ORIGINAL ARTICLE

Chromatography–mass spectrometry analysis of tianeptine in urine

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Abstract

Introduction: Coaxil (tianeptine) is a new generation antidepressant with selective serotoninergic action. Recently, a significant increase in non-medical use of the atypical tricyclic antidepressant drug, tianeptine, has been observed in Ukraine. According to domestic narcologists and psychiatrists, there is information on tianeptine abuse by drug addicts. In literature, there is information that this medicine combines characteristics of opium, heroin, and cocaine in it. Receiving of tianeptine in high dosages (400–600 mg intravenously) leads to a state of euphoria and gives a narcotic effect that is several times stronger than heroin and cocaine. **The aim of the Study:** The aim of the research was to study of tianeptine metabolism products in the human body in urine by gas chromatography–mass spectrometry. **Methods:** The analysis has been carried out on a gas chromatograph Agilent 6890N with a mass-selective detector Agilent 5973 and an injector Agilent 7683 (USA) equipped with a chromatographic quartz capillary geyser of FactorFour Varian (USA). Gas carrier was helium. **Results and Discussion:** The analysis of extracts received as with acidic and with alkaline pH values has shown metabolites of tianeptine MC5 in them. Besides the main metabolite (MC5), there have also been desalkyldesaminotianeptine and desalkyldesaminonortianeptine in the extracts obtained at pH 3–4 and in the extracts obtained at pH 9–10 - desalkyltianeptine and a metabolite, the structure of which has not been established at the moment. Based on the fact that the main metabolite of tianeptine MC5 is isolated from the urine both at acidic pH values (pH = 3–4) and alkaline (pH = 9–10), it is possible to use as acidic as alkaline extracts for the chromatography–mass spectrometry analysis. **Conclusions:** The possibility of using gas chromatography and mass spectrometry methods for metabolites has been shown. The main metabolite of tianeptine can be isolated from the urine at acidic and also at alkaline pH values of the medium.

Key words: Antidepressants, chromatography–mass spectrometry, extracts, metabolites, tianeptine

INTRODUCTION

Coaxil (tianeptine) is a new generation antidepressant with selective serotoninergic action (stimulation of reuptake of serotonin), which has been widely used since its introduction (1988) in the treatment of depressive disorders. Today, Coaxil is the second most commonly used antidepressant (after amitriptyline), which is prescribed in >20% of depression patients receiving treatment in psychiatric clinics. However, data on the usage of Coaxil, as well as practically all new generation antidepressants, in the hospital environment are extremely limited.[1,2]

Recently, a significant increase of the non-medical usage of the atypical tricyclic antidepressant has been observed in Ukraine - Tianeptine (Coaxil®). In medical practice, medicines containing this substance are used in the treatment of the depressive states of different degrees of severity, including those suffering from chronic alcoholism in the abstinence period; anxiety-depressive state weighted with somatic complaints; organically depressed and anxiety disorders; neurasthenia; as well as acute reaction to stress and post-traumatic stress disorder.

It causes severe drug dependence with regular usage of doses that are 10 times higher than medical and only with the

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intravenous way of administration. As a rule, for the purpose of narcotic use, injections of this substance commonly used. Herewith, the clinical picture of intoxication is similar to heroin dependence, which is why many drug addicts replace heroin with it. However, this similarity extends to the withdrawal syndrome. Frequent abuse of handmade tianeptine leads to severe consequences: Blindness, amputation of the limbs, and fast lethal outcome.

