ELSEVIER

Journal of Chromatography B, 719 (1998) 234–238

JOURNAL OF CHROMATOGRAPHY B

Short communication

Analysis of the enantiomers of citalopram and its demethylated metabolites using chiral liquid chromatography

M. Kosel, C.B. Eap, M. Amey, P. Baumann*

Unité de Biochimie et Psychopharmacologie Clinique, Département Universitaire de Psychiatrie Adulte, CH-1800 Prilly-Lausanne, Switzerland

Received 4 May 1998; received in revised form 21 July 1998; accepted 4 August 1998

Abstract

A procedure using a chirobiotic V column is presented which allows separation of the enantiomers of citalopram and its two *N*-demethylated metabolites, and of the internal standard, alprenolol, in human plasma. Citalopram, demethylcitalopram and didemethylcitalopram, as well as the internal standard, were recovered from plasma by liquid–liquid extraction. The limits of quantification were found to be 5 ng/ml for each enantiomer of citalopram and demethylcitalopram, and 7.5 ng/ml for each enantiomer of didemethylcitalopram. Inter- and intra-day coefficients of variation varied from 2.4% to 8.6% for *S*- and *R*-citalopram, from 2.9% to 7.4% for *S*- and *R*-demethylcitalopram, and from 5.6% to 12.4% for *S*- and *R*-didemethylcitalopram. No interference was observed from endogenous compounds following the extraction of plasma samples from 10 different patients treated with citalopram. This method allows accurate quantification for each enantiomer and is, therefore, well suited for pharmacokinetic and drug interaction investigations. The presented method replaces a previously described highly sensitive and selective high-performance liquid chromatography procedure using an acetylated β-cyclobond column which, because of manufactural problems, is no longer usable for the separation of the enantiomers of citalopram and its demethylated metabolites.

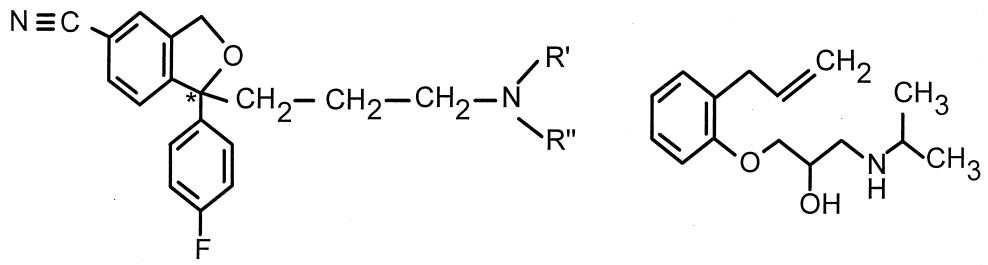
Keywords: Enantiomer separation; Citalopram; Demethylcitalopram; Didemethylcitalopram

1. Introduction

Citalopram (CIT) (Fig. 1), which is only available as a racemic drug, is a widely used antidepressant of the selective serotonin reuptake inhibitor (SSRI) type [1]. The pharmacological effect of racemic CIT resides mainly in the *S*-(+)-CIT enantiomer and, to a lesser degree, in the *S*-(+)-demethylcitalopram (DCIT) enantiomer, whereas *R*-(−)-CIT and *R*-(−)-DCIT can be considered as pharmacologically inactive [2]. We (using cyclobond I 2000 β-acetylated columns) and others have published several reports on the stereoselective analysis, metabolism and pharmacokinetics of CIT and its demethylated metabolites [3–6,8]). We have demonstrated inter-individual variations of the (*S*)/(*R*)-CIT–plasma concentration ratios in CIT-treated depressed patients [4,5]. These variations could be clinically relevant [3] and justify a chiral analysis of CIT and its metabolites. Since the Astec Corporation, the manufacturer of cyclobond columns, has employed a different silica matrix in the manufacturing of their chiral cyclobond I 2000 β-acetylated columns, we

*Corresponding author. Address for correspondence: DUPA – Hôpital de Cery, CH-1008 Prilly-Lausanne, Switzerland.

0378-4347/98/$ – see front matter
PII: S0378-4347(98)00384-3



R' = R" = CH_3 : citalopram

R' = H; R" = CH_3 : desmethylcitalopram

R' = R" = H: didesmethylcitalopram

alprenolol

Fig. 1. Chemical structure of CIT, its metabolites and the internal standard alprenolol. * Asymmetric center.

have been unable to achieve the previous excellent separation of the enantiomers of CIT, DCIT and didemethylcitalopram (DDCIT). It should be mentioned that such a problem has not been reported for the analysis of other substances (E. Beesley, Astec Corp., personal communication).

