



## Case Report

### The Abuse of 3,4-Methylenedioxypyrrolidinobutyrophenone (MDPBP): A Case Report

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#### Abstract

The abuse of new designer drugs available on the black market is increasing worldwide. Their use has, in some cases, led to fatal overdoses in addition to other adverse social and health effects, yet revealing the cause of the potential intoxication has so far proven difficult on account of the absence of appropriate analytical data for the drug in question as well as the lack of specific knowledge of how it is metabolized. The case outlined here concerns abuse of an unspecified drug nicknamed "Funky". Simultaneous general GC-MS screening and ultra-high resolution accurate mass liquid chromatography-mass spectrometry toxicological analysis of the subject's urine confirmed the presence of 3,4 methylenedioxy-pyrrolidinobutyrophenone, one of the new synthetic cathinones. The parent drug in the urine sample was accompanied by a dominant metabolite, which was most likely one of the two possible stereoisomers produced by demethylenation and subsequent O-methylation of the hydroxy group. This was the first confirmation of MDPBP abuse in the Czech Republic supported by the toxicological identification of MDPBP and its dominant metabolite in human urine.

#### Keywords

Designer drugs; Pyrrolidinophenones; Cathinones; Drug abuse; GC-MS Toxicological screening; High Resolution Accurate Mass LC-MS

#### Introduction

Recently, there has been a significant rise in the popularity and abuse of new designer drugs worldwide, represented in part by a group of drugs known as synthetic cathinones. These drugs have emerged on the illicit drug market and are most commonly available in the form of tablets or powders referred to as "bath salts", "legal highs" or packaged with various other labels usually with a formal warning stating "not for human consumption" in order to avoid criminal prosecution. Some of these new drugs have gradually become classified as controlled substances in some countries, yet even in these areas they are still available e. g. *via* internet.

The synthetic cathinone derivatives are stimulants effecting central nervous system and are primarily consumed to achieve

euphoric, cocaine-like or amphetamine-like effects. They have been known to cause agitation, elevated heart rate and blood pressure, seizures and hallucinations, and aggressive behavior [1]. They are most commonly administered via oral ingestion, nasal insufflation, smoking, or intravenous injections. Overdoses can be life threatening [1-9]. The cause of intoxication may be difficult to determine, because these drugs cannot be detected using routine drug immunochemical screening. At the present, the expansion of analytical databases including specific information regarding biotransformation is essential to facilitate the detection of these compounds and their prevailing metabolites in biological samples during toxicological screening. With the availability of control studies on human metabolism of these drugs being restricted due to ethics, it is therefore our intention that the information from case studies such as this may help fill these gaps of knowledge.

The case described herein concerns a 36 year-old man who was admitted requesting treatment for his long-term ethanol addiction but also reportedly had a habit of experimenting with whatever new drugs were available in the local black market shops. The subject claimed to have had stopped ethanol consumption five days before he was admitted and, during those five days, had been snorting an illegal powdered drug that was distributed under the name "Funky" in order to combat the symptoms of ethanol withdrawal. He also reported experiencing subjective amphetamine-like effects after using "Funky". Upon initial exam, the patient was generally physically stable and reported subjective feelings of mild anxiety, along with physical fatigue and excessive sweating. Clinical chemistry results were within acceptable physiological ranges with the exception of rather elevated liver data and slight proteinuria.

The patient's urine was subjected to routine toxicological screening, where immunochemical test results for common drug groups were negative with the exception of cannabinoids and ethylglucuronide, which were anticipated considering the patient history. However, the general gas chromatography-mass spectrometry (GC-MS) screening for unknown drugs in the urine revealed the abundant presence of two unknown substances with similar mass spectra. Subsequent liquid chromatography with ultra high resolution accurate mass spectrometry (LC-HRMS) analysis of the urine sample suggested the presence of 3,4-methylenedioxy-pyrrolidinobutyrophenone (MDPBP) and its potential metabolites. In summary, the accurate identification of MDPBP in this urine sample was subsequently confirmed with high confidence using GC-MS and LC-MS-MS analysis in comparison with a synthesized reference standard. To our knowledge this is the first report of MDPBP abuse in the Czech Republic and the first case of mass spectral characterization not only of the parent drug but also one of its human metabolites in urine.

#### Materials and Methods

##### Chemicals

Solvents and other chemicals used were of analytical grade purity. Bond Elut Certify LRC columns 130 mg were purchased from HPST Prague (Agilent Technologies, Inc.). The reference substance MDPBP was synthesized in the Institute of Chemical Technology in Prague, Department of Organic Chemistry, with 99.2% purity.

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## Urine sample preparation for GC-MS

2 ml of urine were prepared for GC-MS by SPE on Bond Elut Certify LRC 130 mg columns with separate elution of acidic and basic fractions. The resulting extracts of unknown acids and bases were evaporated to dryness and dissolved in 100  $\mu$ l ethyl acetate, from which 5  $\mu$ l was analyzed.

## GC-MS conditions

Agilent HP6890-5973 GC-MS equipped with autosampler, splitless injector, HP5-MS 30 m x 0.25 mm x 0.25  $\mu$ m capillary was used. Carrier gas was helium with constant flow rate 1 ml/min. Analysis was performed in the full scan mode in the range 35-550 m/z in standard EI mode with 70 eV. The GC temperature program for screening of unknowns was in the range 65-300°C (65°C for 1.5 min, following gradient at 20°C/min to 300°C, 300°C ramp for 11.75 min)

## Urine sample preparation for LC-HRMS analyses

2 ml urine was alkalinized 1M NaOH (pH 11-12) and extracted with 4 ml ethyl acetate. 3 ml of organic layer was transferred to a glass tube and evaporated to dryness. The residue was dissolved in 100  $\mu$ l mobile phase and 5  $\mu$ l was injected.

