A Fatality Related to Two Novel Hallucinogenic Compounds: 4-Methoxyphencyclidine and 4-Hydroxy-*N*-methyl-*N*-ethyltryptamine

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In this case report, we present an evaluation of postmortem concentration distribution of the hallucinogenic compound 4-methoxyphencyclidine (4-MeO-PCP) in a fatality principally attributed to this drug. Another hallucinogen, 4-hydroxy-*N*-methyl-*N*-ethyltryptamine was also detected, but was not quantitated. A man—who had a history of recent 'strange' behavior—was found deceased, on his bed, in his locked room. Toxicology testing, which initially screened positive for phencyclidine (PCP) by ELISA, subsequently detected and confirmed the two hallucinogens by gas chromatography–mass spectrometry. 4-MeO-PCP concentrations were then quantified by a specific secondary testing technique. The peripheral blood concentration was 8.2 mg/L compared with the central blood concentration of 14 mg/L. The liver concentration was 120 mg/kg, the vitreous was 5.1 mg/L, the urine was 140 mg/L and the gastric contents contained 280 mg. PCP was not detected, but therapeutic concentrations of venlafaxine, olanzapine, lorazepam and hydroxyzine were confirmed. The cause of death was certified due to acute mixed drug intoxication, and the manner of death was certified as accident.

Introduction

The emergence of novel synthetic psychoactive drugs and their availability through Internet sites has raised concerns about their lack of formal toxicological investigations and potential harms (1). Among the numerous hallucinogenic compounds, drugs related to the dissociative anesthetic phencyclidine (PCP) have been recently described (2). Eticyclidine, MeO-PCP, 3-MeO-PCP and 4-methoxyphencyclidine (4-MeO-PCP) are believed to exert similar behavioral effects on PCP and ketamine (1, 2).

4-MeO-PCP (Figure 1), for example, is reported to have desired effects which include euphoria, empathy, dissociation from the physical body and hallucinations, but may also be accompanied by adverse effects such as dizziness, confusion, psychomotor agitation and cognitive impairment (2, 3). Furthermore, symptoms of toxicity similar to those associated with acute toxicity to methoxetamine—including significant tachycardia and hypertension—may be expected with these PCP analogs (2, 4).

Another group of hallucinogenic compounds, based on structural similarities to psilocin, includes tryptamines such as 4-acetoxy-*N*-methyl-*N*-ethyltryptamine (4-acetoxy-MET). Also known as metacetin or 4-AcO-MET, this compound is a homolog of *O*-Acetylpsilocin (4-AcO-DMT). It is a novel compound with very little history of human use. A related but a lesser known psychedelic drug is 4-hydroxy-*N*-methyl-*N*-ethyltryptamine (4-HO-MET). It is also a structural and functional analog of psilocin as well the 4-hydroxyl analog of methylethyltryptamine (MET). Similarly, this drug has been infrequently encountered in human use (Figure 1).

In this report, for the first time, postmortem concentrations of 4-MeO-PCP are described in peripheral blood, central blood, liver, vitreous humor, urine and gastric contents in a death principally related to this compound. Minor modifications to a previously reported confirmatory analytical procedure were employed (5). Although 4-HO-MET was also detected and confirmed (by gas chromatography–mass spectrometry, GC–MS), it was not quantitated.

Methods

Case report

This 54-year-old man (188 cm, 128 kg) lived in an independent living facility due to his psychiatric illnesses including schizoaffective depressive disorder. He was found unresponsive in bed during a welfare check conducted by the facility manager on the morning of 31 December 2014, initiated by his concerned mother when he did not return her phone calls. His death was confirmed at the scene by emergency medical personnel without resuscitation. His medical history included hypertension and substance abuse. He was known to use alcohol, marijuana, methamphetamine and PCP. According to his mother, he had been sober from drugs and alcohol for many years, but at some point ordered white powder from the Internet she related as '3-MeO-PCP'. A small baggie labeled '#2' containing a fine crystalline residue was found in his room on the dresser. No other drug paraphernalia was found. During an emergency room visit for abnormal behavior on 20 September 2014, he confessed to the staff that he was taking '3-MeO-PCP' to treat his depression. He also admitted to having syncopal episodes while taking the drug.

A complete autopsy was conducted on 1 January 2015 at 10:50 hrs approximately 25 h after he was found, and documented pulmonary congestion and edema (right 840 g; left 880 g), and foam in his mouth and airway consistent with acute drug intoxication. His heart was mildly enlarged (510 g) with mild chamber dilation. There was congestive hepatosplenomegaly and minimal hepatic steatosis. There were no traumatic injuries or other significant findings.

