Forensic Science International 215 (2012) 105–109

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Determination of venlafaxine in post-mortem whole blood by HS-SPME and GC-NPD☆

O. Mastrogianni [a], G. Theodoridis [b], K. Spagou [a], D. Violante [a], T. Henriques [a], A. Pouliopoulos [a], K. Psaroulis [a], H. Tsoukali [a], N. Raikos [a,*]

[a] Greece Laboratory of Forensic Medicine and Toxicology, Faculty of Medicine, Aristotle University, Thessaloniki, Greece
[b] Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University, Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 15 April 2011
Accepted 8 May 2011
Available online 8 June 2011

Keywords:
Venlafaxine
Post-mortem blood
HS-SPME
GC-NPD

ABSTRACT

Venlafaxine is a phenethylamine derivative widely prescribed for the treatment of depression which inhibits both serotonin and norepinephrine reuptake (SNRI). In treatment with antidepressants of patient with depression and other psychiatric disorders there is also increased risk of suicidal thought and behaviour. Several lethal intoxications involving venlafaxine usually among psychotic patients have been reported in the literature. Sample preparation is of the greatest significance for a successful toxicological analysis. The development of simple, effective and rapid extraction procedures of drugs from post-mortem biological samples is a challenge. Headspace-solid phase microextraction (HS-SPME) offers significant advantages such as simplicity, low cost, compatibility with analytical systems, automation and solvent-free extraction. The aim of our work was the optimization of a HS-SPME procedure for the determination of venlafaxine in post-mortem biological samples by gas chromatography (GC) with nitrogen-phosphorous detection (NPD). Venlafaxine was extracted on 100 μm Polydimethylsiloxone Coating-Red (PDMS) SPME fiber and determined by GC-NPD. Salt addition, extraction temperature, preheating and extraction time were optimized to enhance the recovery of the extraction from aqueous solution spiked with venlafaxine.

Finally the developed procedure was applied to post-mortem biological samples of a fatally poisoned woman by venlafaxine. The drug was quantified in post-mortem blood gastric and oesophagus contents of the deceased woman.

A simple and rapid procedure using HS-SPME was developed for sample preparation of venlafaxine in post-mortem biological samples prior to GC-NPD determination. Validation data was satisfactory, thus enabling application in the toxicological analysis of forensic samples.

1. Introduction

Venlafaxine is a phenethylamine derivative widely prescribed for the treatment of depression and its mechanism of action is based on the inhibition of the re-uptake of serotonin and noradrenaline (SNRI). Despite of the pharmacological similarity with selective serotonin re-uptake inhibitors (SSRI), venlafaxine's structure is very distinct. Venlafaxine's efficacy is comparable with tricyclic anti-depressive, however the SNRI has less adverse effects [1]. That is the reason its prescription has been increased the last years. Usual doses range from 75 to 375 mg/day [2]. The symptoms of its overdose may include central nervous system depression, cardiac arrhythmias, hypertension or hypotension, serotonin syndrome, coma and death [2–4]. Buckley and McManus [5] found that venlafaxine has fatal toxicity higher than that of other serotonergic drugs but comparable to that of the less toxic tricyclic antidepressants and proposed "clinicians need to consider whether factors in their patients reduce or compensate for this risk before prescribing venlafaxine". In patients (children or adults) with depression and other psychiatric disorders there is an increased risk of suicidal thought. This risk has been related to antidepressant's half-life [6] (venlafaxine's half life is 3.9 h [7]). Several lethal intoxications involving venlafaxine (usually among psychotic patients) have been reported in which case toxicological analysis should be performed to clarify the cause of death. Prior to toxicological analysis the step of sample preparation remains is crucial.

The development of simple, effective, and quick extraction of the drug from post-mortem biological samples is a challenge. In

☆ 48th Annual Meeting of the International Association of Forensic Toxicologists (TIAFT). Joint meeting with the society of Toxicological and Forensic Chemistry (GTFCh).

* Corresponding author. Tel.: +30 2310 999206; fax: +30 2310 999236.
E-mail address: raikos@med.auth.gr (N. Raikos).

