

Successful quantification of 4'-methyl- α -pyrrolidinohexanophenone (MPHP) in human urine using LC–TOF-MS in an autopsy case

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Received: 8 December 2015 / Accepted: 16 January 2016 / Published online: 4 February 2016
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Abstract The toxicological detection of the new synthetic cathinone 4'-methyl- α -pyrrolidinohexanophenone (MPHP) in urine samples has been impossible, because much of MPHP is metabolized before its excretion into urine. In this study, we successfully quantified unmetabolized MPHP in urine of an autopsy case using a sensitive method by liquid chromatography–time-of-flight-mass spectrometry. The quantification method showed good linearity in the range of 1.00–100 ng/mL, and the limit of detection was 0.5 ng/mL in human urine. In the autopsy case, the concentrations of MPHP in urine, plasma, and liver tissue samples were determined to be 60.1, 32.9 ng/mL, and 63.1 ng/g, respectively.

Keywords 4'-Methyl- α -pyrrolidinohexanophenone · MPHP · Synthetic cathinone · Liquid chromatography–time-of-flight-mass spectrometry · LC–TOF-MS · Human urine

Introduction

The new designer drug 4'-methyl- α -pyrrolidinohexanophenone (MPHP) is a synthetic cathinone classified as an α -pyrrolidinohexanophenone derivative (see reviews [1, 2]). MPHP was first identified in seized products as a drug of abuse in Germany in 2000 [3]. In Japan, MPHP has been detected in many types of seized products including mixed

dried plants called “herbs,” powder-type products called “bath salts,” and liquid-type products called “liquid aroma” because it was first identified in distributed illegal products in 2013 [4].

MPHP is presumed to exhibit stimulant effects and serious toxicity like other synthetic cathinones and amphetamines [2, 5, 6]. There is only one report on acute poisoning with toxic liver damage and rhabdomyolysis after MPHP intake [7]. In this poisoning case, MPHP was found at a concentration of 100 ng/mL in the patient's serum by gas chromatography–mass spectrometry (GC–MS), while it could not be detected in urine because of its metabolism [7], which is consistent with the findings in a study on MPHP metabolites in rat urine [8]. MPHP could not be found in rat urine 24 h after administration with either 1 mg/kg, which corresponds to the common dose of abusers, or 20 mg/kg by GC–MS with a limit of detection of 100 ng/mL [8]. Conversely, the main urinary MPHP metabolite 4'-carboxy- α -pyrrolidinohexanophenone (4'-carboxy-PHP) could be detected in both human [7] and rat [8] urine after MPHP administration. Therefore, to date, the toxicological detection of MPHP in urine by GC–MS seems to be possible only via its metabolites, including 4'-carboxy-PHP, but these compounds are not commercially available [9]. Recently, Minakata et al. [10] reported that a matrix-assisted laser desorption ionization-quadrupole time-of-flight-mass spectrometry enabled the sensitive quantification of MPHP with a range of 2–100 ng/mL, although the samples used consisted of blood from volunteers with MPHP added as a reference. In the present study, we are the first to identify and quantify MPHP in human postmortem urine by using liquid chromatography–time-of-flight-mass spectrometry (LC–TOF-MS) in an autopsy case.

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Case history

A 52-year-old man started to thrash about suddenly while sleeping and had a general convulsion. His wife and two children held down his arms and legs for approximately 20 min until his convulsion calmed down. At this point, his family assumed that the patient had fallen asleep again. They suspected abuse of designer drugs as a cause of his convulsion because he had been suspected of possession of illegal designer drugs and was questioned by the police 4 months earlier. The family then searched the living room for drugs and found eight packages consisting of one black package labeled “Zombie,” three clear packages “Bolt 1G,” “Aladdin pre 1G,” and “Spica 0.2” written on them, and four unlabeled clear packages. His wife returned to the bedroom and found him dead. An autopsy was performed at our department 1.5 days later. He had no notable medical history, but his daughter had seen him thrashing about approximately 1 month earlier.

Alcohol was not detected in either blood or urine samples by GC. Testing of urine samples by a Triage DOA kit (Sysmex, Kobe, Japan) showed negative results. Screening of a forensic toxicology library (MassLynx 4.1 TOF Toxicology Database 1; Waters, Milford, MA, USA) using a LC–TOF–MS system (ACQUITY UPLC–Xevo[®] G2-S QToF; Waters) detected nothing in the blood and urine samples.

Materials and methods

Reagents and materials

MPHP and 4'-methoxy- α -pyrrolidinopropiophenone (MeOPPP) were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Other common chemicals were of analytical grade and purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Blank blood and urine samples were collected from healthy volunteers with no history of drug intake after informed consent was obtained. The blank samples were screened for common drugs of abuse, alcohol, and MPHP and all were confirmed to be negative.

