



## High Performance Liquid Chromatography Determination of Sertraline in Human

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### Abstract

An accurate and sensitive reversed-phase high-performance liquid chromatographic method for determination of sertraline in human serum is described using 4-chloro-7-nitrobenzofurazan as pre-column derivatization agent. The drug and an internal standard (azithromycin) were extracted from serum using a mixture of diethyl ether-chloroform and subjected to the pre-column derivatization with the reagent. Analysis of the resulted derivatives was performed on a Lichrosorb CN (250×4.0 mm) column using a mobile phase composed of methanol and sodium phosphate buffer (0.05 M; pH 3.7) containing 2 ml/lit triethylamine (63:37 v/v). Detector response was monitored at excitation and emission wavelengths of 470 and 537 nm, respectively. The calibration curve was linear over the concentration range of 2 to 640 ng/ml. The lower limits of detection and quantification were 0.5 and 2 ng/ml, respectively. The validation of the analysis was carried out in terms of specificity, sensitivity, linearity, precision, accuracy and stability. The validated method was shown to be accurate, with intra-day and inter-day accuracy from 0.3 to 4.2% and precise, with intra-day and inter-day precision from 2.4 to 15.5%. The drug is detected at concentrations as low as 2 ng/ml in a 0.5 ml serum sample and the described method can be easily applied in human single-dose pharmacokinetic studies of sertraline.

**Keywords:** 4-Chloro-7-nitrobenzofurazan; Determination; HPLC; Sertraline.

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### 1. Introduction

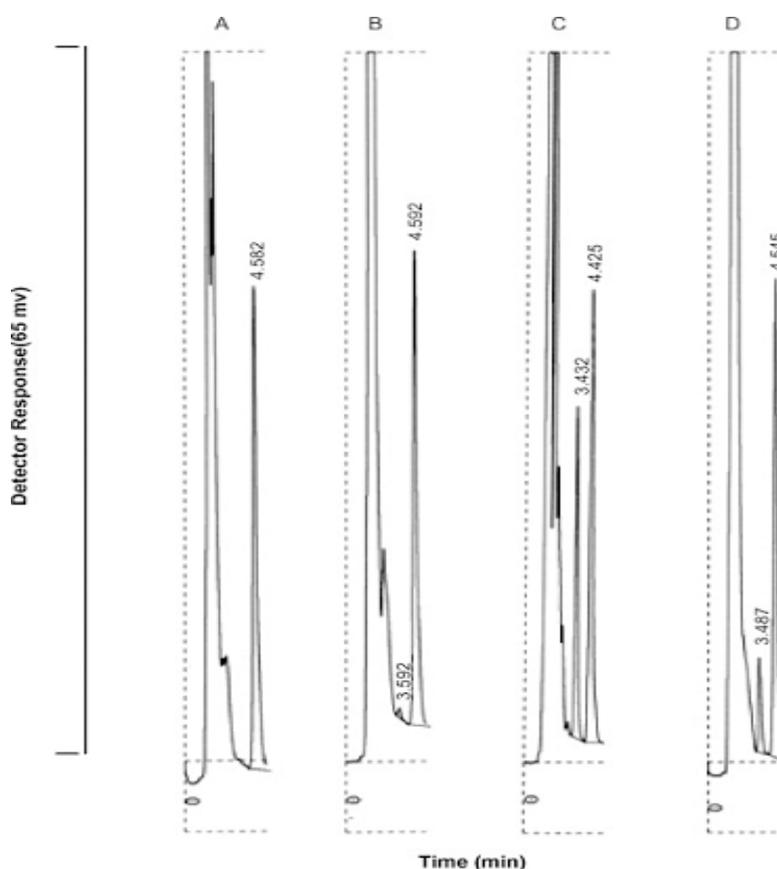
Sertraline hydrochloride [(*cis*-(1*S*,4*S*)-*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine)] is a relatively new selective serotonin reuptake inhibitor

(SSRI) which is administrated in treatment of depression, panic disorder, anxiety and social phobia [1]. Like other SSRIs, it has a wide therapeutic index and seems to be better tolerated comparing to the tricyclic antidepressants [2]. The drug is slowly absorbed with time to peak plasma concentration of about 4-8 h and elimination half life of 22-35 h. Sertraline is *N*-demethylated to norsesertraline

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which eliminated slowly (half-life of 60 to 70 h) and contribute limited pharmacological activity [1]. In common with other antidepressants, the role of therapeutic drug monitoring of sertraline in management of depressive patients is unknown [2], however, as low blood concentrations are achieved following single dose administration, quantification methods of the drug in pharmacokinetic studies need to be sensitive and specific. Several methods including high performance liquid chromatography (HPLC) coupled with UV [3, 4], fluorescence [5] or tandem mass spectrometry [6-9], capillary electrophoresis [10] and gas chromatography (GC) coupled

with mass spectrometry [11] have been reported on the analysis of sertraline in blood samples. This methods, however, are not sensitive enough to measure low drug levels which are obtained in human single dose studies [3-5, 7], or need an expensive instrument which is not available in many laboratories [6-9, 11]. The present study was aimed to develop an accurate and sensitive method to measure sertraline in human serum, using 4-chloro-7-nitrobenzofurazan (NBD-Cl) as labeling agent and test its applicability for analysis of the drug in a healthy volunteers following single dose administration.



