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Detection and identification of atypical quetiapine metabolite in urine

Research Article

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Abstract: Quetiapine fumarate (Seroquel®) is an atypical antipsychotic dibenzothiazepine derivative. Due to its extensive hepatic metabolism and low level of unchanged excretion (< 1%) the routine toxicological drug-screening analyses of urine often leads to false negative results. In the present study, we report that a newly identified metabolite of quetiapine, N-desalkylquetiapine, can be used as an indicative marker of quetiapine-intake in urine using common GC-MS screening procedure. The structure of the mentioned metabolite was solved from the mass-spectrum obtained and the quetiapine presence was proved by consequent HPLC plasma analysis.

Keywords: *Quetiapine • N-desalkylquetiapine • GC-MS screening*

1. Introduction

Quetiapine (quetiapine fumarate, Seroquel®) is an atypical antipsychotic, indicated for the management of psychotic disorders [1-3]. From a chemical perspective, quetiapine (**I**) is a clozapine-like dibenzothiazepine compound. It is rapidly absorbed after oral administration with maximum observed plasma concentration time (t_{max}) of 1 – 1.5 hours in case of 25 mg dose and corresponding concentration (C_{max}) of 53 – 117 ng/mL. The mean apparent elimination half-life time is 3.1 – 5.5 hours [4]. Limited information about the acute overdosage with Seroquel® (quetiapine fumarate) was found in the clinical trial database with estimated doses ranging from 1200 to 9600 mg without fatalities. Generally the reported symptoms result from an exaggeration of the drug known pharmacological effects as drowsiness and sedation, tachycardia and hypotension.

An extensive hepatic metabolism is characteristic for the drug mainly by CYP 3A4 mediated oxidation (89% to the overall metabolism) [5]. The quetiapine metabolism involves oxidation of the alkyl side chain, oxidation of the hydroxyl group to carboxylic acid one, sulfoxidation, desalkylation, hydroxylation of the dibenzothiazepine ring and consequent phase II biotransformations. There are two active drug metabolites - 7-hydroxy-quetiapine and 7-hydroxy-N-desalkyl-quetiapine - but their presence in plasma is lower than 10% of the initial quetiapine amount. The main metabolites of quetiapine are its sulfoxide and carboxylic acid (*c.a.* 30% of quetiapine amount). The elimination of quetiapine and its metabolites is mainly by urine (73%) and faces (21%). The unchanged quetiapine is less than 1% of all excreted forms [4]. The extensive metabolism of quetiapine leads to its low excretion level in urine, which makes the GC-MS analysis uncertain. At the same time the major quetiapine metabolites of Phase I biotransformations (sulfoxide and carboxylic acid, respectively) are not suitable for GC-MS screening analysis because of the thermal instability of sulfoxide and the low volatility of carboxylic acid (a derivatization is required in the last case).

Different procedures for analysis of quetiapine and its metabolites using special sample preparation

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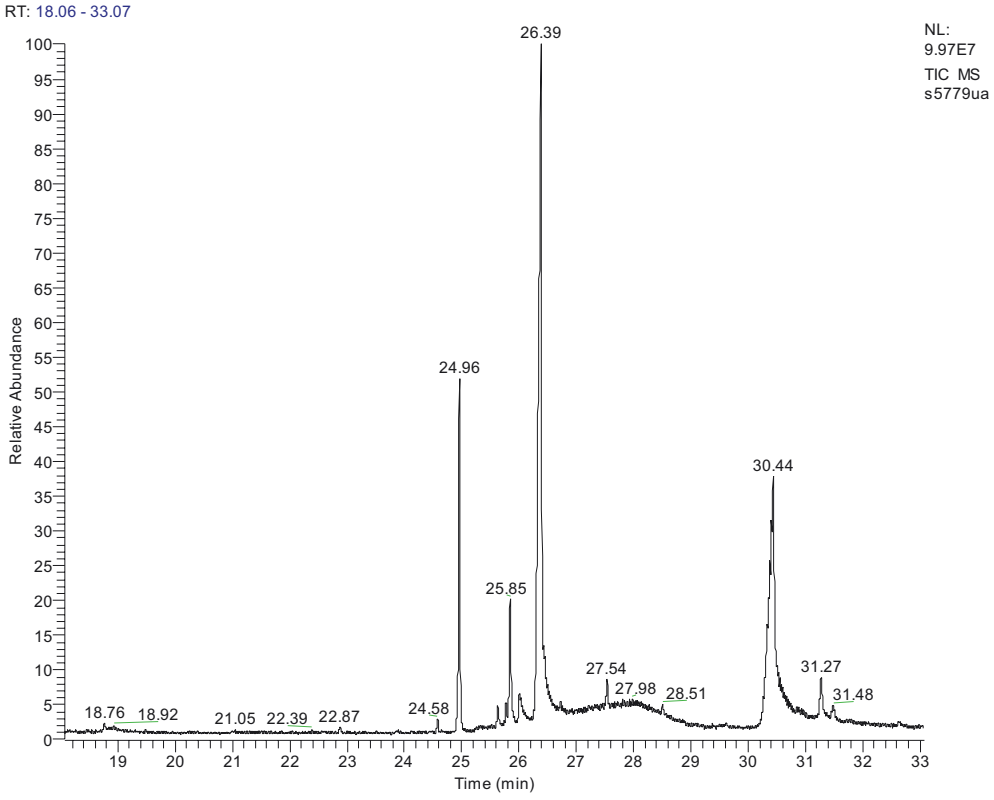
Figure 1. Chromatogram (18-33 min) of urine sample from quetiapine overdosed patient. The retention time of II is 26.39 min.



