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UHPLC analysis of trazodone in biological fluids

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Abstract
A simple and fast, yet effective and precise UHPLC method for qualitative determination of an antidepressant medication trazodone in biological samples has been developed. Both UV (DAD, 254 nm) and fluorescence (Ex. 320, Em. 440 nm) detection modes are determined applicable. Excellent linearity (r > 0.9999) over sufficiently wide concentration range (from 0.15 up to 15 µg mL^{-1}) covering therapeutic, toxic, and comatose blood levels has been demonstrated. High precision (RSD < 1.6%), good recovery (85%) and low detection limits (LOQ 150 ng mL^{-1}) make this relatively rapid method (ca. 30 min for a single run) a suitable asset in identification of acute intoxications, diagnosis refinement and treatment monitoring. The applicability and importance of newly developed method are demonstrated by a clinical case of Trittico self-poisoning and a corresponding treatment course.

Keywords: Trittico, Desyrel, trazodone, overdose, self-poisoning, acute intoxication

Introduction
Trazodone (under the trade name Trittico® in Europe and Desyrel® in USA, among many others worldwide) is a second-generation SARI-class antidepressant of phenylpiperazine type. Chemically, it is 2-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-1, 2, 4-triazolo[4, 3-*a*]pyridin-3(2*H*)-one (Fig. 1). Commonly, trazodone is used in treatment of major depressive disorder [1, 2], anxiety disorder [3] and insomnia [4], although there are positive reports for its application in cases of schizophrenia [5], OCD [6] and alcohol dependence [7, 8]. For analytical toxicology, some important reference blood trazodone concentrations are as follows: therapeutic – 0.8-1.6 µg mL^{-1}; toxic – above 4.0 µg mL^{-1}, and comatose (fatal) – 12.0 µg mL^{-1} and higher [9]. An in-depth review on trazodone's pharmacology can be found elsewhere [10].

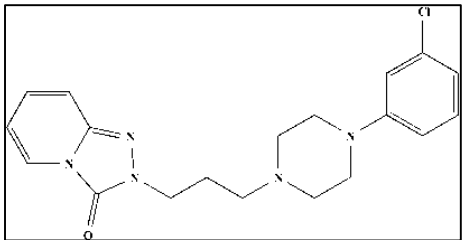

Fig 1: Chemical structrure of trazodone – a phenylpiperazine type antidepressant

Although trazodone is considered safer than TCAs and MAOIs [11] and over dosage is reported to be with good prognosis and low lethality in controlled conditions, combined acute intoxications with other psychotherapeutic agents (usually in cases of suicide attempts) are potentially fatal [12, 13]. Thus, availability of a rapid and precise method for blood trazodone level determination is important in evaluation of detoxification procedures (in cases of acute intoxications) as well as in therapeutic drug monitoring (during the course of treatment).
A modern approach to determination trazodone in biological samples is represented mostly by chromatographic techniques. Identification is usually done by GC-MS for major metabolite 1-(3-chlorophenyl) piperazine (mCPP) [14]. Quantitation adopts HPLC separation combined with UV [15], fluorescence [16], or electrochemical [17] detection, or in tandem with mass-spectrometry [18]. As for clinical use we praise swiftness and reliability over sensitivity and precision, we needed to combine advantages of known techniques and optimize performance for requirements of our Department of Analytical toxicology.

Material and Methods

A series of patients (October 2017 – February 2018) of Clinic for Intensive Treatment of Acute Intoxications and Toxicoallergies, Naval Hospital – Varna, Bulgaria, have been subjects of our study.

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. 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/FLD. The Agilent Chem Station package was used for data acquisition and manipulation. Statistical analysis was done using OriginPro® software.

Pre-analytical procedure begins with 500 µL of initial sample, utilizes alkaline liquid-phase extraction by 3 mL ethyl acetate, followed by evaporation and reconstitution in 500 µL of mobile phase, followed by syringe filtering (0.22 µm, Nylon). Liquid chromatography was done under isocratic conditions with mobile phase consisting of phosphate buffer (pH 2.7; 10 mM) containing 1.5 ml L^{-1} triethylamine – acetonitrile (65:35, v/v) at 20ºC; flow-rate: 1.0 mL/min. Injected sample volume was 20 µL. Detection was done by both UV (diode-array detector, 254 nm) and FLD (Ex. 320 nm, Em. 440 nm). Retention time under described conditions was approx. 4.7 min.