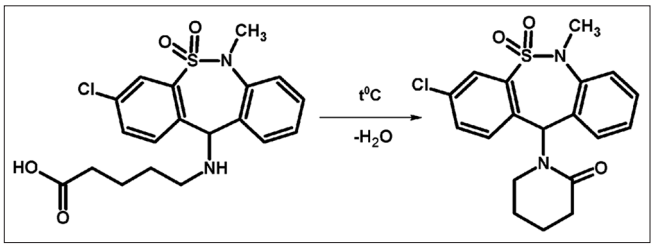
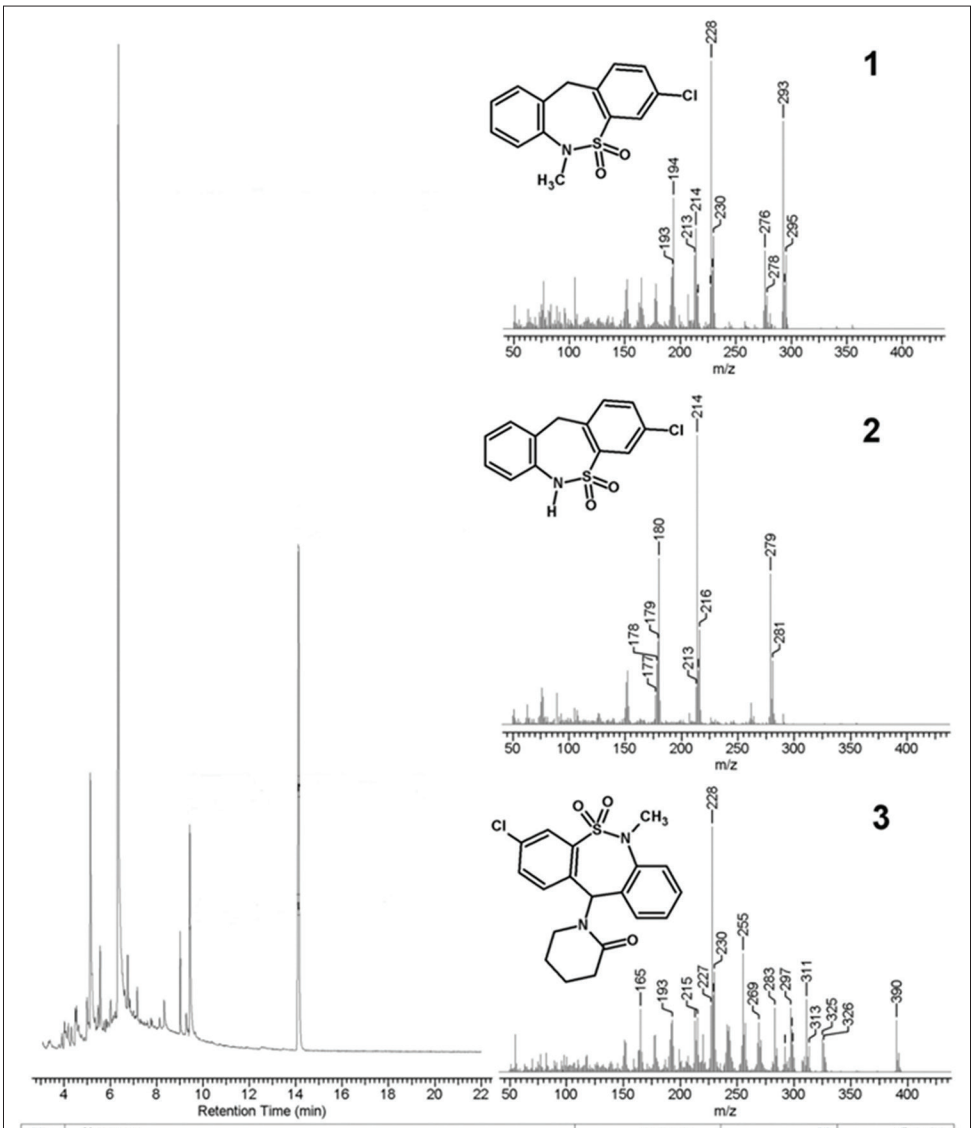


Figure 1: The process of the formation of metabolite MC5 lactone

The purpose of the study is to study the metabolism products of tianeptine in the human body, which are detected in urine during studies by gas chromatography and gas chromatography–mass spectrometry.

Tianeptine (Coaxil®) is an original tricyclic antidepressant of the dibenzothiazepine series. According to literature, the main way of its metabolism in the human body is β-oxidation of the aliphatic substituent at the nitrogen atom, which results in the formation of two active metabolites: With the side chain of propionic acid (the metabolite MC5) and valerian acid (metabolite MC3). In addition, N-dealkylation products of this substance may be found in urine. Not less than 3% of the dose received is released from the body in unchanged form during 24 hours.



No.	Название	Формула	M	tR (min)
1	Дезалкилдезаминотианептин	C14H12ClNO2S	293.028	9.023
2	Дезалкилдезаминонортеанептин	C13H10ClNO2S	279.012	9.429
3	Тианептин-МС5 - H2O	C19H19ClN2O3S	390.080	14.110

Figure 2: Chromatogram of the acid extract of urine and mass spectrum of metabolites

