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Case report

Fatal intoxication with tianeptine (*Stablon*®)

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Abstract

Tianeptine (*Stablon*®), although structurally similar to tricyclic antidepressants, acts by enhancing the reuptake of serotonin. A fatal case is presented involving a 26-year-old man, found lying in bed with a ''mushroom of foam'' around his mouth. Empty blister packs of *Stablon*® and a suicide note were found next to the body. A liquid–liquid extraction procedure with *n*-hexane: ethyl acetate and *n*-hexane: 2-propanol, followed by LC-DAD-MS analysis, using positive mode electrospray ionization was performed. The detection limit was 0.001 μg/mL. The toxicological results revealed the following tianeptine concentrations in the post-mortem samples: blood 5.1 μg/mL; urine 2.0 μg/mL; liver 23 μg/g; stomach contents 22 mg. Femoral blood analyses also revealed an ethanol concentration of 0.53 g/L. The present method was also developed and validated for the other post-mortem specimens, since no previous published data had confirmed the post-mortem distribution of tianeptine. The absence of other suitable direct causes of death (macroscopic or histological) and the positive results achieved with the toxicological analysis led the pathologist to rule that death was due to an intoxication caused by the suicidal ingestion of tianeptine in combination with alcohol.

Keywords: Suicide; Poisoning; Tianeptine; LC-MS; Post-mortem distribution

1. Introduction

Tianeptine (*Stablon*®) is a tricyclic compound structurally similar to imipramine, which has been used as an antidepressant in certain European countries since 1988 [1]. Although, the anxiolytic efficacy profile of tianeptine is also similar to tricyclic antidepressants, it acts by enhancing the reuptake of serotonin. Tianeptine is completely metabolised by the liver and is not subject to first-pass metabolism [2]. Tianeptine differs from most antidepressants in that it is not primarily metabolised by the hepatic cytochrome P450 system, indicating less likelihood of drug–drug interactions [3]. Metabolism occurs mainly by extra renal routes. Its major metabolic pathway is β-oxidation and the principal metabolites are propanoic acid (MC_3, inactive metabolite) and pentanoic acid (MC_5, active metabolite) [1–4] (Fig. 1). The pharmacokinetics is linear to the dosage. Less than 3% of the dose is excreted unchanged in urine and MC_5 half-life is 7.2 h. It is eliminated partly unchanged by the kidneys and undergoes biotransformation [2,5–8].

Several methods have been published for the simultaneous determination of tianeptine and its MC_5-metabolite using high performance liquid chromatography with UV detection [9–11] and fluorescence detection [12].

In this study, we report a fatal case involving tianeptine, using a liquid chromatography associated with photodiode array and the mass spectrometer (LC/DAD/MS) method developed to detect, confirm and quantify this antidepressant in post-mortem samples.

2. Case report

A 26-year-old man was found dead in bed in his apartment by a friend who had not been seen him since the day before. During an investigation of the scene, empty blister packs of *Stablon*® (tianeptine, 12.5 mg tablets) and a suicide note to his family were found next to the body.

At the autopsy, a ''mushroom of foam'' was observed around the mouth, but no signs of traumatic injuries were registered. The internal examination was unremarkable except for some

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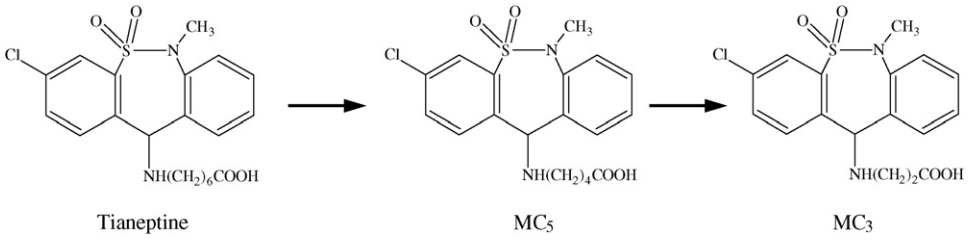

Fig. 1. Metabolic pathways for tianeptine.

tissue congestion and edema. Histological findings showed an exuberant congestion of the cerebrum and lungs. Interstitial edema of the heart was found and some myocytes were slightly wavy and showed contraction bands. These alterations were attributed to eventual arrhythmia, complicated by cardiac and pulmonary failure.

Samples of blood (femoral and cardiac), urine, liver and stomach contents were taken for toxicological analysis.