Postmortem specimen collection

All specimens analyzed were collected at autopsy at the San Diego County Medical Examiner's Office. Peripheral blood (~20 mL) was drawn from the left common iliac vein (blood returning from the leg and visually identified in the pelvis at autopsy) and stored in standard glass tubes containing sodium fluoride (100 mg) and potassium oxalate (20 mg). Central blood was collected directly from the heart and placed into identical tubes. Sections of the right lobe of liver were collected and stored in an opaque plastic 0.118 L container without preservative. Vitreous humor samples were withdrawn from the eyes with a syringe and stored in a glass tube without preservative. Urine was collected into an opaque plastic 0.118 L container without preservative. The entire gastric contents were also collected
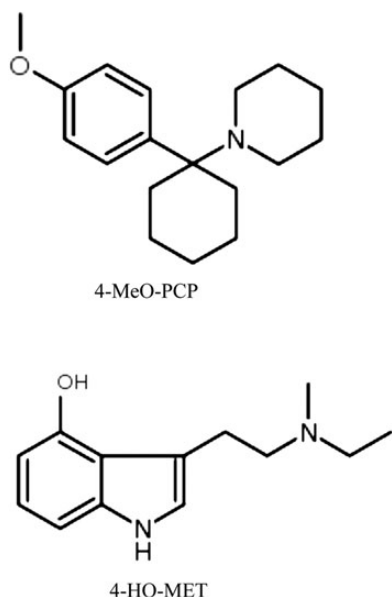
Figure 1. Chemical structures.

into an opaque plastic 0.118 L container without preservative. All samples were stored at 4°C until analyzed within 3 months of collection.

Toxicology

A comprehensive toxicological screening regimen was performed. Postmortem blood was screened for alcohol and volatile compounds (GC-flame ionization detector headspace), 12 drugs of abuse panel by ELISA (cocaine metabolite, opiates, methamphetamine, benzodiazepines, cannabinoids, fentanyl, phencyclidine, oxycodone, methadone, zolpidem, carisoprodol and buprenorphine; Immunalysis, Inc., Pomona, CA, USA), an alkaline drug screen by GC-MS following solid-phase extraction and an acid/neutral drug screen with HPLC-photodiode array detection following specimen precipitation with acetonitrile. An additional screen (GC-MS) was also performed on the urine specimen. Following routine practice, significant positive results were confirmed and quantified by subsequent and specific techniques.

Alkaline blood drug screen (GC-MS)

The drug screening procedure has been utilized by this laboratory for over 7 years, and has been previously described (6). Briefly, it consists of a routine solid-phase extraction technique utilizing SPEWare Trace J extraction cartridges. Two milliliters of calibrators, controls and casework were extracted after the addition of cyclizine (200 μL, 5 μg/mL: internal standard) and ascorbic acid (200 μL, 2% solution). Samples were then precipitated with zinc sulfate (5 mL, 5% methanolic solution) and treated with sodium acetate buffer (4 mL, pH 6.0). SPE cartridges were pretreated with methanol (3 mL), deionized water (3 mL) and sodium acetate buffer (2 mL) before the addition of samples. Following the extraction of samples, the SPE cartridges were washed with deionized water (3 mL), acetic acid (0.1 M; 2 mL) and methanol (3 mL). Cartridges were dried for 3 min and the specimens were eluted with dichloromethane/isopropanol/14.8 M ammonium hydroxide solution (78/20/2). Samples were then

evaporated (30°C, under a stream of nitrogen) and reconstituted with ethyl acetate (150 μL). One microliter (splitless) of each extract was then injected onto the GC-MS system to attain separation and identification of alkaline drugs. A 15 m, 0.25 mm diameter, 0.25-μm film thickness analytical column (Phenomenex Zebron, ZB-5MS) was used with helium as the carrier gas (1.1 mL/min). The inlet temperature of the gas chromatograph (Agilent Technologies, 7890A, Santa Clara, CA, USA) was 250°C, and oven temperature was initially 85°C, ramped 40°C/min up to 170°C (held 4 min), then 40°C/min to 190°C (held 5 min) and finally 10°C/min up to 300°C (held 7 min). The MS Aux was 280°C. The mass selective detector (Agilent Technologies, 5975C) was set in scan mode with a solvent delay of 2.64 min. Peak identification was determined by relative retention time (relative to the internal standard: RRT), and then mass spectral matching from a commercial MS library and/or SWGDRUG mass spectral library (http://www.swgdrug.org; at least 70% match).