Sampling and sample preparation

Heart blood, urine, and liver tissue samples were collected at autopsy. The blood samples were centrifuged at $1600\times g$ for 10 min immediately after autopsy to prepare plasma. The plasma, urine and liver tissue samples were stored at $-20\text{ }^{\circ}\text{C}$ until use.

Liver tissue specimens were homogenized with 4 times (v/w) the amount of ultra-pure water using a bead beater-type shaking machine (TissueLyser II; QIAGEN, Hilden, Germany), and then the homogenate was diluted two-fold with ultra-pure water. Forty-five microliter samples of plasma, urine, or diluted liver tissue homogenate were deproteinized with 100 μL of acetone, and followed by the addition of 5 μL of 1.0 $\mu\text{g}/\text{mL}$ MeOPPP as an internal standard. After centrifugation at $10,000\times g$ for 5 min, the supernatant was used for LC–TOF–MS analyses.

LC–TOF–MS conditions

LC–TOF–MS conditions were exactly the same as described in our previous report [11]. Briefly, LC–TOF–MS was conducted on a Waters ACQUITY UPLC system with a quadrupole time-of-flight MS system (Xevo[®] G2-S QToF; Waters). LC separation was performed with a Waters ACQUITY UPLC HSS C18 column ($2.1\times 150\text{ mm}$, 1.8 mm i.d., 1.8 μm particle size) with an aqueous solution of 5 mM ammonium formate adjusted to pH 3.0 using formic acid (solvent A) and an acetonitrile solution containing 0.1 % (v/v) formic acid (solvent B) for the mobile phases. The gradient elution mode was employed using the solvents A and B as described [11].

Results and discussion

Prediction of the main compound present in the urine samples by LC–TOF–MS

Figure 1a, b shows the total ion current chromatogram (TIC) of LC–TOF–MS obtained from the deceased's urine sample, and the mass spectrum of the main TIC peak at a retention time of 3.63 min, respectively. Elemental composition analysis with the exact mass of the protonated precursor monoisotopic ion (290.1775) acquired with a collision energy of 6 eV predicted the formula $\text{C}_{17}\text{H}_{24}\text{NO}_3$ (calculated 290.1756). These results suggested the main metabolite of MPHP, that is, 4'-carboxy-PHP [8, 12, 13], because MPHP is the main component of “Zombie” and “Aladdin” [14], which were found in the deceased's living room. The fragment patterns of the target ion acquired using collision energy ramp of 10–40 eV (Fig. 1c) indicated 4'-carboxy-PHP by molecular structure analyses (Mass Fragment software; Waters) (Fig. 1d, Table 1).

Identification of MPHP in the urine sample

To examine whether the parent compound MPHP was present in the urine sample, target ions at m/z 260.202 ± 0.010 , which were determined by analysis with

Fig. 1 Total ion current chromatogram (a), mass spectra of the precursor ion (b), and fragment ions (c) of a peak with a retention time of 3.63 min obtained from the deceased's urine by liquid chromatography–time-of-flight–mass spectrometry (LC–TOF–MS). d shows the disconnection sites of 4'-carboxy- α -pyrrolidinohexanophenone predicted in molecular structure analysis for the full-spectrum of the fragment ions (c)