**Figure 1.** HPLC determination of sertraline in human.

A) human blank serum containing the I.S.; B and C) human blank serum spiked with the I.S. and the drug at concentrations of 5.5 and 150 ng/ml, respectively; and D) serum sample obtained at 8 h after a single oral dose of 100 mg from a healthy volunteer containing 45 ng/ml of the drug. Peaks eluted at 4.8 and 7.0 min correspond to sertraline and the I.S., respectively.

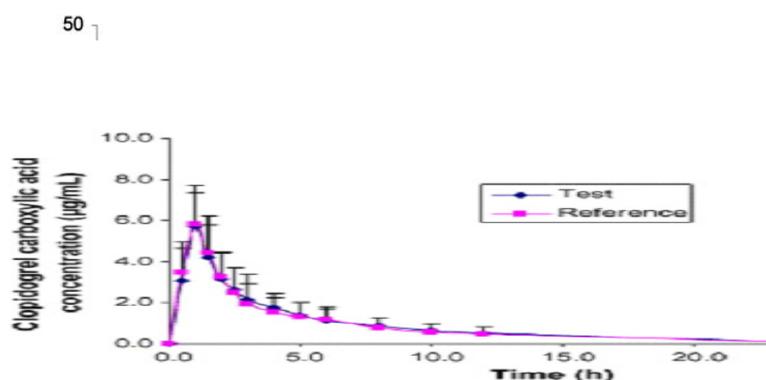
## 2. Material and methods

### 2.1. Chemicals and standard solutions

Sertraline (purity 99.5%) was from Recordati (Milan, Italy) and was kindly provided by Bakhtar Bioshimi Pharmaceutical Company (Kermanshah, Iran). Azithrimycin (internal standard; I.S.) was from Sigma (St. Louis, MO, USA). All reagents used were of analytical grade except methanol which was HPLC grade and were obtained from Merck (Darmstadt, Germany). Water was glass-double distilled and further purified for HPLC with a Maxima purification system (USF ELGA, England). A stock solution of sertraline (1000 µg/ml) was prepared in methanol. Working standards of the drug (0.01 to 6.4 µg/ml) were prepared by serial dilution of the stock solution in methanol. A working standard solution of the I.S. (100 µg/ml) was prepared in acetonitrile. A 10 mg/ml solution of NBD-Cl was prepared in acetonitrile. A phosphate buffer (0.05 M) was prepared in water and adjusting to pH 9.0 with 0.05 M sodium hydroxide solution. All solutions were stored at 4 °C and were stable for at least 3 weeks.

### 2.2. Calibration curve, sample preparation and derivatization

Pooled blank human serum was used for preparation of the calibration curve. After evaporation of 100 µl from each working solutions of the drug, under a gentle stream of nitrogen at 50 °C, the residues were reconstituted in 500 µl of drug-free human serum. An aliquot of 0.5 ml from the blank, calibration or unknown human serum samples was pipetted into disposable glass tubes (100×16 mm). After addition of 100 µl of the I.S., 4 ml of a mixture of chloroform-diethyl ether (1:3 v/v) and mixing for 30 s on a vortex mixer, the samples were centrifuged for 5 min. at 6000 g. The organic phase was then removed and evaporated to dryness under a stream of nitrogen at 50 °C. To the residue, 125 µl of the NBD-Cl solution and 25 µl of the phosphate buffer were added and after mixing for 10 s on a vortex mixer, the samples were kept at 80 °C for 5 min. Alternatively, the mixture can be kept at 60 °C for 20 min. The NBD-Cl derivatives were then analyzed by injection of a 10 µl of the reaction mixture onto the chromatographic column. Calibration curves (weighted regression line) were obtained by linear least-squares regression analysis plotting of peak-area ratio of sertraline to the I.S. versus the drug concentrations.



**Figure 2.** Serum concentrations-time profile of sertraline in a healthy volunteer after administration of a single 100 mg oral dose.

