-free donor and from an extract of a standard containing 308 nmol/l CT, 161 nmol/l DCT and 34 nmol/l DDCT and internal standard, all eluted within 6 min and without interfering peaks.

Fig. 1c shows a chromatogram of an extract of the plasma from a patient who had been given a dose of 60 mg CT daily.

The sample was taken before dose at steady-state.

Standards curves were prepared by adding known amounts of CT, DCT and DDCT to drug free plasma. Peak height ratios between CT, DCT, DDCT and the internal standard were used for the calculation of standard curves and unknown samples from patients.

The standard curves were linear over a concentration ranges of 60–620 nmol/l for CT, 30–320 nmol/l for DCT and 7–70 nmol/l for DDCT, with correlation coefficients between 0.9965–0.9999, 0.9956–0.9997, 0.9933–0.9998, respectively (n= 10).

3.2. Precision and accuracy

Accuracy and within-day precision were calculated by analysis of three different concentrations levels of the standards (n=5) and the day-to-day

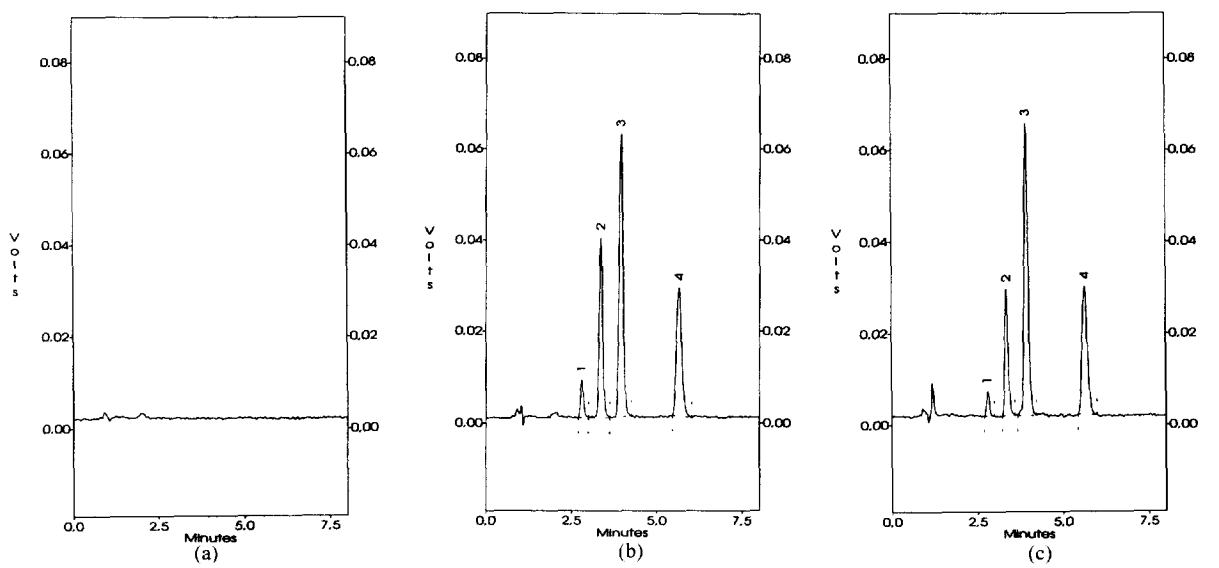

Fig. 1. (a) Chromatogram of an extract from a blank plasma. There are no interfering peaks detected. (b) Extract from a standard in plasma, containing 308 nmol/l citalopram (3), 161 nmol/l desmethylcitalopram (2) 34 nmol/l didesmethylcitalopram (1) and internal standard (4). (c) Chromatogram of a serum sample from a patient at steady state, before dose, receiving 60 mg of citalopram/day, containing 317 nmol/l citalopram, 114 nmol/l desmethylcitalopram and 23 nmol/l didesmethylcitalopram.

precision by analysing controls at three different concentrations ($n=11$) (Table 1).

Within-day precision ranks from 1.2–3.9% for CT, 0.6–3.8% for DCT and 3.9–10.0% for DDCT.

The day-to-day precision ranges from 5.1–7.5% for CT, 7.0–10.9% for DCT and 7.5–12.1% for DDCT.

3.3. Recovery studies and limit of detection

Known mixture amounts of CT, DCT, DDCT and internal standard were added to blank plasma and extracted. By injection of the corresponding amount of the compounds dissolved directly in the mobile phase the recoveries could be calculated.

Table 1
Within-day and day-to-day precision for citalopram, desmethylcitalopram and didesmethylcitalopram

Concentration (nmol/l)	Citalopram	Desmethylcitalopram	Didesmethylcitalopram
Within-day precision ($n=5$)			
Standard I			
Target concentration	62	32	7
Mean	62.6	31.9	7.4
S.D.	0.8	0.2	0.8
C.V. (%)	1.2	0.6	10.0
Accuracy (%)	101.0	96.6	105.6
Standard III			
Target concentration	308	161	34
Mean	303.6	159.6	33.9
S.D.	9.20	6.01	1.38
C.V. (%)	3.0	3.7	4.0
Accuracy (%)	98.6	99.1	99.7
Standard V			
Target concentration	616	322	67
Mean	621.3	322.3	66.3
S.D.	24.8	12.3	2.6
C.V. (%)	3.9	3.8	3.9
Accuracy (%)	100.9	100.1	99.0
Day-to-day precision ($n=11$)			
Control I			
Target concentration	92	48	10
Mean	84.5	50.8	10.8
S.D.	6.3	4.3	1.3
C.V. (%)	7.5	8.5	12.1
Accuracy (%)	91.8	105.8	108.2
Control II			
Target concentration	277	145	32
Mean	265.5	160	32.2
S.D.	13.5	11.1	2,4
C.V. (%)	5.1	7.0	7.5
Accuracy (%)	95.9	110.2	107.4
Control III			
Target concentration	555	290	61
Mean	518.4	306.8	61.5
S.D.	38.8	33.3	5.5
C.V. (%)	7.5	10.9	9.0
Accuracy (%)	93.4	105.8	100.9

