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RESEARCH ARTICLE

Quantitative Determination of Citalopram and its Metabolite Desmethycitalopram in Plasma by High Performance Liquid Chromatography

Zuhal UCKUN*, H. Sinan SÜZEN*°

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Summary

Citalopram has by far the highest selectivity for serotonin re-uptake and is used for the treatment of depression. Therapeutic drug monitoring is clinically important for psychiatrists in terms of therapy success. In this work a simplified, rapid, sensitive, selective and validated high performance liquid chromatographic method for the determination of racemic citalopram and its main metabolite desmethylcitalopram in human plasma using protriptyline as internal standard was developed. The compounds were extracted with solid phase extraction using C-18 extraction cartridges. Calibration curves were linear over a working range 10-150 ng ml^{-1} for citalopram and 5-75 ng ml^{-1} for desmethylcitalopram. High percentage recovery values were obtained from compounds. Precision data as well as accuracy were satisfactory. Therefore, the method can be used for therapeutic drug monitoring of desmethylcitalopram and citalopram in patients treated with citalopram.

Key Words: *Citalopram; Desmethylcitalopram, solid phase extraction, HPLC.*

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Sitalopram ve Metaboliti Desmetilsitalopramın Plazmada Yüksek Performanslı Sıvı Kromatografisi ile Kantitatif Tayini

Özet

Sitalopram serotonin geri alımında oldukça seçici olup depresyon tedavisinde kullanılır. Terapötik ilaç izlenmesi psikiyatristler için klinik açıdan önemlidir. Bu çalışmada plazmada rasemik sitalopram ve başlıca metaboliti olan desmetilsitalopramın tayini için internal standart olarak protriptilin kullanılarak basit, hızlı, hassas, seçici ve doğrulanmış bir yüksek performanslı sıvı kromatografik yöntem geliştirildi. Bileşikler C-18 kullanılarak katı faz ekstraksiyonu ile ekstrakte edilmiştir. Kalibrasyon eğrileri sitalopram için 10-150 ng ml^{-1}, desmetilsitalopram için 5-75 ng ml^{-1} çalışma aralığında doğrusaldır. Elde edilen geri kazanım yüzdeleri yüksek olup kesinlik ve doğruluk açısından iyi sonuçlar alınmıştır. Böylece geliştirilen metod sitalopram ile tedavi olan hastalarda desmetilsitalopram ve sitalopramın terapötik ilaç izlenmesi amacıyla kullanılabilir.

Anahtar Kelimeler: *Sitalopram, desmetilsitalopram, katı faz ekstraksiyonu, YPSK.*

* Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100 Ankara, TURKEY
° Corresponding Author E-mail: suzen@pharmacy.ankara.edu.tr uckunzuhal@yahoo.com

INTRODUCTION

Citalopram (1- [3- (dimethylamino) propyl] -1- (4- fluorophenyl) -1,3-dihydroisobenzofuran-5-carbonitrile) is a selective and potent serotonin reuptake inhibitor that is used for the treatment of depression. It offers similar therapeutic efficacy and a more favourable tolerability profile than the tricyclic antidepressants (1). The therapeutic range for plasma citalopram (CIT) is 30-200 ng ml^{-1} and it becomes toxic when the plasma CIT concentration is greater than 1000 ng ml^{-1} (2). CIT has been used to treat not only major depression but other mood disorders, such as anxiety, panic disorders, obsessive-compulsive disorders and alcohol dependence, and seems to be suitable for the treatment of pathological laughing and crying (3).

CIT and its metabolites are racemic compounds with both S- (+) and R- (-) enantiomers. The S- (+) enantiomer of CIT is pharmacologically active in relation to inhibition of serotonin reuptake, whereas the R- (-) enaniomer of CIT is pharmacologically inactive (4). CIT is usually administered at daily doses ranging from 20 to 40 mg day^{-1} (and also maximum dose of 60 mg day^{-1}). Orally administered CIT is well absorbed from the gastrointestinal tract. The elimination half-life of CIT is about 33 h. CIT is metabolised by the hepatic cytochrome P450 (CYP) system and thus occur demethylated metabolites such as *N*–demethylcitalopram (d-CIT) and *N,N,*-didemethylcitalopram (dd-CIT) (3).

The structures of S- (+) and R – (-) enantiomers of citalopram are shown in Figure 1.