techniques [6-10] or special analytical instruments application [7,11,12] are reported. In the present study we describe the quetiapine-intake identification in urine and blood samples of patients intoxicated with Seroquel®. The results are based on mass-spectral detection and identification of unknown up to now quetiapine metabolite as *N*-desalkylquetiapine (**II**).

2. Material and Methods

2.1. Sample preparation

6 mL urine sample is placed in test-tube and mixed with 1 mL 500 mM NaOH followed by single extraction with 6 mL ethyl acetate at 1 min vortexing. After centrifugation (3 min at 4 500 rpm) the organic layer is separated and dried with 100 mg anhydrous magnesium sulphate. The dry organic phase was concentrated in water bath under nitrogen flow (the temperature not exceeding 45°C) to a final volume of *apx* 50 µL.

After identification of quetiapine-metabolite in the urine sample the quetiapine-intake and its presence in plasma was proved using HPLC method. An aliquot (200 µL) of heparin plasma sample was deproteinated with acetonitrile (1.0 mL) and two-fold basic (200 µL 500 mM NaOH) extraction was performed with 3 mL ethyl acetate (1 min at vortexing). After centrifugation and organic layer separation the combined extracts were dried with 100 mg anhydrous magnesium sulphate and evaporated to dryness. The sample was reconstituted with 100 µL mobile phase and after sonication was filtered through 0.45 µm nylon filter. The aliquots (20 µL) were injected in HPLC instrument. The quantification of the drug was determined using external calibration with recovery of 86%. All reagents used were purchased from Merck (Darmstadt, Germany).

2.2. Instrumental analysis

GC-MS analysis was performed using Trace GC (Thermo Electron) instrument equipped with a quadruple MS-detector DSQ (Thermo Electron). The separation was carried out on capillary fused silica column DB-5 MS 30 m x 0.25 mm ID x 0.25 µm film (J & W Scientific, USA), helium as carrier gas and split injection of the sample. The mass-spectrometric detector operates in *scan*-mode (33 – 460 m/z).

HPLC determination of quetiapine in blood serum was performed on Perkin Elmer liquid chromatography system equipped with quaternary pump LC 410 and UV-Vis detector LC 95. Reversed-phase ODS column - Purospher® STAR, 125-3, 5 µm particle size (Merck) and mobile phase containing 20% (v/v) acetonitrile (Merck)

Figure 2. Mass-spectrum of N-desalkylquetiapine, II.