Given the importance of carrying out further studies on the pharmacokinetics of citalopram and its interactions with other drugs, we have developed the present method.

2. Experimental

2.1. Reagents

CIT hydrobromide, D-CIT hydrochloride and DDCIT L-tartrate were obtained as racemates or enantiomers from Lundbeck (Copenhagen, Denmark). These compounds were dissolved in methanol at a concentration of 1 μg/μl base (racemates or enantiomers), as stock solutions, and in 0.01 *M* hydrochloric acid at 10 ng/μl base, as working solution, and stored at −20°C.

The internal standard (I.S.) *S*-alprenolol was purchased from Sigma (Buchs, Switzerland). It was dissolved in methanol at a concentration of 1 μg/μl, as stock solution, and in 0.01 *M* hydrochloric acid at 2 ng/μl, as working solution, and stored at −20°C until use.

All other reagents were of analytical or HPLC grade.

2.2. Extraction conditions

Extraction conditions were slightly modified, compared to those previously reported [3]. To 1 ml of heparinized plasma was added 200 ng of *S*-alprenolol, 100 μl of 1 *M* sodium hydroxide and 6 ml of heptane–isoamyl alcohol (98.5:1.5, v/v). The mixture was shaken for 15 min and centrifuged at 3000 *g* for 8 min at 8°C. The organic layer was transferred to another tube containing 1.2 ml of 0.1 *M* hydrochloric acid. The solution was shaken for 15 min, followed by centrifugation at 3000 *g* for 8 min at 8°C. The organic phase was discarded, and 1 ml of 1 *M* sodium carbonate buffer (pH 9.4) was added to the aqueous phase. The mixture was extracted with 150 μl toluene–isoamyl alcohol by shaking for 15 min. This solution was then centrifuged at 3000 *g* for 4 min at 8°C, and the organic layer transferred to an injection tube. After evaporation to dryness of the toluene–isoamyl alcohol under a stream of nitrogen at 40°C, the residue was redissolved in 130 μl of the mobile phase.

2.3. Instrumentation and chromatographic conditions

Analyses were performed on a Hewlett-Packard HP 1100 high-performance liquid chromatography (HPLC) system equipped with an injector loop of 100 μl. A chiral analytical column (Chirobiotic V, particle size 5 μm, 150×4.6 mm from Astec (ICT, Basel, Switzerland) was coupled with a fluorimetric detector (Perkin-Elmer LC 240, Perkin Elmer, Le

