Two Fatal Intoxications with the New Designer Drug Methedrone (4-Methoxymethylcathinone)

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Abstract
We report two fatalities involving the new designer drug methedrone, 4-methoxymethylcathinone. Blood was extracted with ethyl acetate after the addition of sodium hydroxide followed by evaporation and derivatization with TFAA before gas chromatography–mass spectrometry analysis. Hair was decontaminated and cut into segments, and after overnight extraction with acetonitrile/methanol/20 mM ammonium formate buffer (pH 3) (10:10:80), samples were analyzed by liquid chromatography–tandem mass spectrometry. The first case was treated in hospital, and blood was collected for drug screening. The concentration of methedrone in antemortem blood was 13.2 µg/g and in postmortem femoral blood 8.4 µg/g. The second case presented with 9.6 µg methedrone/g femoral blood, and in a hair sample, methedrone was detected in five short segments suggesting exposure to the drug during the months prior to death. In living abusers, the blood concentration range was 0.2–4.8 µg/g (n = 11). We conclude that use of methedrone may result in accidental death owing to its toxic properties and that the blood concentrations found in the two cases are close to those seen in the living. This suggests a rather narrow “therapeutic” window and emphasizes the danger in taking this kind of drug for recreational purposes.

Introduction
There are numerous psychotropic substances, so-called designer drugs, available through the internet. Even though governments work steadily for the scheduling of these substances to reduce their spread, providers of these drugs are obviously still ahead of the authorities. Over the years, a number of non-controlled substances have hit the internet drug market as a “safe” alternative to scheduled drugs (1) that occasionally lead to deadly results (2,3) or resulting in intoxications ending up in the emergency room (4). The substance mephedrone was until recently widely used in Sweden (2), and numerous cases were reported to the Swedish Poison Center in 2008 until several deaths occurred, resulting in mephedrone being scheduled as a narcotic (5). After this, the demand for mephedrone decreased somewhat, and fewer cases that arrived at the National Board of Forensic Medicine were found positive for the drug. Figure 1 shows the number of positive cases for mephedrone during parts of 2008 and 2009. However, during 2009, a new unscheduled drug began to be distributed. At first this was apparent when calls from hospitals concerned methedrone instead of mephedrone, and later during the summer it was confirmed in drug seizures made by the police and debated in the news. Here, we report two deaths related to the cathinone analog methedrone (4-methoxymethylcathinone). The structure of methedrone and some related substances are shown in Figure 2. There is little but anecdotal information available on methedrone. According to users, the doses of methedrone range from as low as 50 to 500 mg, and the duration of effects range from 45 min to 2 h.

Autopsy cases
Case 1
A 23-year-old man (166 cm, 70 kg) became sick at a party after ingestion of a recreational drug. Upon arrival to the emergency department at the hospital, he was unconscious with a body temperature of 42°C (107.6°F). Despite resuscitative efforts, he suffered complete organ failure and died the following day, 16 h after admission. At the hospital, a blood sample was collected and submitted for drug screening. An autopsy was performed three days after death. The postmortem examination

Figure 1. Mephedrone-positive cases analyzed at the National Board of Forensic Medicine during parts of 2008 and 2009. The first positive case was found in June 2008.
documented pulmonary edema and acute pulmonary congestion. The right and left lungs weighed 934 and 922 g, respectively. Postmortem samples, including cardiac and femoral blood, urine, and vitreous humor, were collected during autopsy and submitted for toxicological analysis. The ante-mortem blood sample contained 13.2 µg/g of methedrone and in the postmortem femoral blood 8.4 µg/g was found. In addition, diazepam (0.05 µg/g), midazolam (0.02 µg/g), and lidocain (0.6 µg/g) were detected, although these substances were administered at the hospital.

Case 2
A 19-year-old man (185 cm, 81 kg) was found unconscious at home in an empty bathtub. During ambulance transport, he developed seizures and became lifeless. Upon arrival at the hospital, he had stopped breathing and heart activity was negligible. Resuscitation was unsuccessful. Body temperature was not recorded. Six days later, an autopsy was performed, and the internal examination documented pulmonary edema and congestion (right and left lungs, 866 and 747 g, respectively). Femoral blood, urine, vitreous humor, and hair samples were collected during autopsy and submitted for toxicology analysis. The only positive finding was 9.6 µg methedrone/g blood. In five short segments of hair, the concentrations of methedrone were evenly distributed (segment one: 37 ng/mg, segment two: 33 ng/mg, segment three: 29 ng/mg, segment four: 29 ng/mg, and segment five: 36 ng/mg) suggesting a chronic intake of methedrone over the previous months before death. In addition to this, a concentration of 0.037 g/dL of acetone was found in the urine.