4-MeO-PCP confirmation analysis

Materials

All solvents and chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA) and were of analytical grade or better. Borosilicate glass test tubes were used for all phases of the extraction (VWR International, Radnor, PA, USA). The 4-MeO-PCP drug standard used in the calibration formulations and the PCP internal standard were both purchased from Cerilliant Corporation (Round Rock, TX, USA).

Extraction

4-MeO-PCP was confirmed and quantitated utilizing minor modifications of a previously described procedure for basic drugs using GC coupled with a nitrogen phosphorous detector (NPD) (5). The analysis included whole-blood (porcine) calibrators (0.10, 0.25, 0.50, 0.75, 1.0 and 2.0 mg/L), liver homogenate (porcine) calibrators (0.25, 0.50, 0.75, 1.0, 2.0 and 3.0 mg/kg), case samples (whole blood, liver, urine, vitreous and gastric) and positive and negative controls that were subjected to an alkaline liquid/liquid extraction procedure. To 1 mL of calibrators, controls and casework, deionized water (5 mL) was added and vortexed. Next, working internal standard (100 μL PCP, 10 mg/L) was added and vortexed. Samples were made alkaline by the addition of concentrated ammonium hydroxide (1 mL) before being vortexed again. 1-Chlorobutane (6 mL) was added and tubes were capped and mixed on a mechanical rocker for 30 min. Samples were then centrifuged for 5 min at ~2,400 g. Approximately 200 mg of sodium sulfate was added to each tube to suppress emulsions and the tubes were centrifuged for another 5 min at 2,400 g. The top organic layer was then transferred to new test tubes. About 1.0 N hydrochloric acid (3.5 mL) was added and the tubes were mixed for 30 min. The tubes were then centrifuged for 5 min at 2,400 g before the top organic layer was aspirated to waste. The remaining aqueous portion was made alkaline with concentrated ammonium hydroxide (1 mL) and vortexed. 1-Chlorobutane (3 mL) was added and tubes were again mixed for 30 min. Samples were then centrifuged for 5 min at 2,400 g before the top organic layer was transferred to new test tubes. The organic layer was dried under a stream of nitrogen at 30°C. Samples were

reconstituted with methanol (100 μL) before being transferred to autosampler vials for analysis by GC-NPD.

Chromatographic conditions

The following GC-NPD conditions were used in the analysis. The samples (1 μL) were injected splitless into a GC (7890A; Agilent Technologies) equipped with a capillary column (DB-17, 15 m, 0.25 i.d., 0.25 μm; Phenomenex, Torrance, CA, USA). The injector temperature was 250°C and the detector was set to 280°C. The initial oven temperature was 50°C and was ramped at 35°C/min to 275° C and held for 7 min. Total runtime after injection was 13.5 min. Hydrogen was used as the carrier gas at a constant rate of 2 mL/min. Retention times for PCP and 4-MeO-PCP were 5.2 and 6.0 min, respectively.

Validation

The limit of detection was 0.05 mg/L and limit of quantitation, determined from the lowest calibration concentration, was 0.10 mg/L for whole blood and 0.25 mg/kg for liver. Control samples, prepared independently at 0.50 and 1.5 mg/L in whole blood, measured 0.47 ± 0.03 mg/L (mean $\pm$ standard deviation; $N = 4$) and 1.28 ± 0.06 mg/L (mean $\pm$ standard deviation; $N = 4$), respectively. Matrix effects were evaluated by extraction and analyses of comparable control specimens (0.5 and 1.5 mg/L) prepared in water

and a liver homogenate. Levels of 0.47 ± 0.02 mg/L (mean $\pm$ standard deviation; $N = 3$) and 1.34 ± 0.18 mg/L (mean $\pm$ standard deviation; $N = 5$) were determined for water, and 0.45 ± 0.06 mg/kg (mean $\pm$ standard deviation; $N = 4$) and 1.47 ± 0.11 mg/kg (mean $\pm$ standard deviation; $N = 4$) were determined for the liver homogenates. Both extracted blank (extract containing no additives) and negative control (extract containing only internal standard) specimens confirmed a lack of interference and/or contamination.