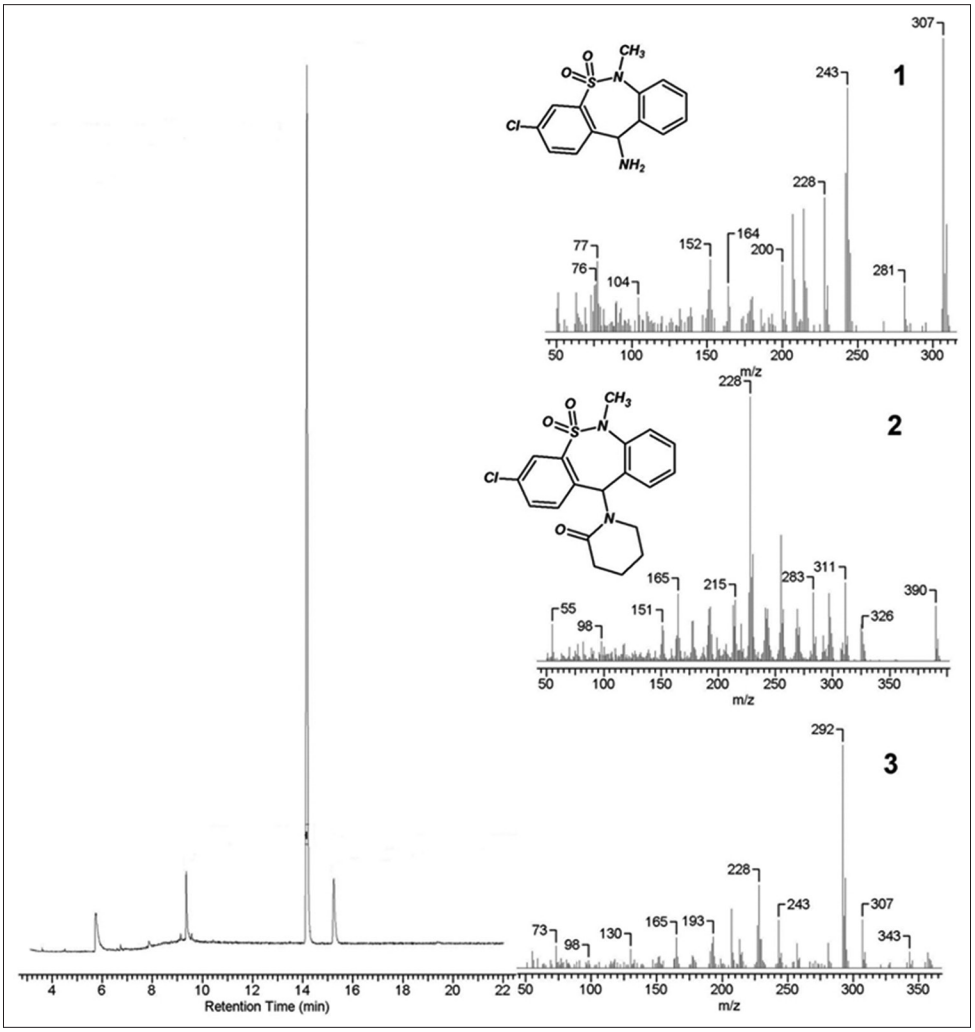


Figure 3: Chromatogram of the alkaline extract of urine and mass spectrum of metabolites

Table 1: Peak area of tianeptine metabolites at different methods of sampling preparation (GC/MS analysis) usage

pH Metabolite	1-2 (*n*=10)	3-4 (*n*=10)	7 (*n*=10)	9-10 (*n*=10)
MC5	52.75±1.79	84.4±2.44	114.79±1.41	98.73±1.41
Metabolite 1	0	0	0	11.61±2.63
t(*P*=0,95)		*t*2 і 3–9.81 *t*2 і 4–21.85 *t*2 і 5–18.77 *t*3 і 4–9.29 *t*3 і 5–5.03 *t*4 и 5–6.25		

There are chemical groups of acid and alkaline nature, as well as a long lipophilic, aliphatic fragment in the tianeptine molecule. It has fairly large molecular mass. All of these factors create difficulties in analyzing bioliquids for the presence of tianeptine by gas chromatography and gas chromatography–mass spectrometry. For a successful study of this substance by these methods, usually, derivatization of active groups with one or more reagents is carried out.[3-21] However, as practice shows, in case of using tianeptine for the purpose of obtaining a narcotic effect, urine contains products of its metabolism or disintegration.

METHODS

The analysis was carried out on a gas chromatograph Agilent 6890N with a mass-selective detector Agilent 5973 and an injector Agilent 7683 (USA) equipped with a chromatographic quartz capillary geyser of FactorFour of Varian (USA) 30 m of length, an internal diameter of 0.25 mm, and 0.25 μm phase thickness. The gas carrier is helium, the gas flow rate through the geyser was 1.2 ml/min; a linear velocity was 41 cm/s. The temperature of the evaporator was maintained at 250°C. The programming temperature of the

