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DETERMINATION OF SERTRALINE AND ITS METABOLITE BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY IN PLASMA

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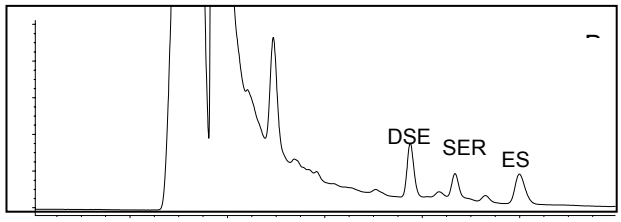
A fast, simple and sensitive high-pressure liquid chromatography (HPLC) method with UV detection was developed for frequently prescribed antidepressant, sertraline (SERT) and its main metabolite *N*-desmethylsertraline (DSERT), in human plasma. SERT and DSERT were extracted by an optimized solid phase chromatographic (SPE) method using C-18 cartridges and Clomipramine was used as external standard (ES). The analytes were separated on C18, 4.6 mm × 150 mm, 5 µm column at 50 °C with a mobile phase of 45% acetonitrile + 55% NaH_2PO_4 at a flow rate of 0.4 mL/min. Detector responses monitored at 4 different wave-lengths; 200-205-210-215 nm. The method proved to be rapid and effective for the plasma sample analyses of therapeutic drug monitoring for sertraline treated patients.



INTRODUCTION

High rates of poor compliance, considerable genetic variability in metabolism, and the clinical heterogeneity of depression are the main problems for the practical application of selective serotonin reuptake inhibitors (SSRI).[1] Therapeutic drug monitoring (TDM) of these agents aims the elimination of such drawbacks of treatment with SSRIs. There are four major clinical rationales for the use of TDM with SSRIs in the management of depression, i.e., achievement of therapeutic ranges, identification of potentially toxic blood concentrations, confirmation of subtherapeutic concentrations in treatment non-responsive patients, and monitoring after overdose. Sertraline hydrochloride (*cis*-(1*S*,4*S*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine) is a widely used SSRI which is administered in treatment of depression, panic disorder, generalised anxiety disorder, and social phobia.[2] Like other SSRIs, it has a wide therapeutic index and seems to be better tolerated than tricyclic antidepressants.[3] The drug is slowly absorbed with a time to peak plasma concentration of approximately 4–8 h and an elimination half life of 22–35 h. Sertraline (SERT) undergoes biotransformation via *N*-demethylation *in vivo* to form the desmethyl metabolite as given Fig. 1 (4). Desmethylsertraline (DSERT) or norsertraline is eliminated slowly (half-life 60–70 h) and contributes limited pharmacological activity.[2] In common with other antidepressants, an exact relationship between sertraline plasma levels and its efficacy in depressive patients has not yet been established. In common with other antidepressants, the role of TDM of sertraline in management of depressive patients are unknown, however, as low blood concentrations are achieved

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following single dose administration, quantification methods of the drug in pharmacokinetic studies need to be sensitive and specific.[3]

Several methods including high-pressure liquid chromatography (HPLC) coupled with UV, fluorescence or tandem mass spectrometry, capillary electrophoresis and gas chromatography (GC) coupled with mass spectrometry have been reported on the analysis of SERT in blood samples.[5-13] Because low blood concentrations are achieved after single-dose administration, methods for quantification of the drug in pharmacokinetic studies must be sensitive and specific.[14] The purpose of this study is to develop a fast and reliable analytical method to determine SERT and its metabolite DSERT in plasma with UV detection for the use of TDM. The method was validated by internal (recovery, linearity, accuracy, precision, interferences) and external quality control (Table 1, 2).

