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DEVELOPMENT OF LC-MS METHOD FOR DETERMINATION OF THE QUETIAPINE IN HUMAN URINE

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Introduction Quetiapine (QT)– dibenzothiazepine derivative, is an atypical antipsychotic agent which is widely used in the treatment of schizophrenia, bipolar mania and other psychotic disorders. Chemical it is2-(2-(4-dibenzo[b,f][1,4]thiazepine11-yl-1-piperazinyl)ethoxy)ethano (Figure 1). Quetiapine was developed by Astra Zenecaand approved by the FDA in 1997

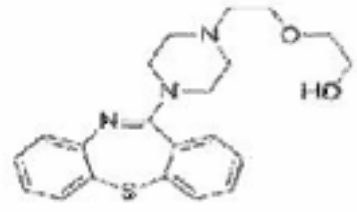

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Figure 1: Structure of Quetiapine The mechanism of action of Quetiapine is unknown. However, it has been proposed that the efficacy of it in schizophrenia and its mood stabilizing properties in bipolar depression and mania are mediated through a combination of dopamine type 2 (D_2) and serotonin type 2 (5HT) antagonisms.

Toxicity – The adverse reactions to Quetiapine therapy include somnolence, hypertension, dizziness, dry mouth and dyspepsia. Acute overdose with 1.2-9.3 g of the drug in 6 adult patients produced only sedation, tachycardia and hypotension. A young man ingested 4.7 g QT and 0.6 g fluoxetine, but manifested only drowsiness, disorientation and intermittent agitation; his plasma agent concentration was 0.18 mg/L at 60 hrs post-ingestion. A 26 years old woman who ingested 10 g of QT in a suicide attempt exhibited coma and tachycardia, but responded to therapy; an admission serum specimen contained 13 mg/L of the drug .

Risk assessment – Quetiapine intoxication is associated with predictable dose-dependent CNS depression ranging from sedation to coma and a characteristic risk of tachycardia. Mild hypotension is so metimes observed; it may be profound with massive ingestion. Co-ingestion of ethanol or other se da ti ve-hypnotic agents increases the risk of coma and loss of airway protective reflexes .

Several analytical methods have been employed and published for the determination of QT in biolo gi cal fluids. There was suggested HPLC method for the determination in human plasma: mobile phase containing a mixture of aceto- nitrile and 0.02 M phosphate buffer (506"50) at pH=5.5, UV detection at 254 nm . Was published different HPLC method with chemiluminescence detect ion and time of flight mass spectrometry (TOF-MS). For qu antification of Quetiapine fumarate in biological samples have been applied electrochemical de termination. Voltametric measurements were performed using a μ-Autolab potentiostat controlled by GPES-4.9 software .For determination of quetiapine fumarate in spiked human urine was used extractive spectro- photometric method. For identification of its, known and unknown metabolites in urine were identified using LCQTOF in combination with hydrolysis .

However, most of these methods are time-consuming, solvent-usage intensive, expensive and involved tiresome sample preparations and frequently suffer from poor selectivity.

The objective of this current work was to develop the sensitive and selective method for qualitative and quantitative determination of Quetiapine in human urine, for the needs of forensics analysis. Sample handling was performed by liquid-liquid extraction procedure. Experimental *Material and Methods:* European Pharmacopoeia Ref- erence Standards of Quetiapine fumarate (CAS number 111974-72-2) and Risperidone (CAS number 106266-06-2)the internal standard (IS) was obtained from SIGMA-ALDRICH. Organic solvents of HPLC grade were from Scharlab,S.L. (Spain) and Merck (Darmstadt, Germany). Water passed through a