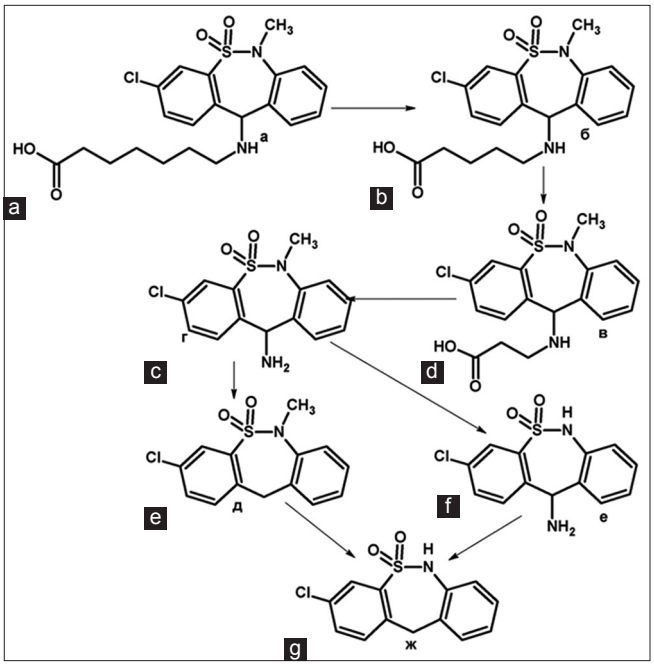


Figure 4: Metabolism of tianeptine (a) tianeptine, (b) metabolite MC5, (c) metabolite MC3, (d) desalkyltianeptine, (e) dezalkyldezaminotianeptine, (f) desalkyldesaminotianeptine, (g) desalkyldesaminonortianeptine

geyser thermostat: 110°C - 1 min, from 110°C to 290°C was heated at the rate of 25°C/min and maintained at 7.8 min at final temperature. Total time for analysis is 16 min. The introduction of the sample was performed in split mode 1:20. The volume of the entered sample is 1 µl. The temperature of the quadrupole was 150°C; the temperature of the ion source was 230°C. Ionization with an electron impact at 70 eV was used in the scan mode of full ion flow (SCAN) in the range from 50 to 550 m/z.

Urine samples of 2 ml were adjusted to pH values in the 1–2 range; 3–4; 7; and 9–10 and 3 ml of diethyl ether were added. To create pH in the interval from 1 to 4, 2 g/l of hydrochloric acid *R* was used, and for pH 9–10 - 5 g/l of potassium hydroxide was used. After shaking (10 min at 120 rotations per minute) and centrifugation (10 min at 3000 rotations per minute), the upper organic layer was carefully collected with a disposable plastic pipette and transferred to a glass conical tube of 10 ml for evaporation. Evaporation was carried out at a room temperature in a stream of cold air to a dry residue. A dry residue of 200 µl was dissolved in 10 ml of 96% ethyl alcohol and transferred to glass inserts. As an internal standard, 20 µl of 0.05% methanolic diphenylamine solution of "Sigma" (USA) was added to the final extract.

RESULTS

The analysis of extracts received with both acidic and alkaline pH values showed the presence of tianeptine MC5 metabolites in them. Interesting fact is that in the process of analysis, the metabolite MC5 (namely its lactone derivative) is detected, and the tianeptine itself and its metabolite MC3 are not detected.

We assumed that the process of introducing the sample into the injector of the chromatograph, as well as the process of chromatographic separation of the components, is carried out at relatively high temperatures, the MC5 metabolite in these conditions loses water with forming of lactone derivative MC5-H_2O. The scheme of the process for the formation of lactone is presented in Figure 1.

This formula is confirmed by the fact that the processing of urine extracts, both acidic and alkaline, with vinegar or trifluoroacetic anhydride, in alkaline medium does not result in decrease or complete disappearance of the metabolite.

In addition to the main metabolite (MC5), in the extracts received at pH 3–4, there are also dezalkyldezaminotianeptine and dezalkyldezaminonortianeptine and in the extracts received at pH 9–10 - dezalkyltianeptine and a metabolite, the structure of which has not been established at the moment. In general, the output of the metabolite MC5 with the usage of sampling preparation with the creation of alkaline pH values of urine is usually greater than with acidic values. The results are shown in Table. 1.

DISCUSSION

In some samples of urine, after sample preparation at alkaline pH values, another set of metabolites is detected, the structural formula of which has not been established to date. The chromatogram of the acid extract of human urine, human who received Coaxil® and the mass spectra of the metabolites are presented in Figure 2, similarly for an alkaline extract - in Figure 3.

Based on the received data, we presented a possible scheme of tianeptine metabolism, which is presented in Figure 4.

It is possible to use as acidic as alkaline extracts based on the fact that the main metabolite of tianeptine MC5 is isolated in the urine both at acidic pH values (pH = 3–4) and alkaline (pH = 9–10), for the chromato-mass spectrometric analysis. In practice, the alkaline extracts are used to detect tianeptine degradation products by thin-layer and gas chromatography or chromatography–mass spectrometry.

CONCLUSIONS

The possibility of using gas chromatography and mass spectrometry methods for metabolites is shown. The main metabolite of tianeptine may be isolated from urine at acidic and also at alkaline pH values of the medium.

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