ADHABSTRACT
Purpose: To develop and implement a UHPLC method for quantitative determination of sertraline in biological samples – mostly human blood and urine.
Material&Methods: Blood and urine samples available from Laboratory of analytical toxicology and Clinic for intensive treatment of acute intoxications and toxiocoallergies were used during method validation and case monitoring. Analytical identification of sertraline and/or metabolites was done by GC-MS. Gas chromatography coupled with flame ionization detection was used for alcohols/volatiles screening of clinical samples. Ultra high-performance liquid chromatography system in tandem with diode-array detector has been used as the main quantitative instrument.
Results: After critical consideration of available reference data a carefully set of experimental conditions for sertraline extraction and UHPLC determination were adopted and optimized. Preliminary liquid-phase sample purification was applied. Zorbax Extend-C18 column (150 x 4.6 mm, 5 µm) was used under isocratic conditions with phosphate buffer (pH 2.7; 10 mM) containing 1.5 ml L−1 triethylamine – acetonitrile (65:35, v/v) at 20 oC, at the flow-rate of 1.0 mL/min and UV detection at 220 nm. This method was validated for the determination of sertraline in human plasma/serum samples (70% recovery).
Conclusions: A simple yet sensitive and reliable method for sertraline determination was introduced. Linearity over 20-1000 ng mL−1 range was shown; LOQ was 20 ng mL−1. The method was clinically applied for monitoring the blood sertraline levels during a course of detoxication of a female patient.

Keywords: sertraline, Zoloft, HPLC determination, drug monitoring.

INTRODUCTION
Sertraline (brand names: Zoloft, Setaloft, etc.) is a SSRI-class antidepressant, (1S,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine (Fig. 1). Commercially available after 1991, it is administered in the treatment of depression, panic disorder, anxiety and social phobia [1] as well as in the adjunctive treatment of chronic schizophrenia [2].

Recently, sertraline became a popular psychiatric medication; on the USA retail market, it appears to be one of the most prescribed antidepressants nowadays [3]. In common with others SSRIs it possesses a good therapeutic efficacy similar to that of traditional TCAs; however, it claims better toxicological profile [4]. Adverse effects usually include diarrhoea, nausea, and sexual dysfunction, diarrhoea being the most frequent, compared to other SSRIs, especially when prescribed at higher doses [5]. Acute overdosage is often manifested by emesis, lethargy, ataxia, tachycardia and seizures; however, its toxicity in overdose is considered relatively low [6, 7].

Pharmacokinetics of sertraline includes slow absorption (per os 4-8 h peak plasma concentration time), 13-45 h biological half-life [8], and demethylation to nor-sertraline, its major metabolite of limited pharmacological activity [1] and half-life estimated to 60-70 h. Therapeutic blood concentration has been estimated to 50-250 ng mL−1, toxic – to 290 ng mL−1 and above and coma-tose – to 1600 ng mL−1 and greater [9].

Various methods for quantitative determination of sertraline in human plasma are available. Usually, HPLC methods are reported to give excellent results. Reverse-phase liquid chromatography and ultraviolet [10-14] or fluorescence [15] detection are commonly applied. Using
pre-column derivatization very low concentration levels (LOQ of 2 ng mL$^{-1}$) could be reached [16]. Visible light spectrophotometry [10, 17] as well as GC-MS techniques [18] are also possible.

However, in order to fit both our equipment and needs, we developed a slightly different approach. We set our goal to develop a simple sample preparation and UHPLC determination procedure that is simple, fast and robust, yet specific and sensitive enough to accommodate emergency cases needs and to help therapeutic drug monitoring for sertraline intoxicated patients.

**MATERIALS AND METHODS**

Deionized water (0.067-0.100 µS cm$^{-1}$, TKA™ Pacific water purification system), HPLC grade solvents, and chemicals of analytical grade or better were used. In preparation of spiked samples, we used already available human blood from controlled stationary patients of Naval Hospital – Varna. The UHPLC analysis was done by means of Agilent 1260 Infinity Binary LC featuring Zorbax Extend-C18 column (150 x 4.6 mm, 5 µm) and 1260 Infinity DAD. The Agilent ChemStation package was used for data acquisition and manipulation. Statistical analysis was done using MS Excel™ and OriginPro® software.