0379-0738/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2011.05.008

intoxications, fatal or not, the determination of the drug concentrations in blood is vital. In fatal cases plasma or serum cannot be acquired from post-mortem blood, thus whole blood has to be analyzed. A useful alternative in this aspect is the utilisation of solid phase microextraction (SPME) [8–15]. This solvent-free sample preparation technique combines extraction, preconcentration, and sample introduction in a single step. SPME has been applied successfully in environmental, pharmaceutical, food and toxicological analysis. Headspace-SPME (HS-SPME) provides a powerful alternative for the sampling and pre-treatment of various biological samples such as urine, whole blood, plasma, oral fluids and hair. SPME has been applied for the determination of serotonin reuptake inhibitors in environmental water and in urine [16–18] and for the determination fluoxetine and norfluoxetine in plasma (direct immersion SPME) [19] and hair (HS-SPME [20]). To the best of our knowledge there are no references on the utilisation of SPME for the analysis of venlafaxine (or other SNRIs and SSRIs) from post-mortem blood.

The aim of this work was the optimization and development of a HS-SPME procedure for the determination of venlafaxine in whole blood by gas chromatography (GC) with nitrogen-phosphorous detection (NPD) and the application of the developed procedure to post-mortem samples of a fatally poisoned woman.

2. Materials and methods

2.1. Reagents

All the reagents used were of analytical grade: methanol, potassium carbonate (K_2CO_3), sodium bicarbonate ($NaHCO_3$) (Merck, Darmstadt, Germany) and hydrochloric acid (HCl) and sodium hydroxide (NaOH) (Panreac Quimica, SA, Barcelona, Spain). Venlafaxine was purchased from LGC Standards GmbH, Germany. The volume of the clear glass vials is 9 ml. They were sealed with silicone septa and aluminum caps (Altech, Deerfield, IL, USA).

The thermostatic water bath was a Tembloc appliance. SPME devices and 100 μm Polydimethylsiloxone Coating-Red (PDMS) and 85 μm Polyacrylate Coating-White (PA) assemblies were purchased from Supelco (Bellefonte, PA, USA). The calibrated pH-meter was a Cyberscan 1000.

2.2. Toxicological analysis

Blood sample was analyzed for alcohol and tricyclic antidepressants (TCAs) by fluorescence polarization immunoassay (FPIA) on an Abbott AXSYM™ system and after liquid–liquid extraction [15] for cannabinoids, opiates, cocaine, barbiturates, benzodiazepines and amphetamines by enzyme multiplied immunoassay technique (EMIT) on a RxL Dimension Siemens, ex-Dade Behring, system. Blood, stomach and oesophagus contents samples were analyzed by thin layer chromatography (TLC) after liquid–liquid extraction for common neutral, acidic and basic drugs. The identification and quantification of venlafaxine was achieved by TLC and gas chromatography in combination with a nitrogen phosphorus detector (NPD) after HS-SPME.

2.3. Liquid–liquid extraction

Two milliliters of the biological sample, 5 ml of ethyl acetate and 0.5 ml sodium hydroxide (NaOH, 1 M) were added in a centrifuge tube (10 ml). The mixture was vortexed for 5 min and centrifuged in high speed for 10 min. The organic layer was evaporated to dryness at room temperature and the dry residue was resuspended in 0.2 ml of methanol.

2.4. TLC

TLC was performed on TLC aluminum sheets covered with silica gel 60 (20 cm × 20 cm, 0.2 mm layer thickness) purchased from Merck (Darmstadt, Germany). A mixture of methanol–28% aqueous ammonia (98:2, v/v) was used as the development mixture of solvents. Visualization of the venlafaxine spots was achieved by spraying with acidified iodoplatinate reagent.