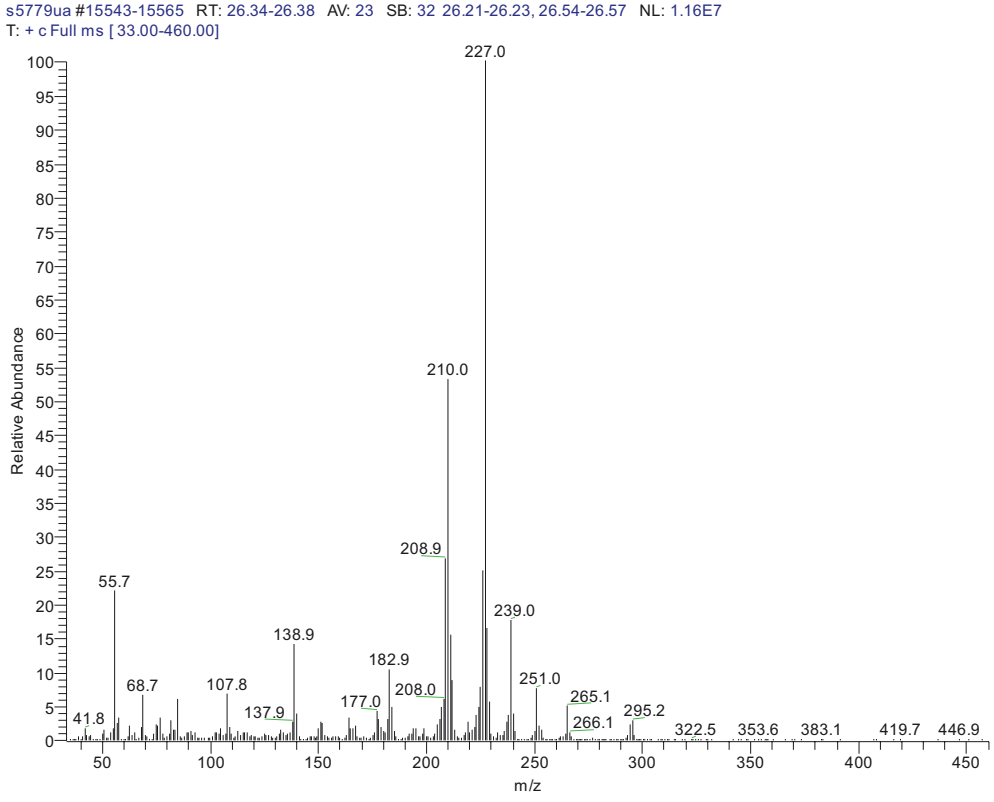
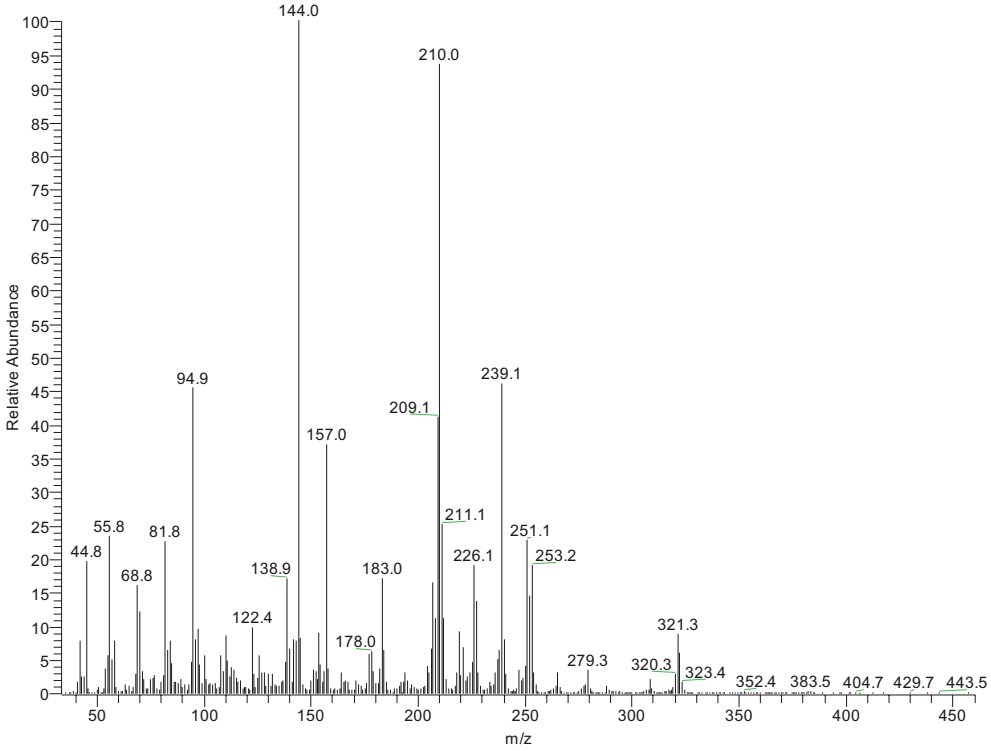
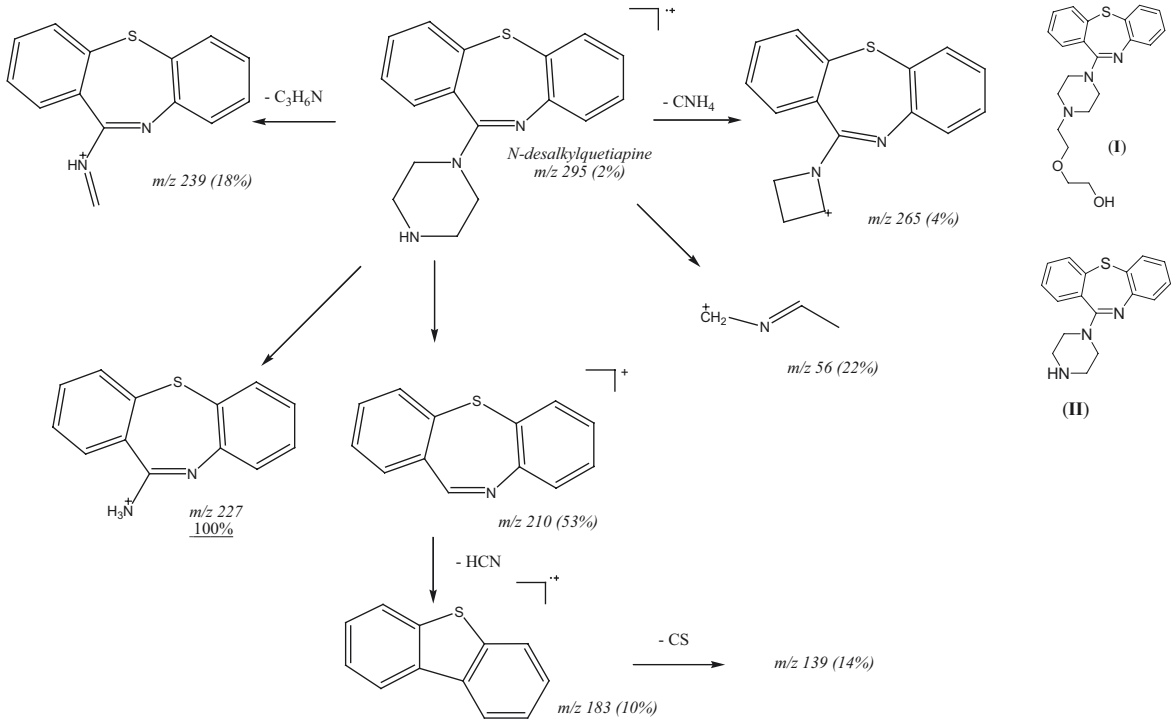