**RESULTS AND DISCUSSION**

**Experimental procedure**

Analysis of plasma samples consists of two steps: pre-analytical (sample preparation) and analytical (liquid chromatography). The pre-analytical procedure follows the algorithm described below:

1. Centrifuge blood sample at 4000 rpm for about 10 minutes and/or take 500 µL of blood plasma/serum;
2. Add 500 µL 1 M NaOH;
3. Add 1.5 mL of acetonitrile and vortex mix for 1 minute;
4. Add 3 mL of ethyl acetate, vortex mix for 1 minute, centrifuge at 4000 rpm for 2 minutes and carefully collect the upper (organic) layer. Repeat this step twice and combine organic phases to achieve approximately 5-6 mL total extract.
5. Add approx. 200 mg of anhydrous MgSO$_4$ and vortex mix for 1 minute, centrifuge at 4000 rpm for 1 minute and decant the clear solution, discarding the precipitate;
6. Evaporate to dryness at 40-60°C under nitrogen flow;
7. Reconstitute in 500 µL of the mobile phase, filter through syringe filter (0.22 µm, Nylon) and prepare resulting solution to UHPLC.

Liquid chromatography was done under isocratic conditions; Zorbax Extend-C18 column (150 x 4.6 mm, 5 µm) was used. As a mobile phase we chose phosphate buffer (pH 2.7; 10 mM) containing 1.5 ml L$^{-1}$ triethylamine – acetonitrile (65:35, v/v) at 20°C; flow-rate: 1.0 mL/min; UV detection at 220 nm. Injected sample volume was 20 µL. Retention times were between 5.25 and 5.35 min.

**Method validation**

Because of the stability of UHPLC system and ease of application we decided to follow the method of external calibration. A calibration curve was constructed upon an array of progressive dilutions, made by adding appropriate volumes of mobile phase to aliquots of stock sertraline solution (1 µg mL$^{-1}$). Each successive concentration level was analysed in triplicate, tracking record of instrument responses (peak areas) and retention times. Predefined acceptance criteria were: retention time difference less than 2% and signal-to-noise ratio below 3. Although all of these criteria are successively met even at 20 ng L$^{-1}$, we cancelled further dilution steps, as concentration fell under therapeutic levels and lost toxicological importance. The linearity of the model was demonstrated over 20-1000 ng mL$^{-1}$ region and LOD was established at 20 ng mL$^{-1}$. Analyzing spiked blood samples a 70% recovery was determined. It is worth mentioning that if ethyl acetate extraction procedure is executed only once, recovery is still very good (approx. 65%). The linear fit (Pearson’s $r = 0.9993$) of the calibration curve, along with regression coefficients and 95% prediction band is shown in Fig. 1.

**Clinical case**

A female patient (S. M.) at the age of 36 was admitted to the Clinical toxicology unit after oral abuse with 14 tablets of Zoloft with suicidal purpose. GC-MS screening confirmed sertraline and its metabolites presence in urine samples. The patient was somnolent, elementary contact with periods of psychomotor agitation. Rhythmical tachycardia – 130 bpm, blood pressure – 100/60 mmHg. Due to the central nervous system toxicity and cardiovascular toxicity, monitoring the blood sertraline concentration was mandatory for treatment clarification (Fig. 2). As blood sertraline level was initially determined to correspond to moderate values, the only general procedure has been followed: gastric lavage, forced diuresis, antidote treat-
ment with nootropil. The patient was discharged from the hospital on the 4th day, without having any toxicological problems.

**Fig. 3.** Blood sertraline level monitoring during the course of detoxication

![Blood sertraline level monitoring](image)

**REFERENCES:**


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**DISCUSSION**

As a reference method for positive identification of sertraline in blood samples, a gas-chromatography with mass spectrometry was applied. As no other analytical technique for quantitative comparison for sertraline was available, HPLC analysis of reference material samples was carefully done, showing excellent reproducibility and precision. Clinical case studied confirmed that proposed procedure is applicable for therapeutic drug monitoring.

**CONCLUSIONS**

A simple and precise method for liquid chromatography determination of sertraline (Zoloft) in blood samples has been adopted and validated. It has been demonstrated that chosen approach combines advantages of known techniques with the simplicity of use, matching the equipment currently available in Laboratory of Analytical Toxicology at Naval Hospital - Varna. Developed method gives excellent results in short time frame (total time of single analysis under 30 minutes), yet sensitivity is high enough to make monitoring of therapeutic concentrations possible.

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