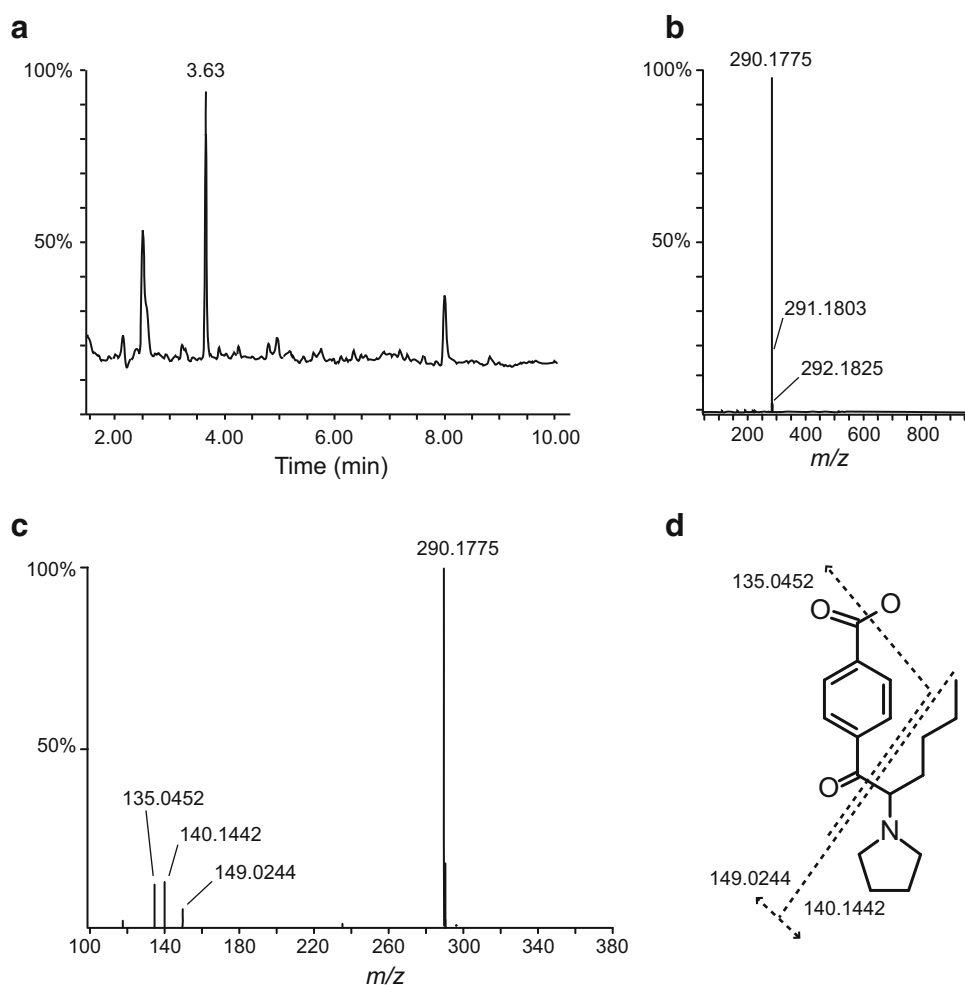


Table 1 Molecular structure analyses with the fragment pattern of a target ion obtained from the deceased's urine sample

Fragment mass (Da)	Theoretical mass (Da)	Score ^a
149.0244	149.0239	1.0
140.1442	140.1439	1.0
135.0452	135.0446	1.5

^a An index of the energy required for disconnecting the bond

the purchased reference standard of MPHP (right panel in Fig. 2b), were extracted (left panel in Fig. 2a). The retention time of the extracted ion chromatogram (XIC) (7.05 min in Fig. 2a), the exact mass of the protonated precursor ion (260.2007) acquired using a collision energy of 6 eV (Fig. 2b), and the full-spectrum of the fragment ions acquired with a collision energy ramp of 10–40 eV (Fig. 2c) in the urine sample (left panel in each figure) coincided with those of the reference standard (right panels in Fig. 2). These results demonstrated the identification of the parent compound MPHP in the deceased's urine.

Similarly, MPHP was also identified in the plasma and liver tissue samples (data not shown).

Quantification of MPHP in urine, plasma, and liver tissue samples

The quantification of MPHP was conducted for the three samples taken from the deceased. Linear calibration was achieved in the range 1.00–100 ng/mL for urine ($y = 0.0210x + 0.000480$; $r = 0.9991$). The limit of detection (signal-to-noise ratio ≥ 3) for MPHP in urine was 0.5 ng/mL. The limit of quantification was defined as the lowest point of the calibration curve (1.0 ng/mL). Recoveries at two different concentrations together with intraday precision and accuracy in the urine samples are shown in Table 2. All parameters were within the acceptable range. The regression equation, limit of detection, and limit of quantification for plasma were $y = 0.0202x + 0.00328$ ($r = 0.9966$), 1.0 ng/mL, and 1.0 ng/mL, respectively. For quantification with the liver tissue samples, the standard addition method [15] was adopted considering the greater matrix effects in the

Fig. 2 Extracted ion/total ion current chromatogram (a), mass spectra of the precursor ion (b) and fragment ions (c) of a peak with a retention time of 7.05 min obtained from the deceased’s urine (left panels) and 4'-methyl- α -pyrrolidinohexanophenone reference standard together with an internal standard 4'-methoxy- α -pyrrolidinopropiophenone (MeOPPP) (right panels) by LC-TOF-MS. XIC extracted ion chromatogram at m/z 260.202 \pm 0.010; TIC total ion current chromatograms; asterisk peak of the internal standard MeOPPP