3. Materials and methods

3.1. Chemicals and reagents

Tianeptine and protriptyline (used as internal standard) were supplied by Sigma–Aldrich–Chemie GmbH (Steinheim, Germany). Each standard solution was prepared in acetonitrile (1 mg/mL) and stored at 4 °C. All solvents were analytical or HPLC-grade and were purchased from E. Merck (Darmstadt, Germany). Deionised and purified water was obtained using a Milli-Q system (Millipore, Molsheim, France). The carbonate buffer (pH 10.5) was prepared by dissolving 4.2 g of $NaHCO_3$ into a 1000 mL volumetric flask and brought to volume with deionised water. An ammonium acetate buffer 10 mM (0.771 g/L) was prepared with deionised water with 0.1% of formic acid. The mobile phase was filtered through a 0.20 μm filter (Schleicher & Schuell) and degassed in an ultrasonic bath for 15 min just before use. All samples were filtered through a 0.45 μm Millex®-HV filter (Millipore, Bedford, MA) before injection into the LC/DAD/MS system.

3.2. Instrumentation

The chromatographic system used was a Waters 2695 Alliance System and a XTerra MS™ C_{18} reversed-phase column (2.1 mm i.d. × 150 mm, 5 μm). The mobile phase consisted of acetonitrile and 10 mM ammonium acetate buffer with 0.1% formic acid (pH 3) (30:70, v/v) pumped at a flow rate of 0.3 mL/min. The column temperature was maintained at 30 °C. The injection volume was 5 μL.

A Waters 996 photodiode array detector (DAD) was operated on a 210–400 nm wavelength scans with a 1.2 nm resolution. The UV absorbance was measured at 220 nm.

Instrument control, data acquisition and processing were achieved using Waters Empower software (Milford, MA).

Mass spectrometry detection (MS) was carried out on a Waters ZQ 2000 single quadrupole mass spectrometer with electrospray ionization (ESI) performed in positive mode. Full-scan spectra were recorded from *m/z* 130–700, at a scan time of 1 s and an interscan delay of 0.1 s. The mass spectra were represented in centroid mode. The other main instrument settings were: capillary voltage 3.5 kV; cone voltage 40 V; extractor 5 V; ion energy 0.5; source temperature 150 °C; desolvation temperature 350 °C; cone gas (N_2) flow rate 0 L/h and desolvation gas (N_2) flow rate 350 L/h.

Quantitation employed the selected ion-recording mode (SIR) using the most abundant characteristic ion $[M + H]^+$ at *m/z* 437 for tianeptine and *m/z* 264 for the internal standard (protriptyline).

3.3. Sample preparation

Control and calibration samples were prepared by spiking drug-free blood post-mortem specimens with standard solutions.

A 1 mL aliquot of whole blood or 1 g of tissue (cut into small pieces) was spiked with 30 μL of internal standard (10 μg/mL). Then, 0.5 mL of carbonate buffer (50 mM, pH 10.5) was added to bring the sample to alkaline conditions. The samples were vortex mixed, and 6 mL of an *n*-hexane:ethyl acetate (7:3, v/v) mixture was added. The samples were extracted by rotation for 15 min following centrifugation at 2000 × g for 20 min. The organic layer was removed and re-extracted with 6 mL of *n*-hexane: 2-propanol (99:1, v/v) mixture. The collected organic layers were evaporated to dryness under a nitrogen gas flow at 40 °C. The sample residue was reconstituted with 250 μL of acetonitrile and an aliquot (5 μL) was injected into the LC/DAD/MS system.

4. Results and discussion

The blood and urine post-mortem samples were initially subjected to a qualitative analysis. Screening was performed for basic, acidic, and neutral drugs and volatiles using standard chromatographic methods. These methods included gas chromatography, liquid chromatography and enzyme immunoassay. The blood alcohol result (analysed by a headspace GC/FID technique) was positive (0.53 g/L). No other drugs were detected in post-mortem blood.

The calibration curve for tianeptine in blood was linear, ranging from 0.01 to 10 μg/mL ($r^2 = 0.9993$, seven calibration points, in triplicate). The detection limit of tianeptine in blood was 0.001 μg/mL (LOD, S/N = 3) and the lower limit of quantification (LOQ, S/N = 10) was 0.01 μg/mL. Analytical recovery was tested at concentration levels 0.1, 5 and 10 μg/mL and was determined by comparing the representative peak areas of tianeptine extracted from drug-free blood spiked with the peak area of a methanolic standard of the same concentration. The mean recovery was 76% with a coefficient of variation of ± 5.3%. For intra-day and inter-day precision determinations, five replicate analyses were performed on each of the three concentrations studied. The method proved to be accurate for tianeptine, both in terms of intra-day and inter-day analysis, with coefficients of variation (CV) of less than 10%.

Tianeptine was quantified by selected ion-recording of *m/z* 437 (using *m/z* 292 as the confirmation ion) and *m/z* 409 for the MC_5 metabolite. The SIR mass chromatograms and mass spectrum in SCAN mode (*m/z* 437) of the tianeptine detected in the blood sample of our reported case are shown in Fig. 2.