There are several studies in the literature regarding the determination of citalopram in biological fluids. A few of them report the use of methods that are based on the use of high performance liquid chromatography (HPLC) with fluorescence (2,5-7), UV (7-9), and mass spectrometric (10,11) detectors. Gas chromatography with mass spectrometric detection (GC-MS) was also used (12,13). In the present work we describe a simple and sensitive HPLC method for the therapeutic drug monitoring (TDM) of CIT and d-CIT in human plasma. TDM for CIT and d-CIT and their concentration ratio is necessary in the depression treatment especially in non-responding patients due to drug interactions or other causes which alter metabolism.

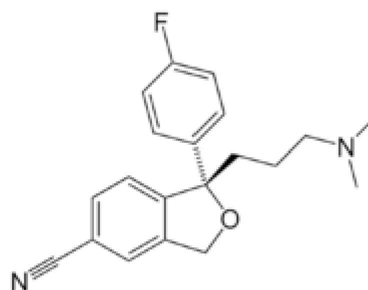
MATERIAL AND METHODS

Chemicals

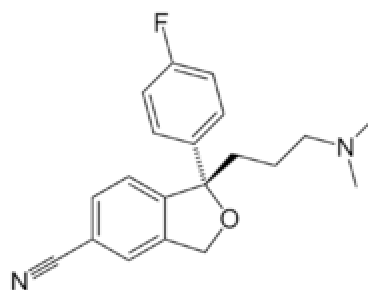
CIT hydro bromide and d-CIT hydrochloride were obtained from Lundbeck (Copenhagen, Denmark). Protriptyline used as internal standard (IS) was purchased in Sigma- Aldrich. Acetonitrile, methanol, and perchloric acid were from Sigma- Aldrich (USA). All other reagents and chemicals were of either HPLC or analytical grade. Ultrapure water was obtained using a MilliQ apparatus by Millipore.

Apparatus and Chromatographic Conditions

As the chromatographic system; an Agilent 1100 HPLC system (Germany) with degasser (G1379A), quatpump (G1311A), auto-sampler (ALS) (G1313A), column oven (G1316A) and an Agilent 1200 series fluorescence detector (FLD) (G1321A), software (Chem Station Rev A 10.2



(a) The structure of S - (+) - Citalopram.



(b) The structure of R -(-) -Citalopram.

Figure 1. The structures of enantiomers of citalopram.

(1757) Copyright® Agilent Technologies 1990-2003) was used. The separation was performed on a INERTSIL ODS3 C18, 250 x 4.6mm, 5 µm particles (Hichrom, UK) column.

The mobile phase consisted of acetonitrile-10 mM potassium dihydrogenphosphate buffer (2:1 v/v), pH of the mobile phase was adjusted to 4.0 ±0.1 with 0.1 N HCl. The flow-rate was 1 ml min^{-1} at 25°C. The fluorescence detector settings were as follows: excitation wavelength 250 nm, emission wavelength 325 nm. Injection volume was 50 µl.

In the isolation of analytes from plasma, solid-phase extraction (SPE) was carried out using C-18 extraction cartridges (50 mg, 1 ml), on a Vac Elut (Supelco) apparatus.

Solutions

Stock solutions of CIT, d-CIT, IS (1 mg ml^{-1}) were prepared in methanol. All the stock solutions were stored in such a way to be protected from light at 4°C. The working solutions were prepared by diluting appropriate volumes of stock solutions with 0,1 N HCl and used to obtain plasma concentrations between 10-150 ng ml^{-1} for CIT and between 5-75 ng ml^{-1} for d-CIT. IS working solutions were prepared by diluting appropriate volumes of stock solutions with 1% potassium bicarbonate, providing finally a plasma concentration of 10 ng ml^{-1} IS.

Plasma obtained

Drug free human plasma was obtained from Ankara University, Medicine Faculty, Blood Center. Plasma was centrifuged at 2000 g for 5 min before stored at –20°C until HPLC analysis.