Millipore system was used for sample dilution and in the mobile phase. Blank human urine of healthy volunteers (negative for substances of abuse like THCA, benzoylecgo nine, methadone, amphetamines including MDMA, opiates, and benzodiazepines with immuneassay and for buprenorphine and norbuprenorphine with ELISA (HumaLyzer 3000) were used. *Preparation of stock and working standard solutions*: Stock solutions of Quetiapine and IS (Risperidone) having concentration 1 mg/mL in methanol. Stock solutions were stored at -10°C. Cali bra tion standards with concentration 10, 20, 30, 40, 50 and 60 ng/mL; were prepared by spiking the working standard solutions of Quetiapine into human urine. Dilutions were used to prepare three levels of Quality Control Solutions (QCs), 100, 500, and 1000 ng/mL in urine. QCs were stored at -20°C. *Sample preparation:* The 20 mL urine samples were weighted prior to analysis as a standard procedure in the laboratory. Respective volume of standard solution was added to blank urine (sample A). *Liquid-Liquid extraction:* To 20 mL of *sample A* added conc. NH OH up to pH 9.0 and 3 mL liquid for ex tra ction: Ethylacetate-Heptane-Dichloromethane-Isopropanol (50:20:15:5). Mix/vortex on Stuart SB3 rotator and ultrasonic bath for homogenization. Centrifuge for 10 min at 3500 rpm. Evaporate to dry ness below 40°C. 4 *Preparing for Analysis*: evaporate reconstitute with 200 µL mobile phase, mix/vortex vigorously for 30 seconds and inject volume 100 µL into chromatograph. *Apparatus and conditions:* The system was consisted AGILENT TECHNOLOGIES 1290 Infinity AGILENT TECHNOLOGIES 6460 Triple quad LC-MS. Separation was performed by isocratic elution on Zorbax Eclipse plus C18 (250´4.5 mm, 5.0 µm) column, equipped with pre-column: UHPLC GUARD Zorbax Eclipse plus C18 (5´2.1 mm, 1.8 µm); column tempera ture was 35°C. Io ni za tion was performed by using electrospray in the positive mode (ESI+), detection method - Total Ion Cur rent (TIC). The mobile phases consisting of 0.1 % water solution of formic acid HCOOH (H O) : 0.1 % aceto nit rile solution of formic acid HCOOH (CH 2 CN)– 70: 30 (v/v).The flow rate was 0.200 mL/min, and the column tem pe ra tu re was 35°C. Results and discussion 3 In the given conditions the retention time of QT is 2.068 min (Figure 2). The expressivity of the developed method is shown with very short run time - 2.5 minutes. The identity of the product to Quetiapine is proved with mass spectrum of the sample (Figure 3). After identification of the product was done creation of the calibration curve in comparison to IS, for following determination of analyte (Figure 4). The linearity (R =0.9947)of the curve gives us oppor tunity to determine QT with high sensitivity in human urine, after its liquid-liquid extraction. In pro po sed condition the extraction rate is 94%.

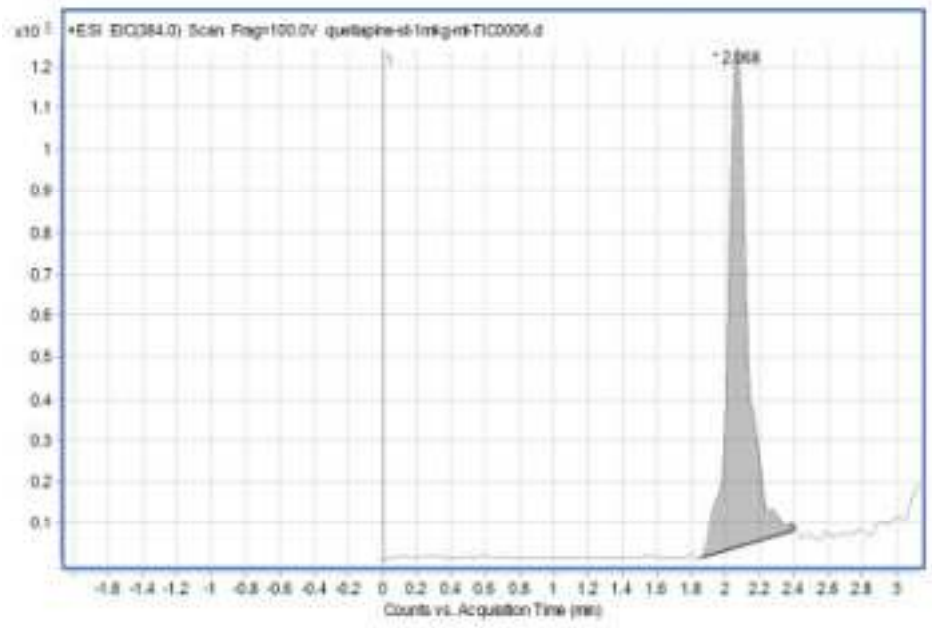

Figure 2: Quetiapine LC-MS chromatogram (TIC)