Results and Discussion

Method Validation

The method of external calibration was used for calibration. Calibration curve was constructed upon an array of progressive dilutions, made by adding appropriate volumes of mobile phase to aliquots of stock trazodone solution (15.0 µg mL^{-1}). Each successive concentration level was analyzed in triplicate, tracking record of instrument responses (peak areas) and retention times. Predefined acceptance criteria were: retention time difference less than 1% *and* signal-to-noise ratio below 3. Although all of these criteria are successively met even at 0.15 µg L^{-1}, further dilution steps were cancelled, as concentration fell well under therapeutic level (0.8 µg mL^{-1}) and lost toxicological importance. Linearity of the model was demonstrated over 0.15-15.0 µg mL^{-1} region and LOQ was established at 0.15 µg mL^{-1}. Analyzing spiked blood samples recovery was determined to be 85%. Excellent inter-day precision (RSD of 1.4% and 1.6%) and linearity (Pearson's r = 0.9999 and 0.9997) are demonstrated for UV and FLD, respectively.

Although both method of detection are proven fit, there are some differences that should be mentioned. UV detection has shown a greater precision and intra-day reproducibility, whereas fluorescence detection offers far better reliability in trazodone identification and lower detection limits (if needed). Hence, for a routine work one may safely prefer DAD detection and going FLD in tough cases for extra sensitivity and security.

Clinical Case

Female patient, K. L., 17, was admitted at the Clinical toxicology unit after oral abuse with 20 tabl. of trazodone, 20 tabl. hydroxizine and 5 tabl. quetiapine with suicidal purpose (anamnesis data). The patient was in good general condition, conscious and comfortable. Vital signs are stable and within normal limits; blood pressure – 110/60 mmHg. Due to the CNS and cardiovascular toxicity of drugs taken in overdose, the monitoring the blood trazodone levels was mandatory for treatment clarification (Fig. 2). As there is no specific antidote for trazodone, a plain supportive treatment has been followed: gastric lavage and forced diuresis; nootropil, vit. group B and Intralipid infusions.

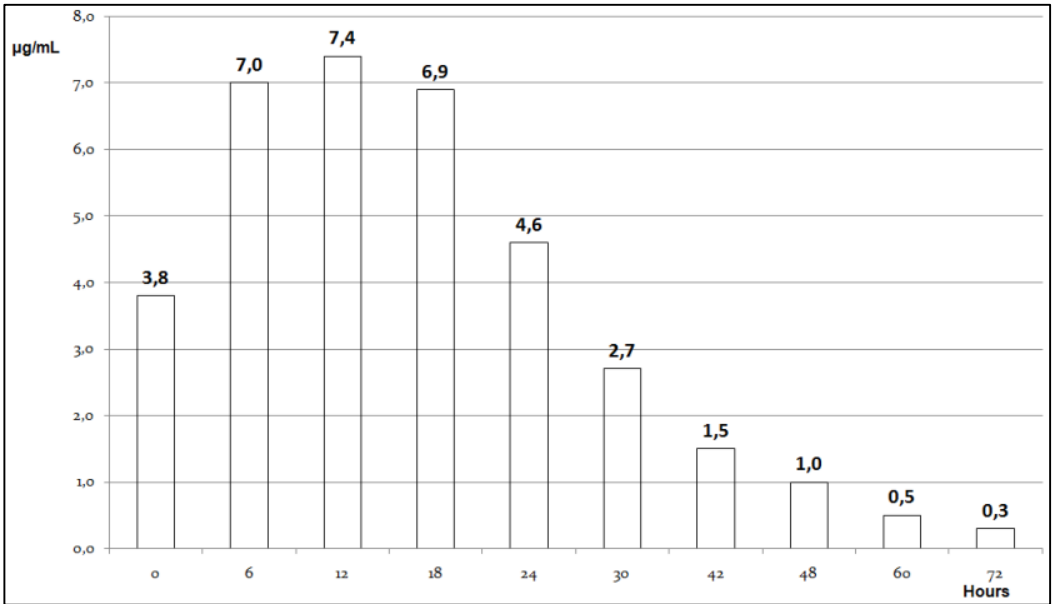

Fig 2: Monitoring the blood trazodone levels during a treatment of acute intoxication

Dynamically analyzing analytical toxicology data (trazodone concentration profile on Fig. 2) it became obvious that initial gastric lavage did not bring the desired efficiency, as trazodone concentration continued its growth during first 12 hours. Second gastric lavage was applied to reveal yet additional tablets residue in vomitus. Treatment continued in before mentioned way. Within 24 hours blood trazodone concentration fell below the toxic level, and, consequently, on the 3rd day – below the therapeutic zone. The patient was discharged from the hospital on the 5th day, without having any toxiological problems.

Conclusion

Acute intoxications, caused by combination of trazodone and other psychoactive drugs, may lead to unexpectedly severe ongoing and, although with a good prognosis, are potentially fatal. A HPLC technique for trazodone identification and determination has been proposed and validated. The method has been applied for diagnosis refinement and treatment monitoring. It is shown that in some cases an initial gastric lavage may not guarantee a successful stomach purge.

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