RESULTS AND DISCUSSION

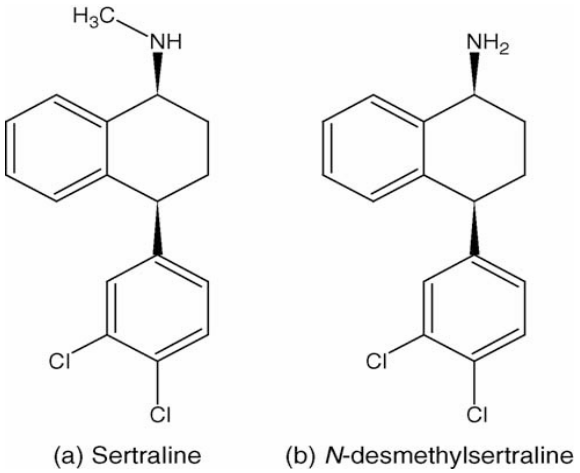
Optimization of the method

First step of optimization was choosing the best stationary and mobile phase selected to develop a chromatographic method for the separation of SERT and its metabolite DSERT. The SERT peaks which were obtained from 15 cm C18 column were closer to the Gaussian shape compared to 25 cm C18 column. Retention time was lower and the separation of SERT peak was also better in C18 15 cm column compared to 25 cm C18 column. Therefore, a C18 reversed phase column 15 cm in length was chosen. Acetonitrile/KH_2PO_4 (0.025M, pH=7) and acetonitrile/NaH_2PO_4 (0.02M, pH=3.8) mobile phases are experimented and preferably better separation of sertraline and its purities were obtained with acetonitrile/NaH_2PO_4 (0.02M, pH=3.8).

The optimization of the HPLC method was carried out by evaluating different chromatographic conditions such as, flow rate (0.3, 0.4, 0.6, 0.8, and 1 mL/min), column temperature (25°C, 40°C, and 50°C) and organic/aqueous solvent percentage (v/v; 60:40, 55:45, 50:50, 45:55, and 40:60). The best chromatographic conditions were identified as follows: C18, 4.6 mm × 150 mm, 5 µm column at 50 °C with a mobile phase of 45% acetonitrile + 55% 0.02M NaH_2PO_4 at a flow rate of 0.4 ml/min. Detector responses monitored at 4 different wavelengths; 200-205-210-215 nm. 200 nm was chosen as detection wavelength for the reasons of maximum absorbance.

Chromatographic separation and selectivity

Typical chromatogram of human blank serum is presented in Fig. 2A. It was observed that there was no SERT, DSERT and ES peaks in the drug-free plasma samples and also there were no interfering peaks close to the SERT, DSERT and ES retention times. In Fig. 2B the chromatogram of human blank serum spiked with SERT, DSERT and ES is presented. Lastly, Fig. 2C show chromatogram of serum sample obtained from a major depression patient who has been taking 50 mg/day sertraline medication. The optimal retention time is defined for each molecule: DSERT (7.7 min), SERT (8.6 min), ES (10.0 min).



(a) Sertraline (b) *N*-desmethylsertraline

Fig. 1 – Chemical structures of (a) sertraline, and (b) *N*-desmethylsertraline or norsertraline.

