International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 801-804

Received: 25-05-2018
Accepted: 26-06-2018

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A simple method for quantitative determination of venlafaxine in blood by high-performance liquid chromatography

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Abstract
Although venlafaxine is generally considered as a relatively safe antidepressant, in unfavorable circumstances it could lead to unexpectedly severe intoxications. Examples are intentional over dosage cases, especially when combined with other psychotropic medications. That is why the main purpose of this work was to develop and optimize an express yet precise method for blood venlafaxine determination that will be a valuable asset to analytical toxicology lab at any emergency toxicology department. The developed method uses RP-HPLC on C18 column (150×4.6 mm, 5 µm) under isocratic conditions at 25 °C. As a mobile phase a 22:78 v/v acetonitrile: phosphate buffer (pH 5.2) mixture at flow rate 1.0 mL/min has been chosen. Fluorescent detection (ex. 228 nm; em. 300 nm) has proven to give best results. Validation of the method in human plasma has shown an 86% yield, a good linearity (Pearson's $r > 0.9997$) over a wide concentration range (0.01 – 10.0 µg mL^{-1}), and an excellent precision (RSD 0.60%). LOQ for venlafaxine has been estimated to 10 ng mL^{-1}. The method has been used for treatment monitoring in a clinical case of combined acute intoxication (venlafaxine and valproic acid). Severance of manifested toxic symptoms, not in correspondence to medications' blood levels, has been pointed out. Drug interaction has been assumed as a possible explanation for mutual toxicity escalation.

Keywords: HPLC, venlafaxine, valproic acid, polyfarmacy intoxication

Introduction
Venlafaxine, sold under the trade names Effexor, Velaxin, Dalium and others [1], is a SNRI class antidepressant of phenyl cyclohexyl ethylamine family [2, 3] and is not structurally related to to any of the conventional antidepressant drugs (Fig. 1). It is one of the most commonly prescribed antidepressant, used in treatment of major depressive disorder, generalized anxiety disorder, panic disorder, and social phobia [4], especially for patients unresponsive to antidepressants of adjacent classes (e.g. SSRI and TCA) [5].

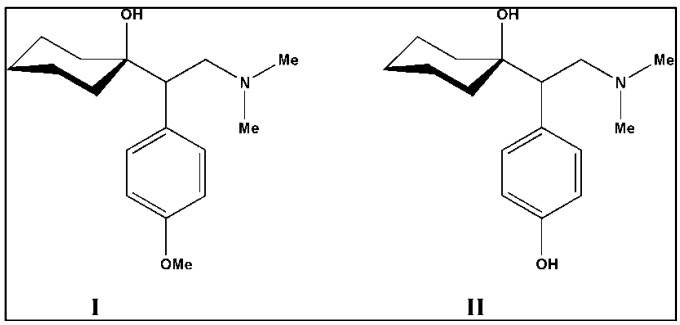

Fig 1: Venlafaxine (I) and its major (active) metabolite, *O*-desmethylvenlafaxine (II)

Reference values for plasma venlafaxine levels are as follows: therapeutic 0.2-0.4 µg mL^{-1}, toxic 1.0-1.5 µg mL^{-1} and comatose above 6.6 µg mL^{-1} [6]. Venlafaxine overdosing is generally associated with high survivability rate even in comatose concentrations, however fatalities are reported as well [7]. There have been reports for an increase in risk of suicide behavior, associated with venlafaxine treatment course [5]. Such a behavior often leads to suicide attempts involving acute self intoxication by combination of venlafaxine with other psychotropic drugs [8] that results in surprisingly severe clinical picture. Drug interaction

between venlafaxine and seizure threshold lowering drugs (such as bupropione and tramadol) has been reported [9]. Coadministration with monoamine oxidase inhibitors should also be avoided, as it can cause serotonin syndrome with fatal outcome [10]. A combination of valproate with venlafaxine requires a cautious dosing and therapeutic drug monitoring [11]. Determination of venlafaxine in biological samples are usually done by HPLC, applying UV [12-15] or fluorescent [16-18] detection, although liquid chromatography in tandem with mass spectrometry has been also reported to give excellent results [19, 20].