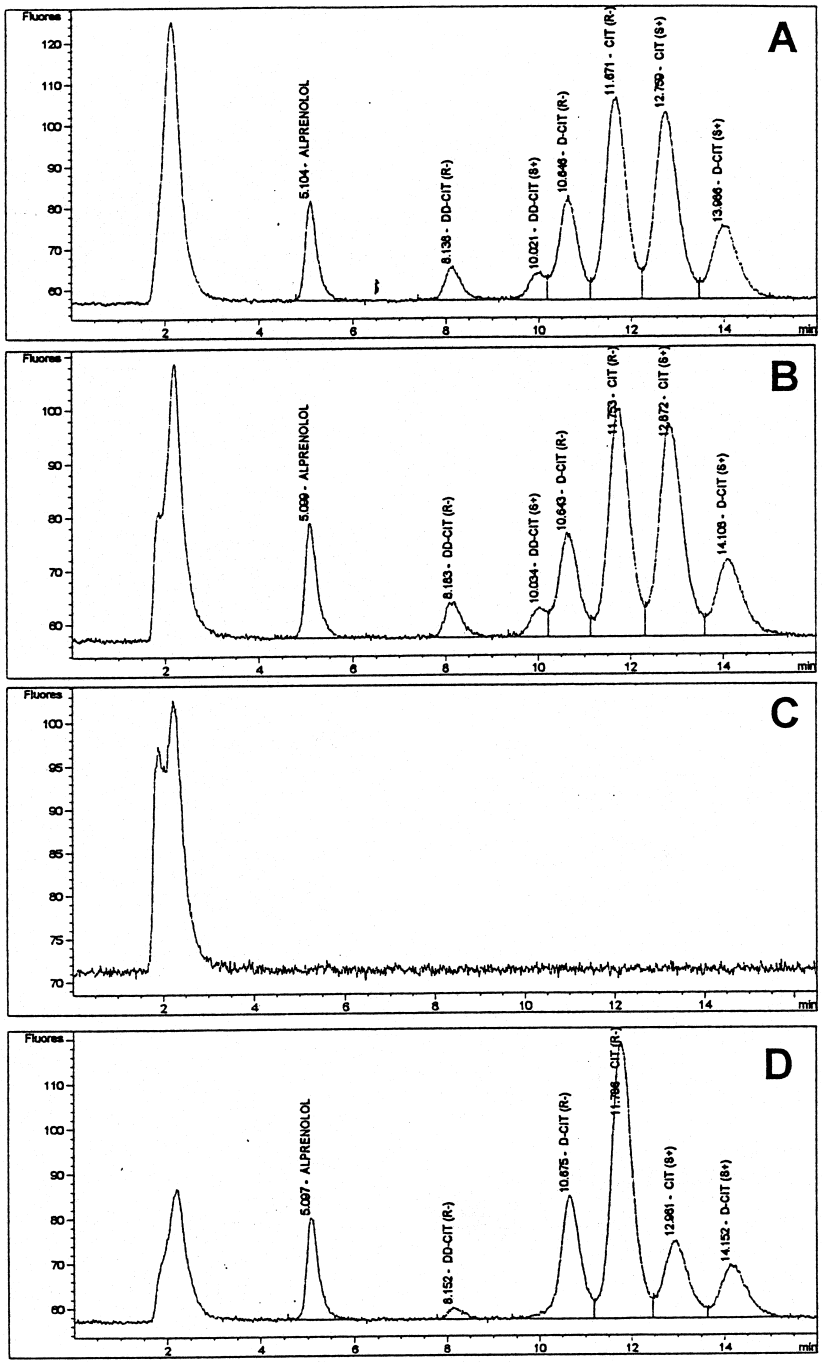


Fig. 2. Typical chromatograms of extracts of (A) a spiked mobile phase; (B) a spiked plasma sample (200, 100 and 60 ng/ml of racemic CIT, DCIT and DDCIT, respectively); (C) a blank plasma sample; (D) a plasma sample of a patient treated with 140 mg/day CIT. Measured concentrations (ng/ml): *R*-(−)-CIT: 129, (*S*)-(+)-CIT: 40.2, *R*-(−)-DCIT: 60.4, *S*-(+)-DCIT: 34.5, *R*-(−)-DDCIT: 11.2, *S*-(+)-DDCIT: not detected.

Mont-sur Lausanne, Switzerland) set at 240 and 296 nm for excitation and emission, respectively. The column flow-rate was 1 ml/min, the mobile phase was methanol–acetic acid–triethylamine (99.9:0.055:0.060, v/v).

3. Results and discussion

For the separation of the enantiomers of CIT, DCIT and DDCIT, different cyclobond I 2000 β-acetylated columns coated with silica from different batches, as well as a cyclobond I 2000 β-sulfated column in combination with several pre-columns (Nucleosil C_6H_5, C_{18}, CN) were screened in reversed- and normal-phase conditions. No satisfactory separation could be obtained.

We finally succeeded in separating CIT and its metabolites with a chirobiotic V column which yields a separation of the seven different compounds in a total run time of 15 min (Fig. 2). The *R* enantiomers were eluted before the corresponding *S* enantiomers, and the primary amines preceded *R*-DCIT, the tertiary amines, which are grouped together, and *S*-DCIT. This is in contrast with the chromatographic data obtained with the previously described cyclobond column [3], by which the *S* enantiomers were firstly eluted. The amines were eluted in the order:

Table 1
Statistical data on the analytical procedure

	CIT		DCIT		DDCIT		Alprenolol
	S	*R*	*S*	*R*	*S*	*R*	
Typical retention time (min)	12.6	11.5	13.7	10.5	9.7	8	5.1
Extraction recoveries							
Theoretical concentration (n=6) (ng/ml)	5	5	5	5	7.5	7.5	200
Recoveries (%)	86.4	83.4	71.9	67.0	43.6	39.9	71.1
S.D.	5.6	8.2	9.7	4.1	2.9	3.0	4.3
Theoretical concentration (n=6) (ng/ml)	250	250	150	150	30	30	400
Recoveries (%)	77.1	77.1	68.1	67.3	33.5	30.1	62.4
S.D.	1.5	1.4	1.6	2.1	2.0	1.4	4.3
Intra-day precision and accuracy							
Theoretical concentration (ng/ml)	25	25	15	15	15	15	
Measured concentration mean (n=7)	23.2	22.7	14.7	14.8	15.1	15.8	
S.D.	0.7	0.5	0.5	0.5	1.3	1.5	
Theoretical concentration (ng/ml)	100	100	50	50	30	30	
Measured concentration mean (n=7)	92.8	92.2	48.6	48.6	29.9	29.3	
S.D.	2.1	2.5	1.4	1.4	1.9	1.7	
Inter-day precision and accuracy							
Theoretical concentration (ng/ml)	25	25	15	15	15	15	
Measured concentration mean (n=7)	23.1	23.3	13.7	13.7	13.5	13.6	
S.D.	1.8	2.0	0.6	0.8	1.7	1.7	
Theoretical concentration (ng/ml)	100	100	50	50	30	30	
Measured concentration mean (n=7)	92.5	92.1	45.9	46.0	28.9	29.0	
S.D.	6.3	5.4	3.4	3.1	2.5	1.9	
Limit of quantification							
Theoretical concentration (ng/ml)	5	5	5	5	7.5	7.5	
Measured concentration mean (n=8)	5.9	5.8	5.3	5.2	7.3	7.6	
S.D.	0.3	0.4	0.9	0.6	1.2	1.2	

