Forensic Science International 243 (2014) 68–73

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Toxicological findings in a fatal multidrug intoxication involving mephedrone☆

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ARTICLE INFO

Article history:
Available online 6 May 2014

Keywords:
Mephedrone
Distribution
GC/MS
Forensic toxicology
Intoxication

ABSTRACT

The distribution of mephedrone in the body fluids and tissues of a subject found dead after the concomitant intake of cocaine and mephedrone is reported. Mephedrone (4-methylmethcathinone) is a designer drug of the phenethylamine family that is able to cause central nervous system stimulation, psychoactivity and hallucinations and that is becoming popular among youth as a recreational drug. Mephedrone has been available in Europe since 2007, and it is sold through the internet and by local shops as bath salt or plant food.

In the case reported here, a 25-year-old man was found dead in the apartment of a friend after a night spent in several local clubs. A fragment of a blue diamond-shaped pill was found in the pocket of the trousers worn by the decedent. During the autopsy, no evidence of natural disease or trauma was found to account for this death. Blood, urine and gastric content samples were collected and submitted for toxicological analysis. Moreover, bile, brain, lung and hair samples were collected as additional matrices. The content of the pill was submitted to a general screening analysis in order to determine its composition.

Mephedrone was detected in the blood, urine, gastric contents and in the additional matrices using an expressly validated GC/MS method. The blood and urine concentrations were 1.33 mg/L and 144 mg/L, respectively. Contextually, cocaine and cocaethylene were found in the blood and urine specimens. The distribution of mephedrone in the body organs was evaluated by analyzing the brain, bile and lung specimens. Hair analysis revealed a past exposure to mephedrone, ketamine, MDMA and cocaine. Sildenafil was identified as the main component of the blue, diamond-shaped pill. The quantitative determination of mephedrone in several body fluids and tissues provides significant knowledge about the distribution of this new drug of abuse in the human body after massive ingestion.

1. Introduction

Mephedrone (4-methylmethcathinone) is a designer drug that is structurally similar to cathinone, which is the active ingredient of the *Khat* plant. Mephedrone was first synthesized in 1929 as a ring-substituted cathinone; its structure is closely related to that of the phenethylamine family [1]. Mephedrone produces stimulant psychoactive effects similar to those induced by amphetamines, methamphetamines, cocaine and MDMA, including hallucinations. For this reason, mephedrone is becoming increasingly popular as a recreational drug, mainly among youths, though its use is reported in several population groups, including young adults, mid-to-late adolescents and older adults [2,3]. Mephedrone is made available from several sources, such as street drug dealers, smart shops and internet suppliers. It is sold as a white or slightly yellowish powder or fine crystals under different names, including '4-MMC', 'bubbles', 'meow meow', and 'M-Cat' [4]. The most common routes for recreational use are inhalation (snorting) and oral ingestion, but due to its high water solubility, mephedrone is also

☆ "The 51st Annual Meeting of the International Association of Forensic Toxicologists (TIAFT)". September 2–3, 2013, Funchal, Medeira, Portugal. Guest edited by Professor Helena Teixeira, Professor Duarte Nuno Vieira and Professor Francisco Corte Real.

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http://dx.doi.org/10.1016/j.forsciint.2014.04.038
0379-0738/

taken by rectal insertion and intramuscular or intravenous injection [5]. Mephedrone abuse is associated with serious side effects, including increasing heart rate, chest pain, agitation, irritability and dizziness [5–8]. Although several cases of acute and lethal intoxication were reported in the literature [5–15], little is still known about the correlation between its blood concentration and its effects. Furthermore, most routine drug screening procedures do not include synthetic cathinones, preventing clear knowledge of the real consumption of these new drugs within the population and the connection between the abuse of new psychoactive substances and road and work accidents [16].

2. Case report

A 25-year-old man (weight 63 kg, height 165 cm) was found dead in the apartment of a friend, where he lay face down in a bed, in the condition of rigor and livor mortis. In the room, an empty glass with alcohol smell and a fragment of a blue, diamond-shaped pill were found; no evidence of violence was observed. The decedent's friend reported to the police that they had spent the previous night together in several local clubs, drinking a high quantity of alcohol and consuming cocaine. He also reported that his friend was a frequent consumer of new designer drugs, and he had with him a white crystal of unknown substance. The white crystal was not found at the crime scene. The death was reported to the public prosecutor who took jurisdiction of the case. To investigate the cause of death, he ordered a post-mortem examination and toxicological analysis.

2.1. Autopsy findings

The autopsy findings were irrelevant, except for a general pulmonary edema and multi-visceral congestion. The body appeared well-nourished, and the internal examination presented no evidence of natural disease or trauma to account for his death. All of the organs were normal. To execute the inherent toxicological analyses, heart blood, urine, gastric contents, brain, bile, lung and hair (length: 3 cm) specimens were collected during the post-mortem examination. Peripheral blood was not collected. All of the samples were stored at −20 °C before the analysis.