Materials and methods

Only analytical or HPLC grade reagents/solvents as well as purified deionized water (0.067-0.100 μS cm^{-1}, TKA™ Pacific water purification system) were used. Agilent Technologies 7890B GC System & 5977A MSD module were used for GC-MS analysis. Agilent 1260 Infinity Binary LC with Zorbax Extend-C18 column (150 x 4.6 mm, 5 μm) and 1260 Infinity DAD/FLD were used for UHPLC analysis. Statistical analysis was done by means of OriginPro® software. Blood samples, taken from a female patient (54) of Clinic for Intensive Treatment of Acute Intoxications and Toxicoallergies, Naval Hospital – Varna, Bulgaria, were object of clinical study.

Results and discussion

Experimental procedure

Qualitative identification of all organic compounds of interest was done by means of GC-MS after liùid-ohase extraction by ethyl acetate (pH >10). Venlafaxine was confirmed at R_t = 23.70 min, with mass spectrum (EI, 70 eV), *m/z*: 58, 134, 91, 119, 179, and (in a clinical case only) valproic acid at R_t = 10.18 min, with mass spectrum (EI, 70 eV), *m/z*: 73, 102, 57.

For HPLC quantitative determination 500 μL of blood serum/plasma is taken. Procedure begins with alkaline liquid-phase extraction by ethyl acetate (2 × 2 mL). Next step are evaporation, reconstitution in 500 μL of mobile phase, and syringe filtering (0.22 μm, Nylon), consequently. UHPLC is done under isocratic conditions. Mobile phase consists of phosphate buffer (pH 5.2; 10 mM) containing 1.5 ml L^{-1} triethylamine – acetonitrile (78:22, v/v) at 25ºC; flow-rate: 1.0 mL/min; sample volume – 20 μL. It is shown that detection could be done by means of UV (DAD, 226 nm); however fluorescent mode (excitation at 228 nm and emission at 300 nm) gives a way better sensitivity. Retention time was approx. 5.4 min.

Method validation

The method of external standard has been applied for calibration.A series of progressive dilutions was prepared to match concentrations of toxicological interest, that is, from 0.01 to 10 μg mL^{-1}. Linearity (Pearson's r > 0.9997), intra-day precision (RSD of 0.6%, n=8), recovery (99.3% at 10.0 μg mL^{-1}, 101,0% at 1.0 μg mL^{-1} and 100.3% at 0.1 μg mL^{-1}, respectively), and reproducibility (RSD of 1.7% over one week period) were evaluated (Fig. 2). LOQ was estimated to be 10 ng mL^{-1}. Analytical yield of 86% was determined.