Extraction procedure for plasma samples (SPE procedure)

The extraction was performed as described previously (2) with minor modification. Plasma was centrifuged at 4000 g for 5 min before used. 0.5 ml of the supernatant was transferred to polypropylene centrifuge tubes including 10 µl of 1000 ng ml^{-1} of IS and added 0.5 ml of acetonitrile. The mixed was vortex for 30 s and was centrifuged at 1500 g for 13 min. The supernatant was applied to a 1.0 ml C18 cartridge which had been previously activated by washing successively once with 1.0 ml of 1 N HCl, twice with 1.0 ml of methanol, and once with 1.0 ml of ultrapure water. The sample was allowed to pass through the cartridge under gravity. The cartridge was successively washed once with 1.0 ml of ultrapure water, once with 1.0 ml of 50% methanol, and once with 1.0 ml of acetonitrile. An aliquot of 0.3 ml of methanol containing 0.5% of 35% of perchloric acid (MPA) was applied to the cartridge. The liquid was then allowed to pass through the cartridge under gravity, and collected in a glass tube. The eluate was evaporated at 65°C, redissolved with 0.25 ml of mobile phase, then a 50 µl aliquot of the eluate was injected into the HPLC system.

RESULTS and DISCUSSION

In the present work, we described the application of HPLC for the determination of CIT and d-CIT in human plasma. CIT emits fluorescence at 325 nm when it is excited at 250 nm. This work has several advantages compared to previously published methods. One of them is that plasma was centrifuged at 2000 g for 5 min before having been stored at –20°C and was centrifuged at 4000 g for 5 min before having been used for HPLC analysis, and this step helps obtain a pure plasma and thus indirectly a cleaner baseline, increasing selectivity. As a second advantage, SPE procedure at 4th and 8th steps was performed under gravity since high vacuum may cause loss of substance. This helps obtain high percentage recovery values. Finally, after eluate was evaporated at 65°C, it was redissolved with 0.25 ml of mobile phase. This enables peaks to be obtained even at very low concentrations and also sharp and big peaks instead of splayed peaks. Thus, peak area can be calculated more accurately (Figure 2). Moreover, with 1 ml min^{-1} of flow rate, analysis time was completed at a short time.

Besides all, system suitability for the proposed method was evaluated. System suitability test results were reported in Table 1. According to Table 1, the results obtained from the system suitability test satisfy the USP requirements.

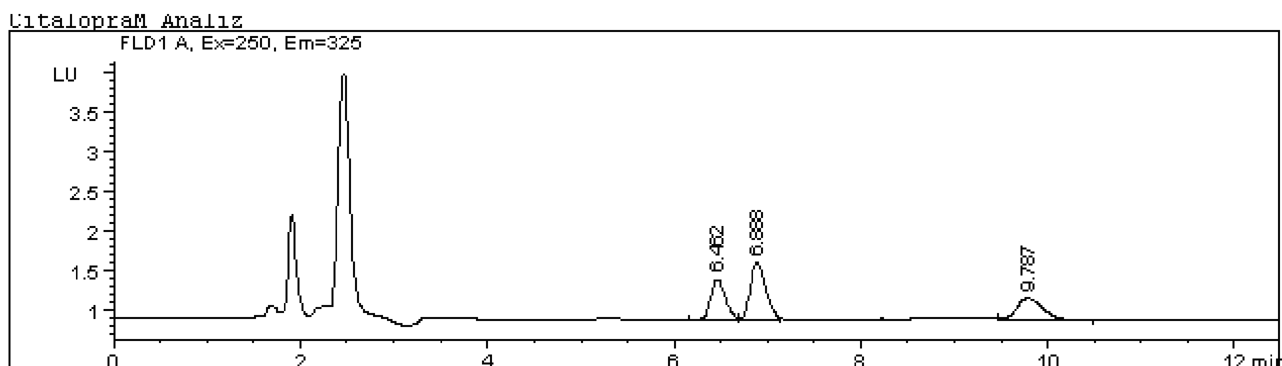


Figure 2. Chromatogram of plasma with d-CIT, CIT and IS (protriptyline).

Table 1. SPE procedure for determination of CIT, d-CIT and IS in human plasma.

Step	Process	Reagent	Times
1	Condition	1 N HCl	1 ml x 1
2	Condition	Methanol	1 ml x 2
3	Condition	Ultrapure water	1 ml x 1
4	Load	800-900 µl plasma (supernatant)	1 ml x 1
5	Washing	Ultrapure water	1 ml x 1
6	Washing	50% methanol	1 ml x 1
7	Washing	Acetonitrile	1 ml x 1
8	Elution	MPA	1 ml x 1

Selectivity

It was observed that there was no CIT, d-CIT, and IS peaks in the drug-free plasma samples and also there were no interfering peaks close to the CIT, d-CIT, and IS retention times.