3. Experimental

3.1. Chemicals and reagents

Mephedrone hydrochloride solution (0.1 mg/L as free base) was provided by the 'Istituto Superiore della Sanità' – National Institute of Health (ISS, Rome, Italy). Amphetamine-D_6 was purchased from LGC Promochem (Milan, Italy). Sodium hydrogen carbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$), potassium phosphate monobasic (KH_2PO_4), methanol, *t*-butyl methyl ether (TBME), trifluoroacetic acid (TFA) trifluoroacetic anhydride (TFAA) and β-glucuronidase (from *Escherichia coli*) were obtained from Sigma–Aldrich (Milan, Italy). Phosphate buffer was prepared by dissolving 4.63 g of KH_2PO_4 and 11.75 g of $Na_2HPO_4{\cdot}H_2O$ in 1 L of deionized water, and the carbonate buffer was prepared by dissolving 2.12 g of Na_2CO_3 and 6.72 g of $NaHCO_3$ in 1 L of deionized water. Deionized water was obtained from a Milli-Q system (Millipore Corporate Headquarters, Billerica, USA).

3.2. Sample preparation for fluids and tissues

General screening analysis was executed on 2 mL of urine, buffered at pH 7.4 with 2 mL of 0.1 M phosphate buffer and deconjugated with the addition of 30 μL of β-glucuronidase from *E. coli* prior to incubating the mixture at 55 °C for 1 h. The sample was subsequently extracted under alkaline conditions (pH 9.6) by adding 2 mL of a 0.1 M carbonate buffer and then 10 mL of TBME. After shaking the mixture in a multimixer for 10 min, the organic layer was separated and dried under a gentle flow of nitrogen. The resulting residue was reconstituted with 50 μL of methanol. Lastly, a 1 μL aliquot was injected (splitless mode) into the gas chromatography/mass spectrometry (GC/MS) system. In addition, the blood sample was screened with an updated method for the detection of more than ninety pharmaceutical drugs and metabolites, including LSD and GHB, routinely employed in our laboratory [17]. For mephedrone quantitation in the blood, urine, gastric contents, bile and homogenized tissues, 2 mL (or 1 g) of samples were added with the internal standard (amphetamine-D_6, final concentration of 250 ng/mL or 500 ng/g) and basified with 2 mL of 0.1 M carbonate buffer and 2 drops of NaOH 1 N. After extraction with 10 mL of TBME, the organic layer was dried under a nitrogen flow, and the dry residue was derivatized with 50 μL of TFAA for 30 min at 65 °C. The resulting residue was reconstituted with 50 μL of TBME, and a 1 μL aliquot was injected (split ratio of 10:1) into the GC/MS system operating in the selected ion monitoring (SIM) mode.

3.3. Sample preparation for hair analysis

Hair analysis was performed on the entire length of the hair lock (3 cm). Approximately 100 mg of hair was twice-washed with dichloromethane and methanol (3 mL each, vortex mixed for 3 min). After complete removal of the solvent wash, the hair was dried at room temperature by a gentle nitrogen flow and subsequently cut with scissors into 1–2 mm segments. For mephedrone quantitation, the hair sample was fortified with 5 μL of an amphetamine-D_6 solution used as the internal standard at a final concentration of 0.5 ng/mg. After the addition of 2 mL of methanol, the sample was incubated at 55 °C for 15 h without stirring. Lastly, the organic phase was collected in a new test tube and 30 μL of TFA was added. The solvent was dried at 55 °C under a nitrogen stream, and the dry residue was derivatized with 50 μL of TFAA for 30 min at 65 °C. The resulting residue was reconstituted with 50 μL of TBME and a 1 μL aliquot was injected (split ratio of 5:1) into the GC/MS system operating in the SIM mode. Moreover, qualitative and quantitative hair analyses for the detection of (1) the most common drugs of abuse and (2) synthetic cannabinoids were performed by means of analytical methods used in our laboratory and described elsewhere [18,19].

3.4. Sample preparation for pill analysis

The blue diamond-shaped pill was subjected to systematic analysis for the detection of drugs and toxic substances. The pill (total weight: 271 mg) was pulverized in a mortar, and 150 mg of the resulting powder was dissolved in 5 mL of methanol. After sonication in an ultrasound bath for 1 h at 55 °C, a 1 μL aliquot of methanolic solution was injected into the GC/MS system with the mass spectrometer acquiring the spectra in the full scan mode (40–650 amu).

3.5. Apparatus and methods

Preliminary screening analyses for amphetamines, tricyclic antidepressants, barbiturates, benzodiazepines, cannabinoids, methadone, cocaine and opiates were performed on urine by the enzyme multiplied immunoassay technique (EMIT, Abbott Laboratories, IL, USA). The ethanol concentration in the blood, urine and gastric contents was determined by headspace-GC–MS. Screening analysis for unknown substances was performed using a 6890 N GC apparatus (Agilent Technologies, Milan, Italy) equipped with a 17 m fused-silica capillary