Results and discussion

4-MeO-PCP was initially detected by ELISA screening for PCP. The screen, established in this laboratory with 5.0 ng/mL of PCP as a reference, provided a positive result with 41% binding compared with a negative sample (100% binding). In the case presented, the central blood demonstrated 2% binding—a clearly positive finding. PCP was not detected by a routine confirmation method. The PCP (GC−MS selective ion monitoring) method had limits of detection and quantitation of 2.0 and 5.0 ng/mL, respectively. 4-MeO-PCP was subsequently presumptively identified in the peripheral blood from the SWGDRUG mass spectral library (http://www.swgdrug.org) following solid-phase extraction using a GC−MS alkaline screening method (6) (Figure 2). It was confirmed with extraction, and a full mass spectral scan
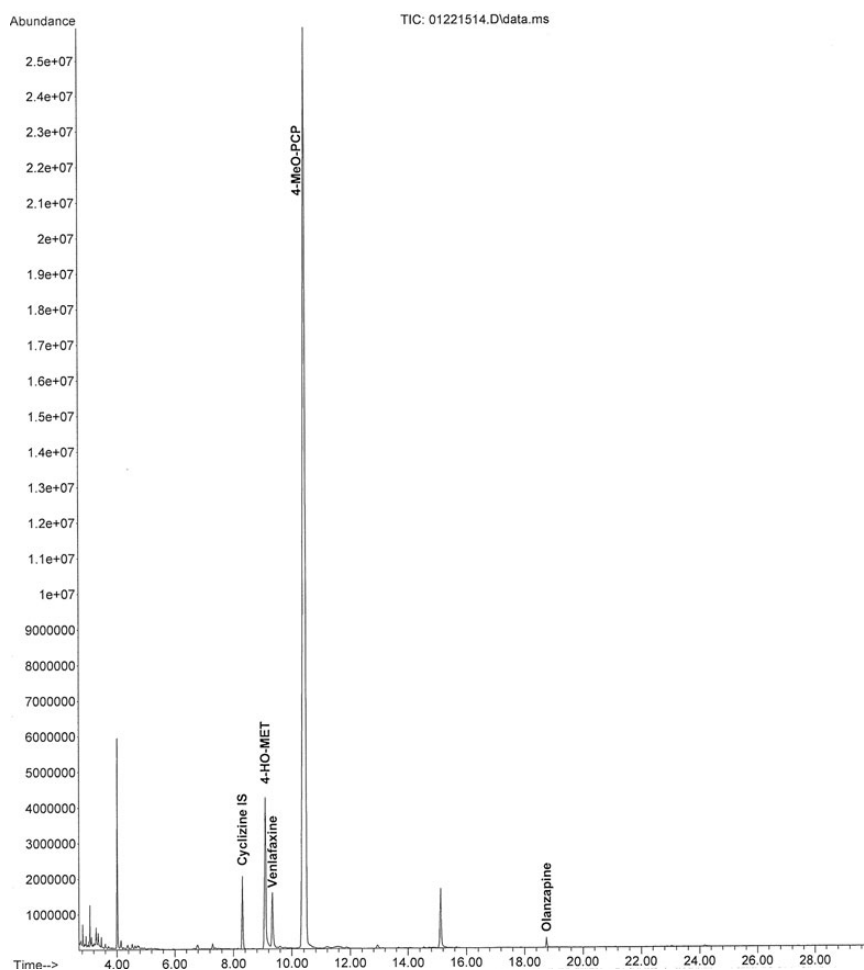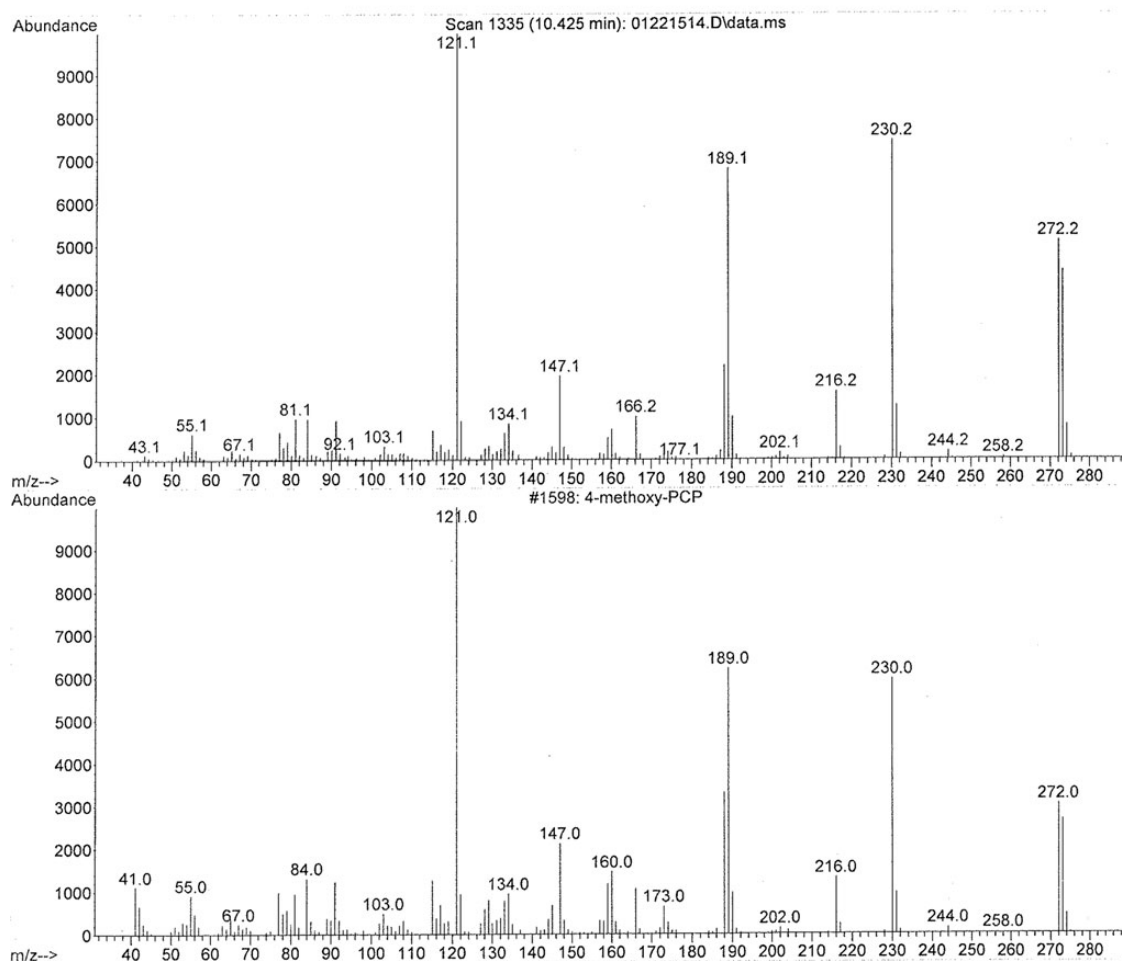