Linearity

Calibration curves were linear over a working range 10-150 ng ml^{-1} for citalopram and 5-75 ng ml^{-1} for desmethylcitalopram. The determination coefficients (r^2) were found to be 0.9951 for d-CIT ($y = 0.0236x + 0.1066$) and 0.9971 for CIT ($y = 0.0188x + 0.221$). Standart errors (SE) of slope and intercept for d-CIT were $5.84x10^{-4}$, 1.898 10^{-2} respectively, and for CIT were $4.528x10^{-4}$, $3.9.10^{-2}$ respectively.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The plasma samples as containing very low level CIT and d-CIT in the presence of IS were analyzed with 3 replications and the peak area ratio; the following standard deviation (SD) of obtained values were calculated. Therefore, LOD and LOQ were found to be 1.14 and 3.8 ng ml^{-1} for d-CIT and 2.5 and 8.4 ng ml^{-1} for CIT, respectively using the following formulas;

LOD (Limit of Detection) = 3xSD/m

LOQ (Limit of *Quantification*) = 10xSD/m

Repeatability (Precision)

Minimum, median and maximum concentrations were selected to be calculated intra-day and inter-day precision for CIT and d-CIT. Four vials were prepared for each concentration (min, median, max) in the presence of IS and each vial was injected into the HPLC system twice. As result of 6-8 injections, the average area ratio (AAR) was obtained. The results are given in Table (2-5).

Accuracy (Recovery)

In the same way as above, minimum, median and maximum concentrations were selected to be calculated accuracy for CIT and d-CIT (Table 1-4). Three vials were prepared for each concentration (min, median, max) and each vial was injected into the HPLC system twice. Three different concentrations of each compound were calculated from their peak areas using the appropriate regression equation.

Table 2. Summary of intra-day data for HPLC assay of d-CIT

d-CIT	5 ng ml^{-1} (min)		25 ng ml^{-1} (median)		75 ng ml^{-1} (max)	
	AAR*	SD	AAR	SD	AAO	SD
Morning	0.1460	0.0004	0.5016	0.0099	1.5813	0.0161
Evening	0.1440	0.0023	0.5285	0.0165	1.5700	0.0681
Average	0.1450	0.0013	0.5151	0.0132	1.5756	0.0421
RSD%	**0.93**		**2.56**		**2.67**	

*AARs shown in table represent the average result of 6-8 injections.

Table 3. Summary of intra-day data for HPLC assay of CIT

CIT	10 ng ml^{-1} (min)		50 ng ml^{-1} (median)		150 ng ml^{-1} (max)	
	AAR*	SD	AAR	SD	AAR	SD
Morning	0.3076	0.0071	1.5066	0.0273	2.3483	0.0751
Evening	0.3108	0.0128	1.6551	0.0366	2.3380	0.1131
Average	0.3092	0.0099	1.5808	0.0319	2.3431	0.0941
RSD%	**3.20**		**2.02**		**4.01**	

*AARs shown in table represent the average result of 6-8 injections.

Table 4. Summary of inter-day data for HPLC assay of d-CIT

d-CIT	5 ng ml^{-1} (min)		25 ng ml^{-1} (median)		75 ng ml^{-1} (max)	
	AAR*	SD	AAR	SD	AAR	SD
Day 1	0.1469	0.0072	0.4724	0.0329	1.5285	0.0709
Day 2	0.1568	0.0005	0.4784	0.0046	1.4837	0.0711
Day 3	0.1466	0.0072	0.4973	0.0151	1.5637	0.0125
Average	0.1501	0.005	0.4827	0.0175	1.5253	0.0515
RSD%	**3.30**		**3.63**		**3.37**	

*AARs shown in table represent the average result of 6-8 injections.

Table 5. Summary of inter-day data for HPLC assay of CIT

CIT	5 ng ml^{-1} (min)		25 ng ml^{-1} (median)		75 ng ml^{-1} (max)	
	AAR*	SD	AAR	SD	AAR	SD
Day 1	0.3095	0.0052	1.6153	0.0265	2.3602	0.1083
Day 2	0.3154	0.02	1.5980	0.0358	2.4451	0.0689
Day 3	0.3473	0.0111	1.6977	0.0568	2.5225	0.0938
Average	0.3241	0.0121	1.637	0.0397	2.4426	0.0903
RSD%	3.73		2.43		3.69	

*AARs shown in table represent the average result of 6-8 injections.