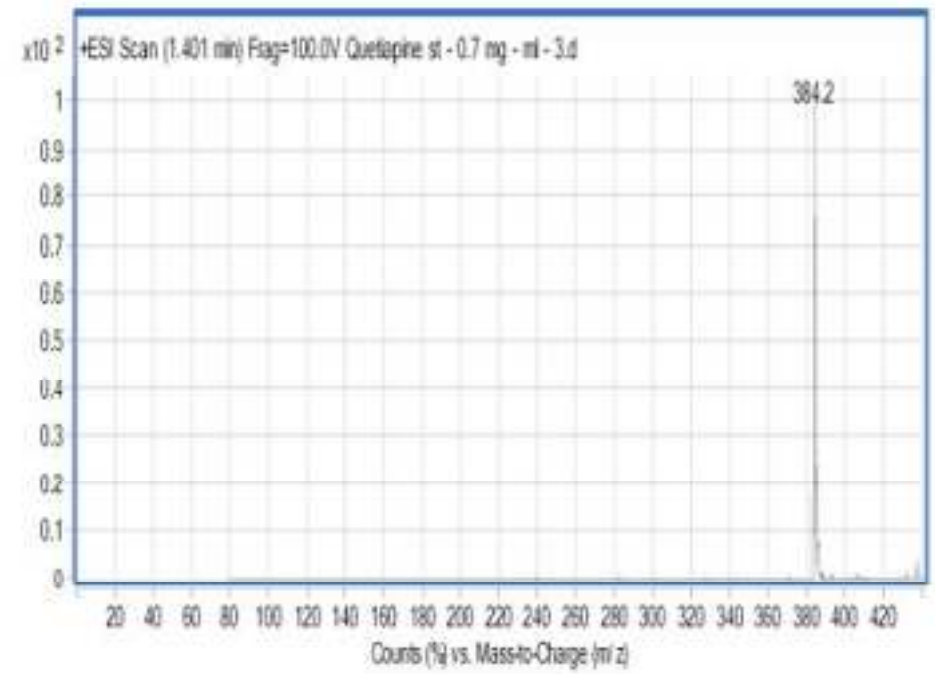

Figure 3: Quetiapine LC-MS spectrum (TIC) Peak area, 10^2 C ng/mL

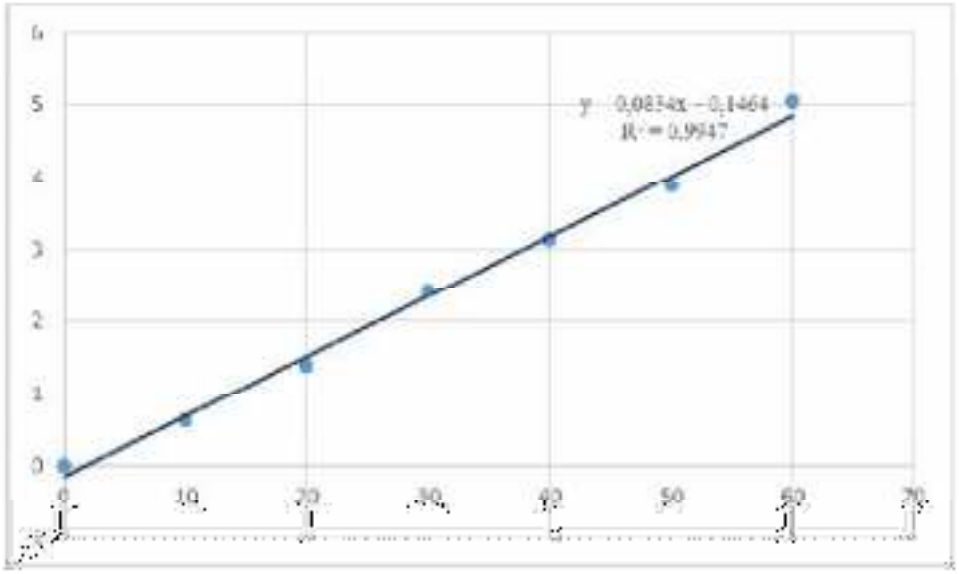

Figure 4: Quetiapine calibration curve in comparison to Internal Standard Risperidone.

Conclusion

During the study was developed selective, sensitive and cost effective LC-MS method for qualitative and quantitative determination of Quetiapine in human urine using Risperidone as an Internal Standard. From the received results, we can conclude that the developed method can be useful for determination of QT of forensic investigation of the intoxication or postmortem cases.