Figure 2. Chromatogram from alkaline blood drug screen (GC−MS).

Figure 3. Library match of electron ionization mass spectra of 4-MeO-PCP in case blood.

of a pure stock of the compound, at 10.4 min (RRT = 1.25; compared with internal standard cyclizine) with significant ions of 121, 189, 230, 272 and 147 (Figure 3).

4-MeO-PCP concentrations were then quantified by a specific GC-NPD method (described here). The peripheral blood concentration was measured at 8.2 mg/L compared with the central blood concentration of 14 mg/L. The liver concentration was 120 mg/kg, the vitreous was 5.1 mg/L, the urine was 140 mg/L and the gastric contents contained 280 mg. The central blood/peripheral blood (C/P) ratio was 1.7 and the liver/peripheral blood (L/P) ratio was 15 L/kg. Based on established C/P drug ratio data (7) and given recent information documenting the L/P ratio as a marker for postmortem redistribution (PMR) (8–10), these data suggest a 'moderate' propensity for 4-MeO-PCP PMR. As this deduction results from a single observation, however, it should be viewed with caution.

Similarly, 4-HO-MET was presumptively identified in the peripheral blood from the SWGDRUG mass spectral library (http://www.swgdrug.org) following solid-phase extraction using the GC–MS alkaline screening method (Figure 2). It was confirmed with extraction, and a full mass spectral scan of a pure stock of the compound

(Cayman Chemical Company, Ann Arbor, MI, USA), at 9.1 min (RRT = 1.09; compared with internal standard cyclizine) with ions of 72, 218, 44, 146 and 117 (Figure 4). 4-HO-MET concentrations, however, were not quantitated in postmortem specimens.

The white powder residue in the baggie collected at the scene (in the decedent's room) was identified by the US Drug Enforcement Agency (DEA) as 4-acetoxy-N-methyl-N-ethyltryptamine (or 4-acetoxy-MET). No other compounds including 3-MeO-PCP or 4-MeO-PCP were identified in the sample. 4-Acetoxy-MET is a hallucinogenic tryptamine: it is the acetate ester of 4-HO-MET; and a homolog of 4-AcO-DMT. Sold as a research chemical by online retailers, this compound has been rarely described in human use. It is expected that it is quickly hydrolyzed into free phenolic 4-HO-MET by serum esterases. Accordingly, there was no evidence of 4-acetoxy-MET in the postmortem blood or the urine of the current case.