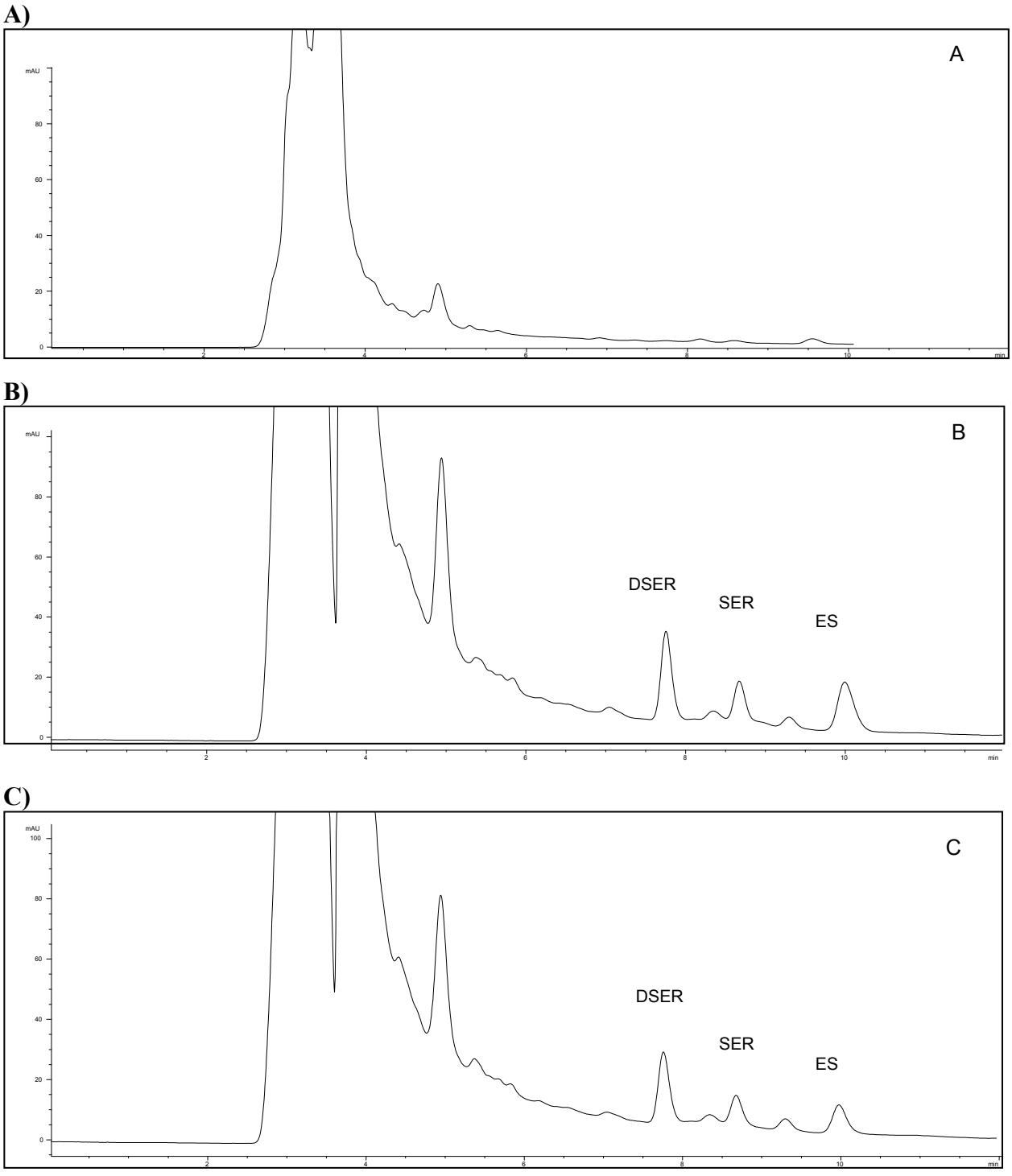


Fig. 2 – HPLC determination of sertraline and its metabolite in human plasma.
(2A) Human blank plasma; (2B) human blank plasma spiked with SERT (40 ng/ml), DSERT (80 ng/mL), and the ES (200 ng/mL); (2C) plasma sample obtained from major depression patient who has 50 mg/day sertraline medication.

Application to patient plasma

To test the applicability of the method to TDM investigations, SERT and DSERT plasma levels of 5 major depression patients receiving 50 mg/day sertraline treatment were determined. None of these samples showed any problem for the quantitation of the analytes, additionally, peak purity showed that no analytical interference was encountered from endogenous substances. The

mean and range of steady-state concentrations of SERT and DSERT were 22.6 ng/mL (18.7-27.2) and 57.8 ng/mL (36.3-77.6) ng/mL, respectively in the patients. The plasma concentrations determined in the patients using the present assay were in the range for recommended reference ranges for TDM.[15] The chromatogram obtained injecting a plasma sample from a patient who was subjected to 50 mg/day sertraline is shown in Fig. 2C.