Figure 3. Mass-spectrum of quetiapine, I.



Scheme 1. Proposed mass-spectral fragmentation of N-desalkylquetiapine, II. The relative abundances of the ions are presented in parenthesis.



and 80% (v/v) 0.01M triethylamine – phosphoric acid (pH 2.7) were used. The detection of quetiapine was carried out at 290 nm.

3. Results

A chromatogram of urine sample from overdosed patient (three cases) in the range of 18 – 33 min is presented in Figure 1. In all the three chromatograms, a compound with retention time (RT) of 26.39 min observed is unknown, thus, it was further analyzed by using its mass-spectrum (Figure 2). The mass-spectrum of quetiapine was registered at the same analytical conditions (Figure 3).

The quetiapine presence in the samples of all patients was also confirmed using HPLC method. The quetiapine plasma level of the patients was determined as follows: 1.2 μg/mL, 2.1 μg/mL and 0.4 μg/mL, respectively. According to the therapeutic and toxic serum concentrations published [13], the first value is very close to the drug therapeutic concentration (lower than 1 μg/mL), the second one exceeds it significantly and the third is in the typical therapeutic range.

4. Discussion

There is no data about the compound with RT= 26.39 min in the NIST Mass Spectral Library (Version 2.0, 2002). The available preliminary information about Seroquel-intake of intoxicated patients directed us to suggest a possible quetiapine-derived compound with unknown structure.