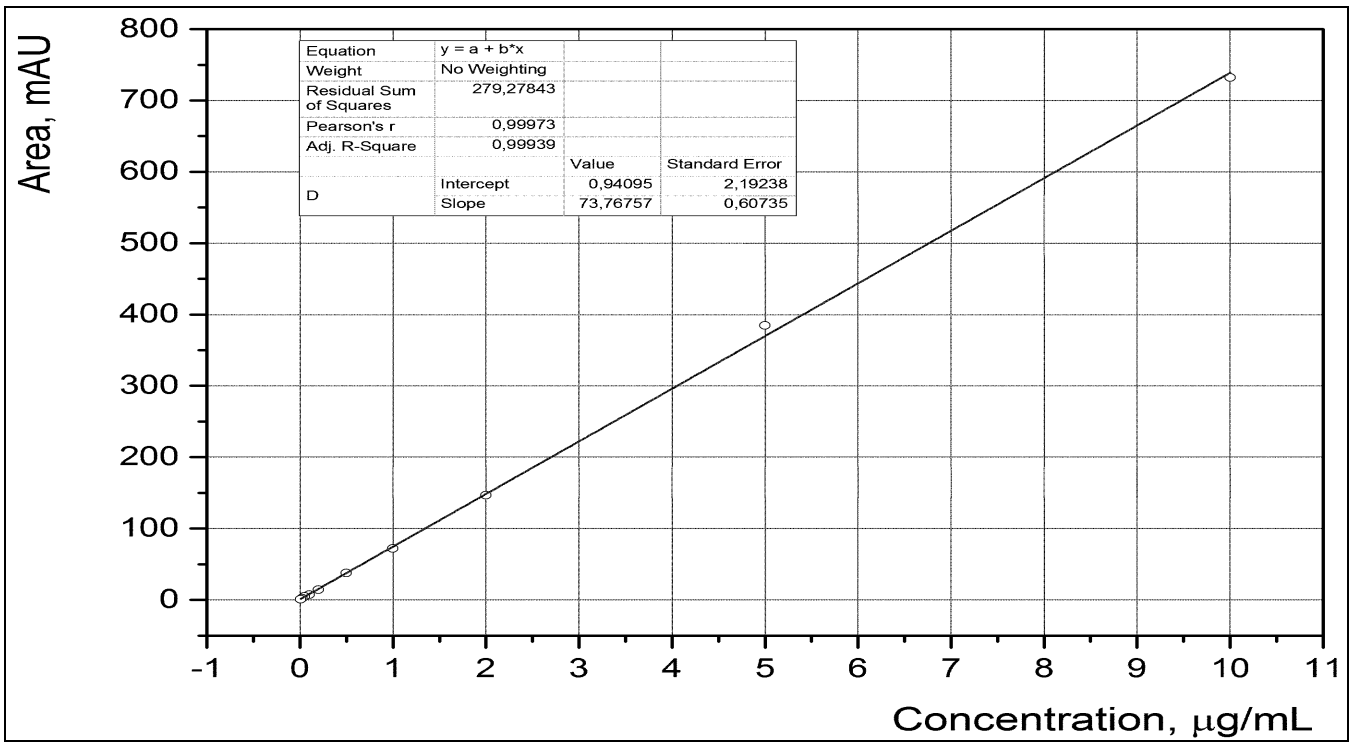

Fig 2: HPLC Venlafaxine determination: calibration curve and corresponding linear fit

Clinical case

Y.S.M., a female patient (54), was admitted at the Clinic of Toxicology on 12.03.2018. By information, given by her relatives, an unknown quantity of Seroquel (quetiapine) tablets were taken approximately one hour earlier in suicidal attempt. The patient suffered from depressive disorder.

Physical examination at admittance: moderately grave general state, unresponsive, passive body stature. Coma I stage. Astenic habitus, cachexia III degree. Pupils with normal size, equal in size, slightly mydriatic; normal reaction to light. Vesicular breathing with normal breath sounds. Rhythmic heart beat, 104/min, arterial blood pressure 40/0 mmHg. Abdomen – soft.

Treatment (detox – depurative and symptomatic): stomach lavage, intravenous infusions of electrolyte, Kabiven i.v., Pyracetam (Nootropil), vitamin B1 and B6, Dopamine.

Analytical toxicology results: GC-MS screening of urine – caffeine, valproic acid, venlafaxine (and metabolites). Both venlafaxine and valproate blood levels were monitored by HPLC during the whole treatment course – as shown on Fig. 3.