### 2.3. Equipments

The HPLC system used consisted of two pumps of Shimadzu LC-10A solvent delivery system, a system controller (SCL 10AD), a spectrofluorometric detector (RF-551) operated at an excitation and emission wavelengths of 470 and 537 nm, respectively, a column oven (CTO-10A), a degasser (DGU-3A) and a data processor (C-R4A) all from Shimadzu, Kyoto, Japan. The analytical column was a Lichrosorb CN (Hibar RT) (Merck KGaA, 64271 Darmstadt, Germany) 250×4.0 mm ID., 5 µm particle size. A mixture of methanol and sodium phosphate buffer (0.05 M; pH 3.7) containing 2 ml/lit triethylamine (63:37 v/v) was used as the mobile phase. The column oven temperature was set at 58 °C and the mobile phase was filtered, degassed and pumped at a flow rate of 1.4 ml/min.

### 2.4. Optimization of the derivatization conditions

To validate the method and optimize derivatization of the analyte with NBD-Cl, quality control samples were prepared with the drug working solutions to make low (2 ng/ml), medium (50 ng/ml) and high (320 ng/ml) concentrations while, the I.S. was reacted with the reagent at the concentration of 10 µg/ml. Concentrations of the NBD-Cl solutions, ranging from 0.5-15 mg/ml, pH of the buffer solutions ranging from 7 to 11 and pH of the mobile phase ranging from 2.2 to 7, were tested to obtain optimal conditions for analysis. The polarity of the reaction solution was optimized using various organic solvents-water proportions, ranging from 1:1 to 10:1 and the reaction was allowed to proceed in a water bath at temperature ranging from 40-80 °C up to 60 min.

### 2.5. Method validation

The limit of detection was defined as a peak height that produces three times of the baseline noise. The lower limit of quantification (LOQ) was estimated as the

lowest concentration that could be quantified with a coefficient of variation of less than 20%. Average recoveries of the extraction procedure for sertraline and the I.S. were estimated by comparing the peak areas obtained from derivatization of an extracted spiked blank sample with those obtained from derivatization of the similarly treated standard. Inter-day variation was measured by assessing different controls in replicates of six. Intra-day variation was based on repeated analysis of the same concentration controls in ten analytical runs performed on different days. 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 procedure except from the addition of the I.S. The selectivity of the assay was verified by checking for interferences by a group of drugs that might be co-administered with sertraline.

## 3. Results

### 3.1. Chromatographic separation

Typical chromatogram of human blank serum containing the internal standard (azithromycin; I.S.) is presented in Figure 1A. Figures 1B and 1C show chromatograms of human blank serum spiked with the I.S. and the drug at concentrations of 5.5 and 150 ng/ml, respectively. Sertraline and the I.S. were eluted with respective retention times of 4.8 and 7.0 min. Endogenous components and excess of the reagent were chromatographed within 3.0 min. and the peaks of the analytes were well resolved from each other. Figure 1D shows the chromatogram of serum sample obtained at 8 h after a single oral dose of 100 mg of the drug from a healthy volunteer.

### 3.2. Optimization of derivatization reaction

The reaction of NBD-Cl with the drug appeared to be highly dependent on the pH of

**Table 1.** Inter- and intra-day precision and accuracy for determination of sertraline in human serum by the HPLC method. Accuracy has been calculated as a percentage of the nominal concentration.

Known concentration (ng/mL)	Concentration found (mean±S.D)	Coefficient of variation (%)	Accuracy (%mean deviation)
<b>Inter-day</b>			
2	2.08±0.32	15.5	4.2
50	50.81±2.80	5.5	1.7
320	321.12±8.30	2.6	0.3
<b>Intra-day</b>			
2	2.12±0.29	13.5	6.0
50	51.2±2.50	4.9	2.4
320	323.25±7.60	2.4	1.0

buffer solution, time, temperature, concentration of the labeling agent and polarity of the medium. NBD-Cl reacts with sertraline in alkaline medium at 80 °C or 60 °C and the reaction efficiently proceeds within 5 or 20 min., respectively. In our method, maximal yield of the derivative was obtained with a NBD-Cl solution of 10 mg/ml, a buffer with pH of 9.0 and a reaction medium containing the buffer-acetonitrile (1:5 v/v).