Since the procedure proved to be sensitive, selective and reproducible, the method developed was applied to the fatal

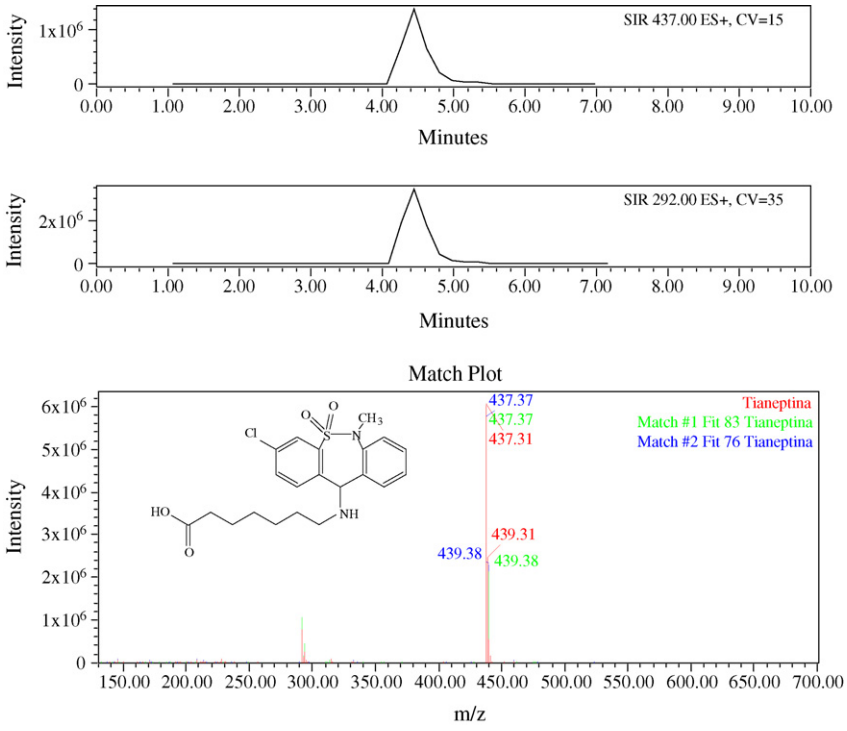

Fig. 2. SIR mass chromatograms and mass spectrum in SCAN mode (*m/z* 437), of tianeptine in post-mortem blood sample (reported case).

case presented. Tianeptine was detected in all the specimens analysed. Toxicological results revealed the following tianeptine concentrations in the post-mortem samples: cardiac blood 5.1 μg/mL, urine ∼2.0 μg/mL, liver ∼23 μg/g and stomach contents approximately ∼22 mg (in a volume of 200 mL). The high concentration in the stomach contents proves acute tianeptine intoxication. The main pharmacologically active metabolite MC_5 was only detected in the urine and the liver, but was not quantified due to the lack of adequate reference standard.

The antidepressant efficacy and favourable tolerability and pharmacokinetic profiles of tianeptine in patients with depression [4], including those with chronic alcoholism, have been shown [13]. Others authors stated that alcohol co-administration could decrease tianeptine absorption rate and lower its plasma levels by about 30% [14]. However, in our case, even assuming the possibility of a decrease in tianeptine's absorption by the presence of alcohol, the concentration found is still high and thus, this absorption decrease percentage would not be relevant. To our knowledge, only a few cases of tianeptine abuse have been reported [15–18]. However, there are no published reports of fatal cases due to this antidepressant. Only a brief statement was found reporting the quantitative results in post-mortem blood samples of three acute tianeptine overdoses. Two of these fatal cases had post-mortem blood tianeptine levels of 4.0 and 4.2 μg/mL (plus 0.8 g/L ethanol) [1]. The tianeptine blood concentrations achieved by these authors, reporting fatal cases with this antidepressant, were lower in comparison with our results Table 1.

The simultaneous analysis and consequent acquisition using two different detectors, photodiode array and mass spectrometer, allowed us to detect this antidepressant with higher selectivity and specificity. The present method was also developed and validated for other post-mortem samples, since no previous published data had confirmed tianeptine post-mortem tissue distribution. In our case, it was proved that tianeptine was quantified in high concentrations in all the post-mortem samples.

The absence of other suitable direct causes of death (macroscopic or histological) and the positive results achieved with the toxicological analysis led the pathologist to rule that death was due to suicidal ingestion of tianeptine in combination with use of alcohol.

Table 1
Selected ions, LOD, LOQ and linearity range of tianeptine in blood samples

Selected ions (*m/z*) and cone voltage		Limits (ng/mL)		Linearity (μg/mL)	R^2
Quantitation	Confirmation	LOD	LOQ		
437 25 V	292 35 V	1 S/N < 3	10 R.S.D. < 20%	0.01–10	0.9993

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