primary amines – secondary amines – tertiary amines. As yet, there is no explanation for the elution order using the chirobiotic V column.

Extraction recovery was studied for two concentrations of racemates (Table 1). It was considered satisfactory for CIT and for DCIT but a lower value was found for DDCIT (30.1 to 43.6%). However, this result did not prevent us from obtaining reproducible results. It was not possible to optimize extraction conditions for the primary and tertiary amines simultaneously. As DDCIT is a metabolite of CIT with minor pharmacological activity, we optimized the extraction conditions for the tertiary amine (CIT). Calibration curves constructed over the concentration ranges 5–250, 5–100 and 7.5–50 ng/ml for the enantiomers of CIT, DCIT and DDCIT, respectively, showed linearity over the whole range of concentrations (r^2>0.991). Intra- and inter-day coefficients of variation (C.V.s) measured at two concentrations for each enantiomer were found to vary less than 12.5%. The limit of quantification defined as the concentration which gives, after extraction and injection, a signal-to-noise ratio of at least 3, and a C.V. of less then 20%, according to Bressolle et al. [7], was found to be 5 ng/ml for each enantiomer of CIT and DCIT, and 7.5 ng/ml for each enantiomer of DDCIT (Table 1).

Chromatograms of extracts of blank plasma, blank plasma spiked with CIT, DCIT and DDCIT, and of plasma from a patient treated with CIT, are shown in Fig. 2.

Up to now, only two reports from other groups deal with plasma concentrations of CIT and its demethylated metabolites, and their enantiomers [8,9]. Compared to the method presented by Sidhu et al. [9], who used a derivatised β-cyclodextrin column (Cyclobond I 2000 DMP, Astec) the present procedure offers some advantages: there is no derivatization step and the run time is much shorter (15 min compared to 50 min). However, both methods are similarly efficient with regard to the lower limits of quantification for the enantiomers of CIT and its metabolites. Finally, another sensitive HPLC method should be mentioned, which allows the separation of *S*-CIT and *R*-CIT using a chiral-AGP column, but which does not take account of the *N*-demethylated metabolites [10]. Moreover, no pharmacokinetic data of CIT in patients treated with this SSRI were reported.

In conclusion, the existence of inter-individual differences in the stereoselective metabolism of citalopram [3,4,8,9] requires a sensitive method as described here for the determination of the enantiomers of CIT and its demethylated metabolites.

Acknowledgements

We thank Astec Corporation for screening of chiral HPLC columns and Dr. Kupferschmidt, ICT Basel, for his technical counselling. We are grateful to Miss K. Powell and Mrs. C. Bertschi for their editorial assistance.

References

[1] P. Baumann, Int. Clin. Psychopharmacol. 11(Suppl. 1) (1996) 5.
[2] J. Hyttel, K.P. Bøgesø, J. Perregaard, C. Sanchez, J. Neural. Transm. Gen. Sect. 88 (1992) 157.
[3] B. Rochat, M. Amey, P. Baumann, Ther. Drug Monit. 17 (1995) 273.
[4] G. Bondolfi, C. Chautems, B. Rochat, G. Bertschy, P. Baumann, Psychopharmacology 128 (1996) 421.
[5] B. Rochat, M. Amey, M. Gillet, U.A. Meyer, P. Baumann, Pharmacogenetics 7 (1997) 1.
[6] B. Rochat, M. Kosel, G. Boss, B. Testa, M. Gillet, P. Baumann, Biochem. Pharmacol., in press.
[7] F. Bressolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[8] J.P. Foglia, B.G. Pollock, M.A. Kirshner, J. Rosen, R. Sweet, B. Mulsant, Psychopharmacol. Bull. 33 (1997) 109.
[9] J. Sidhu, M. Priskorn, M. Poulsen, A. Segonzac, G. Grollier, F. Larsen, Chirality 9 (1997) 686.
[10] D. Haupt, J. Chromatogr. B 685 (1996) 299.