### 3.3. Validation of the method

The calibration curves were linear over the concentration ranges of 2 to 640 ng/ml. The correlation coefficients for calibration curves were equal to or better than 0.9981. Intra- and inter-day reproducibility for calibration curves were determined on the same day in replicate (n=6) and on different days (n=6), respectively, using the same pooled serum sample. The intra-day average slope of the fitted straight lines was 0.852±0.04 ng/ml (C.V.=4.7%) and the mean intercept of the calibration curves was 1.75±0.114 (C.V.=6.5%). The corresponding mean±SD coefficient of the linear regression analysis was 0.9985±0.01 (C.V.=1.0%). For calibration curves prepared on different days (n=10), the mean±SD of results were as follows: Slope 0.881±0.05 ng/ml (C.V.=5.7%), coefficient of the linear regression analysis=0.9981±0.011 (C.V.=1.1%) and intercept=1.44±0.135 (C.V.=9.3%). The LOD and LOQ were found to be 0.5 and 2 ng/ml, respectively. The inter- and intra-day accuracy and precision values of the assay method are

presented in Table 1. The coefficient variation values of both inter- and intra-day analysis were less than 15.5% whereas the percentage error was less than 4.2%. The results of the selectivity study showed that there were no interfering peaks from any of the following drugs: Fluvoxamine, citalopram, fluoxetine, norfluoxetine, amitriptyline, nortriptyline, imipramine, desipramine, clomipramine, doxepine, trimipramine, maprotiline, haloperidol, trifluoperazine, phenytoin, phenobarbital, carbamazepine, lamotrigine, zonisamide, topiramate, primidone, vigabatrin, ethosuximide, clonazepam, acetaminophen, codeine and caffeine. Stock solutions of FL and the I.S. were stable for at least 30 days when stored at 4 °C. Derivatized solutions were found to be stable (>95%) for at least 12 h if the samples were kept at room temperature. Stability of the drug was found to be 100% from the initial value, after 60 days maintenance of the serum at -80 °C and following 3 thaw-freeze cycles. The recoveries of sertraline and the I.S. were found to be 100% and 55%, respectively.

### 3.4. Applicability

To test the applicability of the presented method in pharmacokinetic investigations, a pilot study was done in a healthy volunteer. After an overnight fasting the drug (100 mg) was administered to a 32-year-old man. Blood sampling was carried-out at suitable intervals up to 24 h using disposable glass tubes (100×16 mm). Following centrifugation at 4500 g for 10 min., resultant serum was

separated and stored at -40 °C until analysis. Figure 2 shows the serum concentration-time profile of the drug after a single 100 mg oral dose.

#### 4. Discussion

The present study describes a sensitive and rapid method to quantify sertraline in human serum at levels as low as 2 ng/ml and reports its possible application on pharmacokinetic studies. Comparing to other SSRIs (e.g. citalopram, fluoxetine, norfluoxetine, fluvoxamine and paroxetine), lower blood concentrations are obtained following administration of sertraline either in depressive patients or in human pharmacokinetic single dose studies [1]. Consequently to be applied method in bioequivalence studies of sertraline should be sensitive enough to measure the drug concentrations below 10 ng/ml. NBD-Cl is a suitable labeling agent which reacts with both primary and secondary amines and produces stable adducts with absorption maximum at the visible region. Determination of some SSRIs including fluoxetine [12], norfluoxetine [12, 13] and fluvoxamine [14, 15] by HPLC following derivatization with the reagent have been previously reported but to our knowledge the present paper describes the first report of HPLC quantification of sertraline in human serum using NBD-Cl as fluorogenic reagent.

Several LC-MS/MS methods with sensitivities of 10 ng/ml [7], 0.5 ng/ml [6, 9] and 0.1 ng/ml have been published. However, this technique needs an expensive instrument which is not available in many laboratories. Determination of the drug in serum samples using HPLC with UV detection with different sensitivities (7.5 ng/ml [3] and 10ng/ml [4]) have been reported. However, long analytical run times have been reported in these methods. Meanwhile, lower serum concentrations have to be measured to support single dose human pharmacokinetic studies of sertraline. Simultaneous derivatization of four

SSRIs including sertraline using dansyl chloride as fluorogenic agent has been reported [5]. This method also, is not sensitive enough to measure the drug following its single dose administration (LOQ of 10 ng/ml with 150 µl injection). Furthermore using dansyl derivatives is limited by its poor photo stability and band broadening of the resulted peaks.

Our method, in which simple liquid extraction procedure has been used, is sensitive with LOQ of 2 ng/ml and rapid with run time of about 8 min. Chromatographic conditions for derivatization and analysis of sertraline are similar to those reported for other SSRIs [12-15], however, higher concentrations of the reagent and higher percentage of the organic solvent in the mobile phase are required for analysis of sertraline. To select suitable I.S., several agents which react with NBD-Cl were examined, however, due to high percentage of the organic solvent in the mobile phase most of them were eluted at the first part of the chromatogram. Unlike sertraline, retention behavior of azithromycin is significantly affected by pH and concentrations of triethylamine in the mobile phases. Thus by adjusting the pH and using sufficient amounts of the organic modifier in the eluent, azithromycin is retained on the column and eluted after sertraline.

In conclusion, a new sensitive and rapid HPLC method using pre-column derivatization with NBD-Cl and fluorescence detection has been described to determine sertraline in human serum. The new method was applied in a human single dose pilot study.

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