Validation procedure

The method was validated in terms of specificity/selectivity, Limit of detection (LOD), limit of quantification (LOQ), accuracy [recovery, repeatability (inter day and intraday variations)] and linearity.[16] To examine the possible interferences of endogenous compounds, human serum samples from different volunteers were extracted and analyzed during validation studies. These samples were pretreated according to the sample preparation. The selectivity of the assay was verified by checking a group of drugs such as, fluoxetine, protriptyline, amytriptyline, lercanidipine, trandolapril. The results of the selectivity study showed that there were no interfering peaks from any of the following drugs: fluoxetine, citalopram, amitriptyline, protriptyline, clomipramine. The limit of detection was defined as a peak height that produces three times of the baseline noise and the limit of quantification (LOQ) was estimated as ten times of the baseline noise. The LOD and LOQ for SERT and DSERT were respectively 3.90 ng/mL, 8.78 ng/mL, 4.82 ng/mL, 11.37 ng/mL. Average recoveries of the extraction procedure for sertraline were estimated by comparing the peak areas obtained from of an extracted spiked blank sample with those obtained from the plasma similarly treated with SERT, DSERT and ES. Recoveries were respectively ranged from 82.2-117.4% and 68.8-113.2% of the drug and the metabolite (Table 1).

Intra-day variation was measured by based on the analysis of the same concentration controls in replicates of different controls of 5 analytical runs performed on different times of the same day. Inter-day variation was based on repeated analysis of the same concentration controls in five analytical runs performed on different days. The method was shown to be accurate with intra- and interday % relative standard deviations (RSD %), which were respectively from 3.92 to 6.67% and 2.19 to 3.79% for SERT and were from 2.07 to 4.31% and 1.27 to 3.54% for DSERT (Table 2). Linearity was studied for each compound in the range 10–100 ng/mL for SERT and 20–200 ng/mL for DSERT. Five calibration curves were obtained in one day. Calculating the ratio between the peak area of each compound at the selected wavelength and the peak area of ES at the same wavelength performed quantification of the calibration curve. The calibration curve was linear over the concentration ranges of 10-500 ng/mL for SERT (R^2= 0.9947) and 20-1000 ng/mL for DSERT (R^2= 0.9931).

EXPERIMENTAL

Materials and methods

Sertraline hydrochloride and desmethylsertraline maleate were gifts from Pfizer (USA). Clomipramine was used as an external standard (ES). Methanol and acetonitrile were HPLC-grade; sodium dihydrogenphosphate, potassium dihydrogenphosphate, hydrochloric acid and perchloric acid were of chromatographic purity. Ultrapure water (18.2 MΩ·cm) was produced by means of a Milli-Q apparatus.

Table 1

Accuracy (recovery) data of the method

Compound	Concentration (ng/mL)	Average Amount Found (ng/mL)	% Recovery	BIAS	% RSD
SERT					
	10	11.7442	117.4	-1.74428	4.13
	50	47.4612	95	0.053492	5.48
	100	82.1694	82.17	0.216998	8.53
DSERT					
	20	22.6433	113.2	-0.11674	9.32
	100	93.9645	94	0.064231	5.44
	200	135.5602	67.8	0.475359	1.89

Table 2

Precision (Intra-, Interday) data of the method

	INTRADAY**			INTERDAY***		
	AAR*	SD	% RSD	AAR*	SD	% RSD
DSERT (ng/mL)						
20	0.4534	0.0195	4.31	0.1318	0.0046	3.54
100	1.1518	0.0238	2.07	0.7410	0.0094	1.27
200	2.3977	0.0875	3.65	1.6854	0.0233	1.38
SERT (ng/mL)						
10	0.0862	0.0057	6.67	0.2266	0.0086	3.79
50	0.4091	0.0161	3.92	0.7015	0.0154	2.19
100	1.0522	0.0569	5.41	1.5685	0.036	2.3