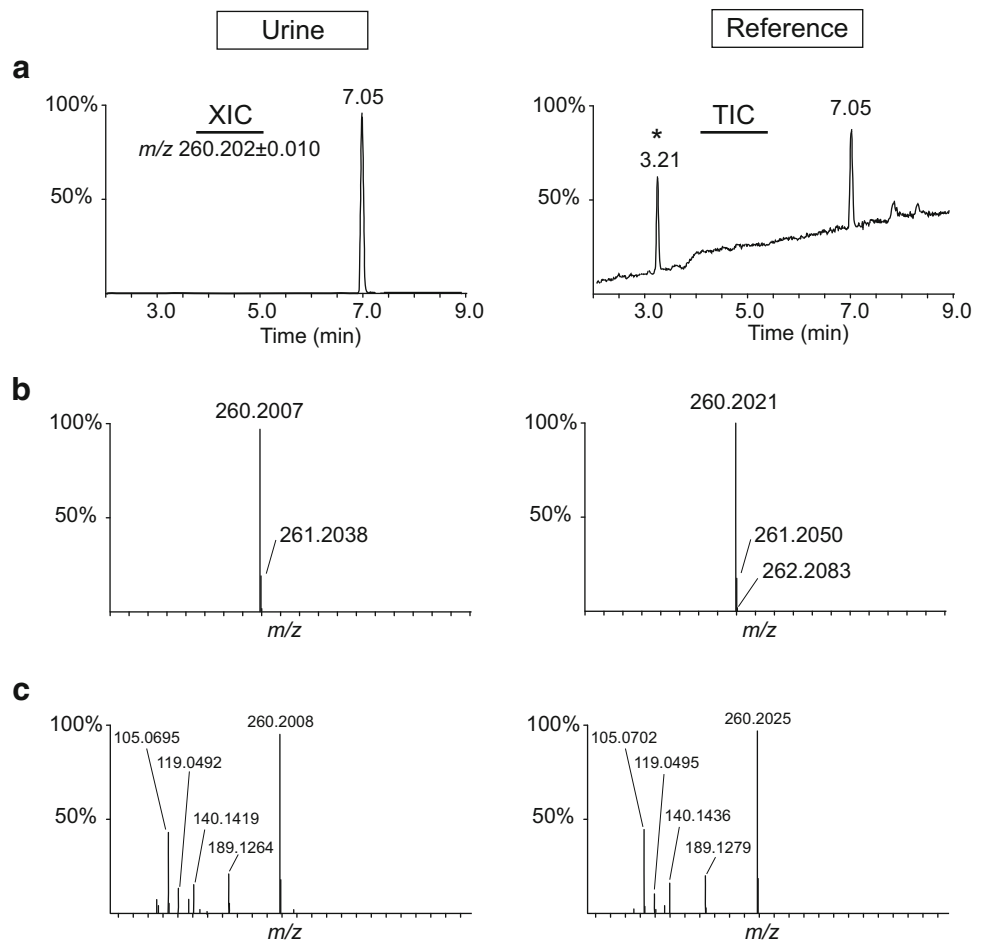


Table 2 Intraday recovery, precision, and accuracy data for determination of 4'-methyl- α -pyrrolidinohexanophenone (MPHP) in urine ($n = 5$)

Spiked concentration (ng/ml)	Recovery (%)	Precision (RSD %)	Accuracy (RE %)
1.0	95.6	9.9	-8.5
100	104	5.9	-5.8

RSD relative standard deviation, RE relative error

Table 3 Concentrations of MPHP and relative amounts of 4'-carboxy- α -pyrrolidinohexanophenone (4'-carboxy-PHP) in the urine, plasma, and liver samples of the deceased

	Urine	Plasma	Liver
MPHP	60.1 ng/ml	32.9 ng/ml	63.1 ng/g
4'-Carboxy-PHP/MPHP (% peak area)	970	35.2	25.5

liver sample than in plasma and urine samples. The regression equation in liver tissue homogenate was $y = 0.00198x + 0.125$ ($r = 0.9996$). The concentrations of MPHP in the urine, plasma, and liver samples were 60.1 ng/mL, 32.9 ng/mL and 63.1 ng/g, respectively (Table 3).

Distribution of the MPHP metabolite 4'-carboxy-PHP in the urine, plasma, and liver

To clarify the distribution of 4'-carboxy-PHP in the urine, plasma, and liver, the relative amount of 4'-carboxy-PHP

to MPHP in each sample was determined using the ratio of the peak area on XIC at m/z 290.178 for 4'-carboxy-PHP against the peak area at m/z 260.202 for MPHP because 4'-carboxy-PHP is not commercially available. The relative amount of 4'-carboxy-PHP in plasma and liver was similar, while that in urine was 28 times more than in plasma (Table 3). Although it was unclear when the deceased took MPHP, these results demonstrated that the majority of MPHP was excreted as its metabolites in urine, which was consistent with the previously reported findings [7, 8].

Conclusions

We reported the first successful quantification of MPHP in an autopsy urine sample using LC–TOF–MS. Although the majority of MPHP was excreted as the MPHP metabolite 4'-carboxy-PHP in urine, the sensitive method of LC–TOF–MS with a limit of quantification of 1.0 ng/ml was able to quantify the remaining unmetabolized urinary MPHP.

Compliance with ethical standards

Conflicts of interest There are no financial or other relations that could lead to a conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all healthy individuals included in the study, who supplied ~1 ml of whole blood and ~10 ml of urine for use as blank materials.

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