Table 6. Recovery values of d-CIT and CIT

Compound	Concentration (ng ml-1)	Amount found	Recovery %	Bias %	RSD %
d-CIT	5 (min)	5.15	103	-3	4.87
	25 (median)	25.6	102.4	-2.4	3.65
	75 (max)	77.8	103.7	-3.7	1.64
CIT	10 (min)	10.5	105	-5	6.1
	50 (median)	52	104	-4	3.3
	150 (max)	151.07	100.7	-0.7	1.78

The percentage recovery values obtained are given in Table 6.

CONCLUSION

In this study, rapid, sensitive, and selective HPLC method was developed and validated for the determination of citalopram and its metabolite in human plasma. In the light of the results obtained, it can be concluded that the proposed method is simple, accurate, precise, and can be employed successfully for the routine determination of CIT and d-CIT in patients treated with citalopram.

ACKNOWLEDGMENT

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REFERENCES

1. Macek J, Ptacek P, Klima J. Rapid determination of citalopram in human plasma by high-performance liquid chromatography. *J Chromatogr B*, 755: 279–285, 2001.
2. Meng Q, Gauthier D. Simultaneous analysis of citalopram and desmethylcitalopram by liquid chromatography with fluorescence detection after solid-phase extraction. *Clin Biochem* 38: 282–285, 2005.
3. Pollock BG. Citalopram: a comprehensive review. Expert Opin Pharmacother 2 (4): 681 – 698, 2001.
4. Bezchlibnyk-Butler K, Aleksic I, Kennedy SH. Citalopram – a review of pharmacological and clinical effects. *J Psychiatry Neurosci* 25 (3): 241-254, 2000.
5. Raggi MA, Pucci V, Mandrioli R, Sabbioni C, Fanali S. Determination of recent antidepressant citalopram in human plasma by liquid chromatography-fluorescence detection. *Chromatographia*, 57: 273-278, 2003.
6. Matsui E, Hoshino M, Matsui A, Okahira A. Simultaneous determination of citalopram and its metabolites by high-performance liquid chromatography with column switching and fluorescence detection by direct plasma injection. *J Chromatogr B Biomed Appl* 668: 299-307, 1995.
7. Kristoffersen L, Bugge A, Lundanes E, Slordal L. Simultaneous determination of citalopram, fluoxetine and their metabolites in plasma and whole blood by high-performance liquid chromatography with ultraviolet and fluorescence detection. *J Chromatogr B,* 734: 229 – 246, 1999.
8. Duverneuil C, De La Grandmaison GL, De Mazancourt P, Alvarez JC. A high-performance liquid chromatography method with photodiode-array UV detection for therapeutic drug monitoring of the nontricyclic antidepressant drugs. *Ther Drug Monit* 25: 565-573, 2003.
9. Frahnert C, Rao ML, Grasmader K. Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. *J Chromatogr B* 794: 35-47, 2003.
10. Gutteck U, Rensch KM. Therapeutic drug monitoring of 13 antidepressant and five neuroleptic drugs in serum with liquid chromatography-electrospray ionization mass

spectrometry. *Clin Chem Lab Med* 41: 1571-1579, 2003.
11. Kollroser M, Schober C. An on-line solid phase extraction-liquid chromatography-tandem mass spectrometry method for the analysis of citalopram, fluvoxamine and paroxetine in human plasma. *Chromatographia* 57: 133-138, 2003.
12. Eap CB, Bouchoux G, Amey M, Cochard N, Savary L, Baumann P. Simultaneous determination of human plasma levels of citalopram, paroxetine, sertraline, and their metabolites by gas chromatography-mass spectrometry. *J Chromatogr Sci* 36: 365-371, 1998.
13. Reymond P, Amey M, Souche A, Lambert S, Konrat H, Eap CB, Baumann P. Determination of plasma levels of citalopram and its demethylated and deaminated metabolites by gas chromatography and gas chromatography-mass spectrometry. *J Chromatogr Biomed Appl* 616: 221-228, 1993.