* Average result of 5 injections ** Average of Morning and Evening test results *** Average of 4 day test results

Human plasma sampling

The blood samples were collected from 5 major depression patients subjected to 50 mg/day sertraline therapy at least four weeks at Psychiatry Department, School of Medicine, Ankara University. The study was approved by the Ethics Committee of the Ankara University and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions. Blood samples were drawn 12 h after the last drug administration. Blood was stored in glass tubes containing EDTA, then centrifuged at 3000 x g for 10 min; the supernatant (plasma) was transferred to polypropylene tubes and stored frozen at - 20^{o} C until analysis. Plasma SERT and DSERT levels in patients were measured less than three months.

Standards

Working standard solutions of the drug and metabolite were prepared by serial dilution of the stock solution with methanol. Concentrations of methanolic stock solutions of SERT, DSERT and ES (Chlomipramine) were 100 mg/l. For analytical procedures, working solutions were prepared to concentrations of between 1000 and 100 ng/ml for SERT; 2000–200 ng/ml for DSERT. A working standard solution of the ES (5000 ng/ml) was prepared in methanol. These solutions were stable at -20◦ C for at least 3 months.

Extraction procedure for plasma (SPE procedure)

Isolation of SERT and DSERT from drug-free plasma is carried out by solid phase extraction (SPE) method with usage Supelco C18 cartridges (50 mg, 1 mL). Plasma was centrifuged at 3000 g for 10 min before used. A 0.5 mL of the supernatant was transferred to polypropylene centrifuge tube and 0.5 mL of acetonitrile was added. The mixture was vortexed for 30 s and was centrifuged at 1500 g for 13 min. After centrifugation, the supernatant was applied to a 1.0 mL C18 cartridge that has 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 is slowly passed through the cartridge under gravity (by mild suction). 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 0.25 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^{o} C, redissolved with 0.25 mL of mobile phase including 200 ng/mL ES, then a 50 µL aliquot of the eluate was injected into the HPLC system.

Chromatographic apparatus and conditions

The HPLC system used is Agilent 1100 HPLC system (Germany) with degasser (G1379A), quatpump (G1311A), auto-sampler (ALS) (G1313A), column oven (G1316A) and an Agilent 1200 series Diode Array Detector (DAD) (G1321A), software (Chem Station Rev A 10.2 (1757) Copyright. The optimized method was validated on a Agilent ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm, 5 µm column (Agilent Technologies, Waldbronn, Germany). The column is eluted at 50 ^{o}C with a mobile phase of % 45 Acetonitrile + % 55 NaH_2PO_4 at a flow rate of 0.4 ml/min. Four different wavelengths (200-205-210-215 nm) UV detection was applied.

CONCLUSIONS

The present assay describes a rapid, selective and sensitive HPLC method for quantification of SERT and DSERT in human plasma. Application of the validated method was successfully implemented to the analysis of plasma samples from patients administered SERT. The acceptance criteria to fulfill the requirements for acceptable bioanalytical method were: % RSD ≤ 15% for accuracy and an intra- and inter-assay % RSD ≤ 15%, for three concentrations of SERT and DSERT, which is in accordance with US Food and Drug Administration (FDA) and ICH requirements.[15, 17] SPE isolation technique applied in this study has some advantages concerning repeatability, rapidity, environmental suitability and extraction yield over previously reported liquid-liquid extraction methods.[7,13,18,19] For modest laboratories, using

LC/MS, LC/MS/MS or GC/MS is difficult to afford compared to HPLC methods. High initial cost of the equipment may deter institutions on a narrow budget especially in developing countries. Therefore, the method presented in this study can be an alternative in TDM analyzes for these types of research institutions. To conclude, this method was successfully validated and applied to the determination of SERT and DSERT in plasma thus can be suitable for the TDM of SERT and DSERT in plasma of depressed patients.

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