Acknowledgment The authors gratefully acknowledge to LEPL Levan Samkharauli National Forensics Bureau, Chemical toxicological laboratory, Tbilisi, Georgia for providing necessary facilities to carry out this work.

Illustrations of the articles in electronic form are available at information - publishing service

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Keywords: LC-MS, liquid-liquid extraction, urine

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ადამიანის შარდში ქვეტიაპინის ანალიზის სითხურ ქრომატოგრაფიული – მას სპექტრომეტრული (LC-MS) მეთოდის შემუშავება

თსსუ, ფარმაცევტული და ტოქსიკოლოგიური ქიმიის დეპარტამენტი; ლევან სამხარაულის სახელობის სასამართლო ექსპერტიზის ეროვნული ბიურო, ქიმიურ-ნარკოლოგიური ექსპერტიზის დეპარტამენტი

ქვეტიაპინი წარმოადგენს დიბენზოთიაზეპინის წარმოებულ ანტიფსიქოზურ საშუალებას, რომელიც ფართოდ გამოიყენება შიზოფრენიის მკურნალობისათვის, ასევე ბიპოლარული და სხვა ფსიქიკური მოშლილობისათვის. ინტოქსიკაცია ასოცირებულია ცნს-ის დათრგუნვასთან სედაციური მოვლენების თანხლებით და კომის განვითარებით, ასევე არსებობს ტაქიკარდიის განვითარების რისკი. ქვეტიაპინის, ეთანოლთან ან სხვა სედაციურ საშუალებებთან ერთდროული გამოყენება ზრდის კომის განვითარების რისკს და სასუნთქი გზების დამცავი რეფლექსის დაქვეითებას.

მოცემული კვლევის მიზანს წარმოადგენდა ადამიანის შარდში ქვეტიაპინის თვისობრივ-რაოდენობრივი განსაზღვრის მგძნობიარე და სელექციური მეთოდის შემუშავება. ***იზოლირებისათვის გამოიყენებოდა სითხე-სითხე ექსტრაქცია***: ნიმუშის შეტუტიანება ხდებოდა 25% NH OH წყლი ა ნი ხსნა რით pH 9. 0- მდე და 3 მლ ექსტრაჰენტი: ეთილაცეტატი – ჰეპტანი – დიქლორმეთანი – იზოპროპანოლი (50:20:15:5). ჰომოგენიზაციისათვის გამოიყენებოდა Stuart SB3 შემრევი და ულტრაბგერითი აბაზანა. ცენტრიფუგირება – 10 წთ-ის განმავლობაში 3500 ბრ/წთ. იზოლირების შემდეგ მიღებულ ორგანულ ფაზას აქროლებენ 40C ტემპერატურაზე. 4 ქრომატოგრაფირებისათვის ვიყენებდით სითხურ ქრომატოგრაფს – მას-სპექტრომეტრთან ტანდემში (LC–MS) - AGILENT TECHNOLOGIES 1290 Infinity AGILENT TECHNOLOGIES 6460 Triple quad LC/ MS. დაყოფა ხდებოდა სვეტზე - Zorbax Eclipse, სტაციონარული ფაზა - C18 (2504.5 მმ, 5.0მ). ხელსაწყო აღჭურვილი იყო წინასვეტით - UHPLC GUARD Zorbax Eclipse, სტაციონარული ფაზა - C18 (5 ′2.1mm, 1.8 μm). სვეტის ტემპერატურა - 35°C.

იონიზაცია მიიღწეოდა ელექტროგაფრქვევით (ESI), დეტექტირება – იონების ჯამური ნაკადით (TIC). $^{+}$ მოძრავ ფაზად შერჩეული იყო სისტემა: 0. 1% ჭიანჭველმჟავას წყალხსნარი HCOOH (H_2O)-0.1 % ჭიანჭველმჟავას აცეტონიტრილის ხსნარი HCOOH (CH_3CN)=70 : 30 (v/v). მოძრავი ფაზის დინების სიჩქარე 0.200 მლ/წთ. ^{3} **2** მოცემულ პირობებში ქვეტიაპინის შეკავების დრო იყო 2.068 წთ.

ექსტრაქციის ხარისხი 94%, საკალიბრო გრაფიკის სწორხაზოვნება R^2 ^{2} =0.9947.