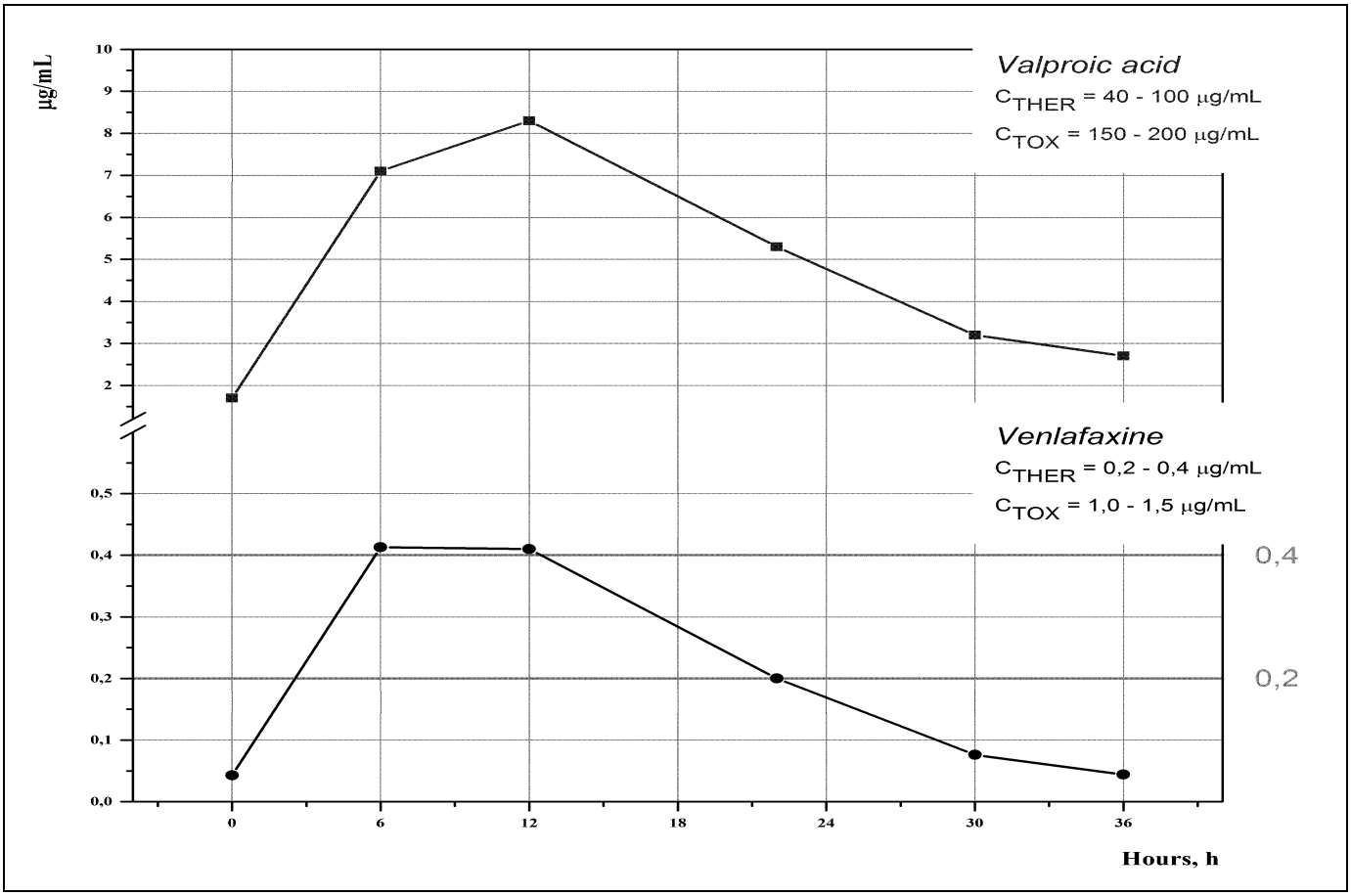

Fig 3: Side by side comparison of blood venlafaxine and valproate levels during the treatment course

Discussion

Analytical toxicology report gives a very low initial blood levels (immediately after admittance in hospital) of identified substances – 43 ng mL^{-1} and 1.7 μg mL^{-1} for venlafaxine and valproic acid, respectively. Although both concentrations were way below and never reached toxic levels, they showed a synchronized rapid growth within first hours, even though a gastric lavage was promptly performed. That points out a recent concomitant abuse, most probably within 1-2 hours before hospitalization. As the subject's body weight was drastically under normal and in heavily damaged condition, reference values for medicines blood content are not particularly applicable. Yet it is clearly shown that even below therapeutic levels venlafaxine and valproate, taken in combination, can produce an unexpectedly heavy clinical symptoms.

Clinical outcome: During the treatment the patient has shown a positive response and was discharged on the 3th day without toxicological problems.

Conclusion

A rapid and precise method for venlafaxin determination in biological fluids was developed. The method has been applied in a clinical case of concomitant drug abuse. Attention has been drawn on the possibility of drug interaction between venlafaxin and valproic acid, which may lead to unexpected aggravation of clinical picture even at low dose.

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