Table 2
Other drugs that do not interfere with the citalopram analysis

Anxiolytics	Neuroleptics and antipsychotics	Antidepressants
Alprazolam	Propiomazin	Clomipramine
Flunitrazepam	Flupenthixol	Desmethylclomipramine
Oxazepam	Levomepromazine	Amitriptyline
Flunitrazepam	Perphenazine	Imipramine
Chlormethiazole	Clozapine	Desipramine
Diazepam	Thioridazine	Lofepramine
Nitrazepam	Haloperidol	Maprotiline
Hydroxizine	Mianserin	Nortriptyline
Zolpidem		Paroxetine
Nitrazepam		
Buspirone		

The recoveries obtained were 98% for CT, 102% for DCT and 96% for DDCT.

The lower limit of detection for the three substances was 3 nmol/l (*S/N* 3:1) plasma when 20 μl out of 100 μl was injected.

3.4. Interference from other drugs

Patients receiving this type of medication may also be taking anxiolytics, neuroleptics or may have been switched from tricyclic antidepressants or other SSRIs.

Table 2 lists substances that do not interfere with the analysis.

4. Discussion

In this paper, SPE with C_2 columns was used to measure CT concentration in plasma. Other antidepressants have been extracted by a similar technique [13] using C_{18} columns instead.

In the washing step with acetonitrile the basic drugs are adsorbed to the column by silanol interactions, a mechanism that has been shown to be a selective way of extracting basic compounds.

After the wash with water the pH is around 7 and a microenvironment is probably formed at the silica surface where ionic interactions hold the basic drugs on to the column. A change in pH, which in order to either suppress the ionization of the basic drug or the acidic surface of the exposed silica makes the drugs available for elution.

1% acetic acid in methanol was used for the elution of the compounds. Another possible elution solvent is ammonia in acetonitrile [16]. Acetic acid in methanol, however, was shown to give the best reproducibility for this method (data not given for ammonia in acetonitrile), perhaps due to the fact that salts of the bases are formed and alleviate losses during evaporation that follows.

A higher concentration of acetic acid (2 and 3%) gave extracts with more interfering peaks in the HPLC analysis. The C_2 column gave a better result compared to C_8 and C_{18} columns probably because it provides a stronger silanol interaction. When comparing C_2 with and without end-capping more interferences were seen in the eluate from the C_2 without end-capping. Obviously the silanol interactions need some suppression for optimal washing with acetonitrile.

Chromatography of basic drugs on C_{18} columns often requires organic amines to reduce secondary interactions and to obtain good performance. Here the chromatographic separation was done on a phenyl column with a mobile phase made of acetonitrile and phosphate buffer. With this column and mobile phase the peak shape was good, without the addition of any organic amine.

Fluorescence detection is an advantage to ultraviolet (UV) detection. Besides high sensitivity, it increases the specificity and selectivity for the compounds of interest and helps to reduce some interfer-

ing peaks. This method is now in use as a routine and we have analysed about 250 patient samples without complication in the extraction procedure or any interfering problems in the chromatography.

References

[1] H. Luo, J.S. Richardson, Int. Clin. Psychopharmacol. SPR 8 (1993) 3.
[2] J. Hyttel, Int. Clin. Psychopharmacol. 9 (Suppl. 1) (1994) 19.
[3] J. Hyttel, K.P. Bogeso, J. Perregard, C. Sánchez, J. Neural. Transm. 88 (1992) 157.
[4] B. Rochat, M. Amey, P. Baumann, Ther. Drug. Monitor. 17 (1995) 273.
[5] B. Rochat, M. Amey, H. Vangelderen, B. Testa, P. Baumann, Chirality 7 (1995) 389.
[6] D. Haupt, J. Chromatogr. B 685 (1996) 299.
[7] E. Øyehaug, E.T. Østensen, B. Salvensen, J. Chromatogr. 227 (1982) 129.
[8] E. Øyehaug, E.T. Østensen, B. Salvesen, J. Chromatogr. 308 (1984) 199.
[9] P.-P. Rop, A. Viala, A. Durand, T. Conquy, J. Chromatogr. 338 (1985) 171.
[10] P.-P. Rop, A. Viala, A. Durand, A. Jørgensen, J. Chromatogr. 527 (1990) 226.
[11] E. Matsui, M. Hoshino, A. Matsui, A. Okahira, J. Chromatogr. B 668 (1995) 299.
[12] O. Vendelin Olesen, K. Linnet, J. Chromatogr. B 675 (1996) 83.
[13] C.B. Eap, P. Baumann, J. Chromatogr. B 686 (1996) 51.
[14] Ph. Reymond, M. Amey, A. Souche, S. Lambert, H. Konrat, C.B. Eap, P. Baumann, J. Chromatogr. 616 (1993) 221.
[15] R.N. Gupta, J. Liq. Chromatogr. 16 (1993) 2751.
[16] M. Josefsson, A.L. Zackrisson, B. Norlander, J. Chromatogr. B 672 (1995) 310.