Reference values for methedrone
During the autumn of 2009, 11 findings of methedrone in blood were reported in suspected petty drug offenders. The blood concentrations are tabulated in Table I. Furthermore, 13 urine samples from suspected petty drug offenders were also found positive for methedrone.

Experimental

Routine analysis of drugs and alcohols
Analysis of medications and drugs of abuse were performed in femoral blood, collected by cutting off the iliac vein using a knife and pressing blood from the femoral vein into a 20-mL plastic tube to which 1% potassium fluoride was added. Hypnotics, tranquilizers, antidepressants, and opioid analgesics were extracted from blood by liquid–liquid extraction and analyzed by gas chromatography (GC) using nitrogen-phosphorus detection (6). Alcohols and acetone were analyzed with headspace GC, according to a previously described method (7). Narcotic drugs were analyzed in urine or blood using immunoassays and in-house GC–mass spectrometry (MS) methods. Hair was screened for drugs of abuse using an liquid chromatography (LC)–MS–MS method previously described (8).

Chemicals and reagents
Methedrone was a gift from the National Laboratory of Forensic Sciences (Linkoping, Sweden) and shown to be 94.7% pure. Amphetamine-d₅ and MDMA-d₅ were purchased from Cerilliant (Austin, Texas). All other chemicals and solvents were of analytical grade or better.

Quantitation of methedrone in blood
Methedrone was extracted from blood (0.2 g) with 3 mL ethyl acetate after adding 0.5 mL 2 M sodium hydroxide (NaOH) and 0.2 µg MDMA-d₅ (internal standard). After centrifugation, the organic layer was transferred to a new tube and gently evaporated under a stream of nitrogen, leaving approximately 50 µL of solvent. After derivatization with 50 µL TFAA at 60°C for 15 min, the samples were evaporated to dryness, reconstituted in 40 µL ethyl acetate, and analyzed with GC–MS.

GC–MS analysis in selected ion monitoring mode (SIM) and electron impact (EI) spectra were performed using an Agilent (Palo Alto, CA) 6890 GC system interfaced to a 5973 mass selective detector. For the injections, a GC PAL from CTC Analytics was used. The column was an Agilent HP-5 MS (5% phenyl-methylsilicone) (30 m × 0.25-mm i.d., 0.25-µm film thickness). Helium was used as carrier gas at a constant flow of approximately 0.9 mL/min. The injections were made in splitless mode with a splitless time of 1.0 min. The volume injected was 1.0 µL.

Full-scan spectra for a positive identification of methedrone in blood extracts were obtained in EI mode, recording from m/z 40 to 550. Comparison of retention time and full-scan spectra with a standard of methedrone was made. Full-scan spectra of extracted urine samples were also performed without derivatization.

The amount of methedrone was determined using the area ratios of m/z 158 from MDMA-d₅-N-TFAA and m/z 135 from methedrone-N-TFAA with m/z 154 and m/z 289 as qualifier ions in SIM analysis.

Reference:

Figure 2. Structures of methedrone and related substances: methedrone (A), mephedrone (B), cathinone (C), MDMA (D), and methylone (E).
Validation for the analysis of methedrone in blood

Working range was verified by analysis of triplicates at seven levels from 0.1 to 10 µg/g blood. The within-day \((n = 5)\) imprecision were estimated by analyzing five replicates at two levels, 0.5 and 5.0 µg/g blood. Calibrators and controls were made by adding standard solutions to drug-free blood. Final calibration levels were at 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 µg/g blood.

Quantitation of methedrone in hair

The hair sample was cut into five segments (three 5-mm segments and two 10-mm segments, 7 to 17 mg) were weighed into glass tubes. The hair segments were washed for 15 min at 37°C with 2 mL isopropanol followed by three 30-min washes in 2 mL 0.01 M phosphate buffer pH 6 and finally again with 2 mL isopropanol.

To the hair samples were added 0.5 mL acetonitrile/methanol/20 mM ammonium formate buffer (pH 3) (10:10:80) and 25 µL of amphetamine-d₅ (internal standard), and the samples were incubated in a water bath (with orbital shaking) at 37°C for 18 h. A 150-µL aliquot was transferred to an autosampler vial, and 1 µL was injected onto the chromatographic system.