In addition to these two novel hallucinogenic compounds, therapeutic concentrations of venlafaxine (0.51 mg/L), olanzapine (0.42 mg/L), lorazepam (<0.05 mg/L) and hydroxyzine (detected) were confirmed in the peripheral blood. Consequently, the cause of death was certified due to acute mixed drug intoxication. The manner of death was certified as accident.

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Library Searched : C:\Database\CaymanSpectralLibrary.L
Quality          : 96
ID               : 4-hydroxy MET
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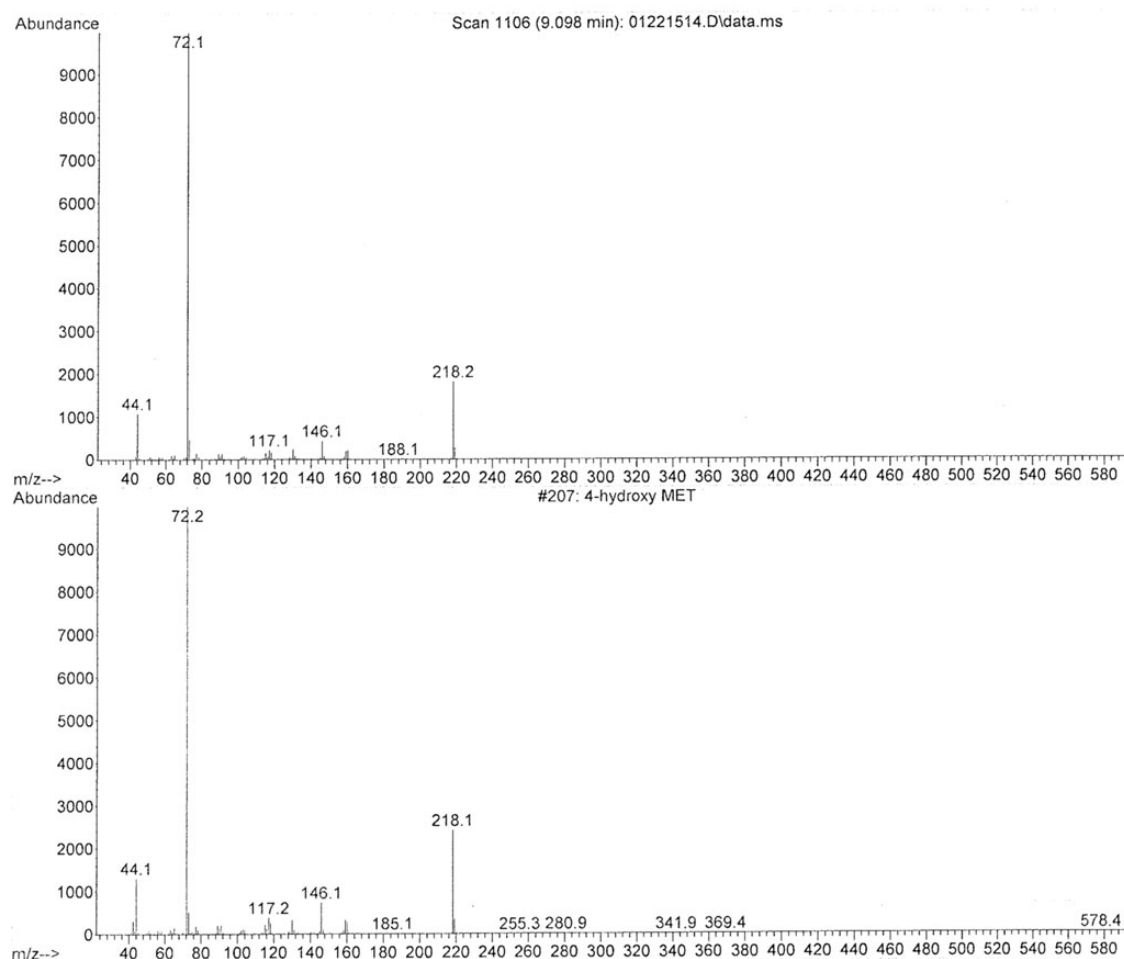

Figure 4. Library match of electron ionization mass spectra of 4-HO-MET in case blood.

Acknowledgments

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