In order to elucidate it the mass-spectrum registered (Figure 2) was thoroughly analyzed and compared with that of parent quetiapine (Figure 3). The presence of common ions (m/z: 239, 210, 183) in both mass-spectra proved that the unknown compound has a quetiapine-related structure and is most probably quetiapine metabolite. The interpretation of the mass-spectrum of this compound attained its structure elucidation (**II**). The identified compound is likely to originate from the parent drug quetiapine due to microsomal oxidation by CYP 450 and was named by us as *N*-desalkylquetiapine.

The fragmentation pathways of *N*-desalkylquetiapine (Scheme 1) show the formation of several characteristic ions which are in agreement with the data obtained in the mass-spectrum of the compound studied. The molecular ion of **II** has low abundance (only 2%). The basic ion with m/z 227 (100%) as well as two derivative ions with m/z 239 (18%) and m/z 265 (4%), respectively, are suggested as resulting from different piperazine

fragmentation pathways. The piperazine cleavage from the molecular ion of **II** affords the formation of the second in intensity ion (53%) with m/z 210. The elimination of hydrogen cyanide results in formation of the ion with m/z 183 (10%) and the ion with m/z 139 (14%) could originate from further CS elimination.

The results presented in this paper show that the newly identified metabolite of quetiapine - *N*-desalkylquetiapine – can be used as an indicative marker of quetiapine-intake in routine GC-MS urine screening analysis although it is not a major metabolite of the parent compound.

References

[1] Goldstein J., Arvanitis L. A., ICI 204,636 (Seroquel): A dibenzothiazepine atypical antipsychotic, Review of preclinical pharmacology and highlights of Phase II clinical trials, CNS Drug Rev., 1995, 1, 50-73

[2] Arvanitis L. A., Miller B. G., Multiple fixed doses of 'Seroquel' (quetiapine) in patients with acute exacerbation of schizophrenia: a comparison with haloperidol and placebo. The Seroquel Trial 13 study Group, Biol. Psychiatry, 1997, 2, 233-46

[3] Caccia S., New antipsychotic agents for schizophrenia: Pharmacokinetics and metabolism update, Curr. Opin. Invest. Drugs, 2002, 3, 1073-1080

[4] De Vane C. L., Nemeroff C. B., Clinical Pharmacokinetics of Quetiapine: An Atypical Antipsychotic, Clin. Pharmacokinet., 2001, 40, 509-522

[5] Hasselstrom J., Linnet K., In vitro studies on quetiapine metabolism using the substrate depletion approach with focus on drug-drug interactions, Drug Metabol. Drug Interact., 2006, 21, 187-211

[6] Flammia D. D., Valouch T., Venuti S., Tissue distribution of quetiapine in 20 cases in Virginia, J. Anal. Toxicol., 2006, 30, 287-292

[7] Hopenwasser J., Mozayani A., Danielson T. J., Harbin J., Narula H. S., Posey D. H., et al., Postmortem Distribution of the Novel Antipsychotic Drug Quetiapine, J. Anal. Toxicol., 2004, 28, 264-268

[8] Langman L. J., Kaliciak H. A., Carlyle S., Fatal overdoses associated with quetiapine, J. Anal. Toxicol., 2004, 28, 520-525

[9] Saracino M. A., Mercolini L., Flotta G., Albers L. J., Merli R., Raggi M. A., Simultaneous determination of fluvoxamine isomers and quetiapine in human plasma by means of high-performance liquid chromatography, J. Chromatogr. B, 2006, 843, 227-233

[10] Mandrioli R., Fanali S., Ferranti A., Raggi M. A., HPLC analysis of the novel antipsychotic drug quetiapine in human plasma, J. Pharm. Biomed. Anal., 2002, 30, 969-977

[11] Anderson D.T., Fritz K.L..Quetiapine (Seroquel®) concentrations in seven postmortem cases, J. Anal. Toxicol., 2000, 24, 300-304

[12] Davis P. C., Wong J., Gefvert O., Analysis and pharmacokinetics of quetiapine and two metabolites in human plasma using reversed-phase HPLC with ultraviolet and electrochemical detection, J. Pharm. Biomed. Anal., 1999, 20, 271-282

[13] Schulz M., Schmoldt A., Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics, Pharmazie, 2003, 58, 437-474