The LC–MS–MS system consisted of a Waters (Milford, MA) ACQUITY UPLC® with a binary solvent manager, sample manager, and column manager. This was connected to an API 4000™ triple-quadrupole instrument (Applied Biosystems/MD Sciex, Stockholm, Sweden) equipped with an electrospray interface (TURBO V™ source, TurboIonSpray® probe) operating in the multiple reaction monitoring (MRM) mode. Ion spray voltage was set to 5500 V. Nitrogen was used as nebulizer gas (345 kPa), heater gas (517 kPa at 500°C), curtain gas (207 kPa), and as collision-activated dissociation gas (set on 5). Ultra-performance liquid chromatography (UPLC) was performed using a Waters ACQUITY UPLC ethylene bridged hybrid (BEH) C₁₈ column (50 × 2.1 mm, 17 µm) preceded by a 0.2-µm column filter operated at 0.6 mL/min with a total run-time of 3 min. Mobile phase A consisted of 0.05% formic acid in 10 mM ammoniumformate and phase B was 0.05% formic acid in acetonitrile. The chromatographic system was run in a linear gradient from 5 to 65% phase B in 2 min, then increased to 95% phase B for 0.5 min, followed by a 0.5 min equilibration with 95% phase A. The injection volume was 1 µL, and the column manager temperature was set to 60°C. Instrument control, integration, and calculation were performed using Analyst™ version 1.4.2 software (Applied Biosystems). Quadratic regression analysis with \(1/x\) weighting was used for the calibration curves. The final MRM method included three transitions 194.2/176.1, 194.2/161.2, and 194.2/146.1 for methedrone and 141.3/124.1 for amphetamine-d₅ with a dwell time of 10 ms for each transition. Criteria for identification was based on a qualifier ratio within 25% of the target ratio.

Validation for the analysis of methedrone in hair

Working range was verified by analysis of triplicates at seven levels from 2 to 100 ng/mg based on a sample weight of 10 mg hair. The within-day \((n = 5)\) imprecision was estimated by analyzing five replicates at two levels, 5 and 80 ng/mg. Calibrators and controls were prepared by adding standard solutions to drug-free hair. Final calibration levels were 2, 4, 10, 25, 50, 75, and 100 ng/mg.

Results and Discussion

A significant finding in these cases was that no other drugs were found even after a comprehensive drug screening. Hair analysis revealed no other drugs in case 2, whereas the detection of methedrone in five segments suggests that the use of methedrone had been ongoing for quite some time prior to the last dose. In addition, none of the cases included any findings

<table>
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<tr>
<th>Case</th>
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<th>Sex</th>
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</tr>
<tr>
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<td>male</td>
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</tr>
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<td>female</td>
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<tr>
<td>Mean</td>
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<th>Accuracy (%)</th>
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<tr>
<td>High (5.0 µg/g)</td>
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<table>
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<tr>
<td>High (80 ng/mg)</td>
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pointing towards suicide but rather that both represent accidental poisonings. This suggests that methedrone alone was responsible for these deaths and that the concentrations in femoral blood actually represent fatal levels. When compared to levels found in living users (Table I), the safety gap seems small. With unknown doses, the range was from 0.1 to 4.8 µg/g blood, whereas the fatalities had concentrations of more than 8 µg/g.

The drug concentrations in blood were comparatively high, and analysis could readily be performed on a small volume of blood using GC–MS and on only a few milligrams of hair using LC–MS–MS. A summary of the validation is presented in Table II. For hair, mean values of triplicates over the working range were within 7% of the target value, and the coefficients of variation at each level were between 0.2 and 19%, depending on the concentration.

For blood, mean values for triplicates over the working range were within 11%, and the variations at each level were between 0.2 and 3.7%.

Analytically, methedrone eluted very close to MDMA both in our GC–MS and LC–MS chromatography and has the same molecular mass and even shares some fragments with MDMA. The LC–ESI-MS positive product ion spectra of the pseudomolecular ion m/z 194.2 gave prominent fragments at m/z 176.1, 161.2, 146.1, and 118.1.

Structurally, methedrone resembles not only MDMA but also methylone (Figure 2), which is another designer drug originally synthesized in mid-1990s as a presumptive antidepressant (9,10). In pharmacological studies of methylone, a metabolite, 3-OH-4-methoxymethylcathinone, resulting from the cleavage of the methylene bridge, was identified (9). This metabolite might also be formed through 3-hydroxylation of methedrone. Methylone and methedrone might also both have N-demethylated metabolites. These pathways were also corroborated by the works of Zaitu et al. (11) that found the corresponding metabolites for β-keto-MDBB and β-keto-MDEA. They also concluded that reduction of the β-ketone was superior to the N-demethylation reaction in humans. In the blood sample from case 1 we could identify a peak that corresponds to the N-desmethylmetabolite (Figure 3). Also, in the urine sample from case 2, a peak with the EI fragments (undervatized) m/z 58, 93, and 121 was found. The corresponding fragments from methedrone were m/z 58, 107, and 135 pointing at an intact side chain with O-demethylation at the para-methoxy group (Figure 4).

Conclusions

We conclude that recreational use of methedrone might lead to accidental death, owing to its toxic properties and that the blood concentrations found in these two fatal cases are close to those
found in the living. This suggests a rather narrow therapeutic window and emphasizes the dangers associated with the use of methedrone. Partly as a result of these two fatalities, the Swedish government decided to schedule methedrone as a narcotic drug effective December 9, 2009.

References


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