2.5. Sample preparation of post-mortem biological samples for HS-SPME

Prior to SPME, biological samples (stomach tissue and content of stomach and oesophagus) were treated as follows: 1 g of biological sample was homogenized along with 2 ml of water using a high speed blender. 100 μl of perchloric acid was added to 0.5 ml of tissue homogenate and the mixture was stirred vigorously. After centrifugation, the supernatant was transferred to the SPME vial. The final volume was adjusted to 3 ml by the addition of Millipore water and the vial was closed and thoroughly shaken in a vortex. The resulting solution was subjected to extraction as described above. 0.5 ml of post-mortem blood were prepared and analyzed with the above described procedure. The chromatograms of post-mortem biological samples obtained from the deceased woman (esophagus and stomach samples were appropriately diluted with distilled water) prior to HS-SPME–GC-NPD analysis.

2.6. SPME procedure

For SPME, 3 ml of an aqueous solution of venlafaxine was transferred into a headspace vial (9 ml volume) and a certain amount of salt was added. The vial was next capped tightly, vortexed vigorously and placed for heating in an aluminum block heater. After a certain pre-heating time, the SPME needle pierced the vial septum in order to expose the fiber in the headspace of the solution. Sampling was performed for a certain time and finally the needle was removed from the vial and inserted into the heated injection port of the gas chromatograph for the desorption step for 2 min.

2.7. Gas chromatography

Initial experiments were conducted to optimize the chromatographic temperature program, and thus, to achieve an adequate resolution of venlafaxine. The final chromatographic conditions selected are presented here: gas chromatographic analysis was performed on a Thermo Finnigan Trace GC with a nitrogen phosphorus detector (Thermoquest Italia, Rodano, Italy). Separations were accomplished on an Alltech EC-5 (30 m × 0.32 mm, 0.25 μm) column. The oven temperature was held at 150 °C for 1 min and then increased to 250 °C at a rate of 15 °C/min, where the temperature was held for 2 min; finally the temperature achieved to 280 °C at a rate of 40 °C/min. The temperatures of the injector port and the detector were set at 230 °C and 300 °C, respectively. Splitless injection mode was used. Helium was used as the carrier gas at a flow rate of 2 ml/min. GC–MS analysis was performed using an Agilent Technologies 7890A gas chromatography with a MS 5975C inrtXL, EI/CI MSD with Triple-Axis detector. Separations were performed on a 30 m Optima-5-MS capillary column, with a film thickness of 0.25 μm and an i.d. of 0.25 μm. The mass spectrometer was operated with electron energy of 70 eV in the electron impact (EI) mode. Detection was done on selected ion monitoring (SIM) mode at 58 and 134 *m/z*.

3. Results and discussion

3.1. SPME optimization

A systematic optimization of the SPME experimental procedure was deemed necessary to achieve higher yields and enhance method sensitivity. Parameters including fiber type, temperature, salt addition, pre-heating and extraction time were step-by-step studied and optimized. For SPME optimization the following experimental procedure was used: 3 ml of an aqueous solution of venlafaxine (1 μg/ml) was transferred into a headspace vial (9 ml volume) and salt was added. The vial was pre-heated in an aluminum block heater at 100 °C for 15 min. Next the SPME fiber was exposed in the headspace of the solution for 15 min and was next inserted into the GC injection port for the desorption step (2 min).

The first parameter studied was the type of fiber. Preliminary experiments revealed much higher signals when using the PDMS fiber compared to the PA fiber. Also, PDMS is a more resistant fiber to high injector temperatures [21]. Hence, PDMS was selected for the study. Salt addition can improve the extraction yield due to the salting out effect. The salts studied in the present work were: K_2CO_3 [22] and $NaHCO_3$ [23]. An amount of 0.3 g of salt was added in the sample and SPME–GC analysis was performed. Addition of K_2CO_3 resulted in higher peak areas and hence to the higher recoveries. This could be well explained taking into account the basic character of the analyte molecules [24,21]. Addition of K_2CO_3 increased the pH of the aqueous solution to 11.55 while addition of $NaHCO_3$ only to 8.14. In higher pH analyte molecules exist in their non-protonated form as basic molecules and cannot participate in ionic interactions. Thus they are more volatile and exhibit higher partition to the fiber. With the addition of 5 M HCl, the pH of the aqueous solution was adjusted to 9.51 and 10.17, respectively. The decrease of pH from 11.55 to 10.17 resulted in a not significant