## LC-HRMS conditions

Dionex UltiMate 3000 LC coupled to Exactive Plus-Orbitrap MS

(Thermo Scientific) equipped with HESI-II source was used. Nitrogen was sheath, auxiliary and collision gas in flow rate 0.5 ml/min. The MS conditions: alternating FULL MS and AIF MS/MS, positive scan mode from 50-500 m/z, resolution 70.000 (scan speed 2 Hz) in full scan and 35.000 for AIF, higher energy collision-induced dissociation (HCD) at 35eV, spray voltage was 3kV, ion transfer capillary temperature 320°C.

LC conditions: Hypersil Gold column PFP (150 x 2.2 mm, 5  $\mu$ m), flow rate 250  $\mu$ l/min, gradient elution with 10mM ammonium formate in 0.1% formic acid as the mobile phase A and acetonitrile 0.1% formic acid as the mobile phase B. Gradient: 0 min 5% B, 5 min 45% B, 18 min 70% B, 20-27 min hold in 95% B.

## Results and Discussion

The initial routine GC-MS screening for unknown basic drugs in the urine sample indicated the presence of two unknown dominant compounds with identical main fragment at m/z 112 in their mass spectra, in the first compound accompanied with observable additional fragments m/z 121 and m/z 149 (Figure 1). Additionally, less abundant substances were also detected having main fragments at m/z 112 in their spectra.

In the next step, the LC-HRMS analysis with accurate mass measurement using Excalibur software revealed the presence of a substance with an exact mass of 262.1438 accompanied in abundance by another substance with an exact mass 264.1594. The LC-HRMS full scan chromatogram and extracted ion chromatograms of these two substances are shown in Figure 2. The peak with the appropriate protonated molecular ion  $[M+H]^+$  and with an exact mass of 262.1438 corresponded to the elemental composition of C<sub>15</sub>H<sub>19</sub>NO<sub>3</sub>. The second and more abundant peak of protonated molecular ion  $[M+H]^+$  with an exact mass 264.1594 corresponded to the composition of C<sub>15</sub>H<sub>21</sub>NO<sub>3</sub>. This information indicated the presence of some of structurally modified designer drug, most likely MDPBP and one of its metabolites.

MDPBP was confirmed unambiguously in the analyzed urine sample using repeated GC-MS and LC-HRMS analysis in sequence with the synthesized reference standard gathering accordant retention and spectral results. The recorded data were then analyzed using Mass Frontier software. This determined the presence of the protonated parent ion  $[M+H]^+$  in addition to the fragments from  $\alpha$ -cleavage and water dissociation and their further fragmentation obtained by LC-ESI-HRMS method. The exact masses of MDPBP and its predominant fragments using HRMS-ESI full scan with HCD fragmentation were calculated with appropriate mass accuracy (mass accuracy error was -0.38 ppm for parent drug and less than 3.5 ppm for fragments). Moreover the accuracy of MDPBP structural identification was confirmed by identical product ion spectra in LC-MS/MS analysis related to the standard substance (Figure 3).

In the GC-MS analysis of the urine sample, the EI mass spectra of the parent drug and supposed metabolites were characterized by the base fragment m/z 112 proposed to be an immonium ion produced by fragmentation of the MDPBP structure. As concerned seized samples, the fragmentation of MDPBP and other  $\alpha$ -pyrrolidinophenones has been studied in detail using GC-MS previously by Westphal et al. [10] and then later by Fornal using LC-HRMS [11]. The spectral fragmentation of the dominant metabolite in the urine with preservation of the immonium ion at m/z 112 along

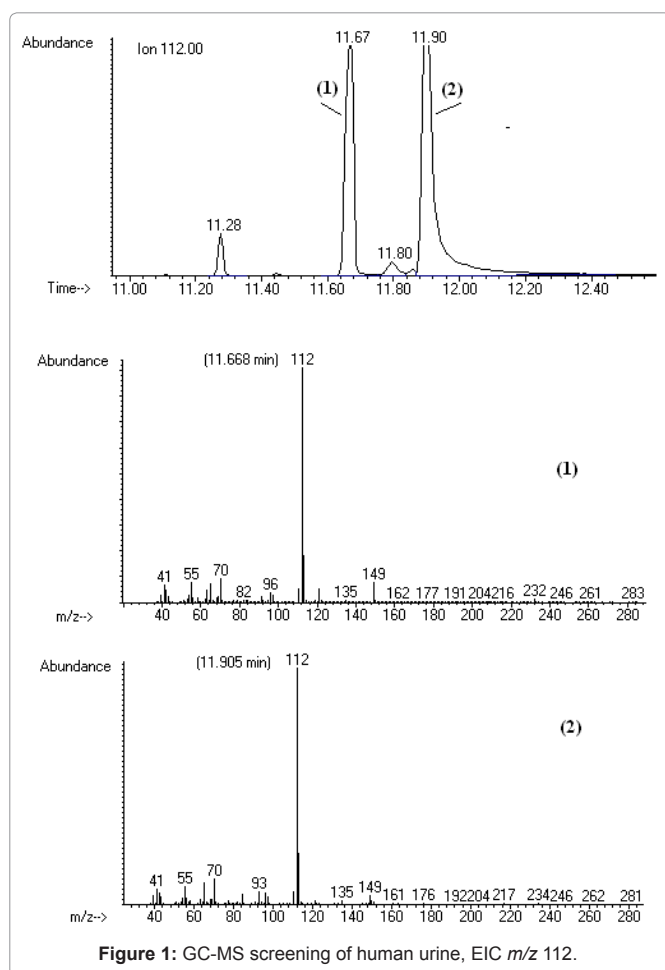
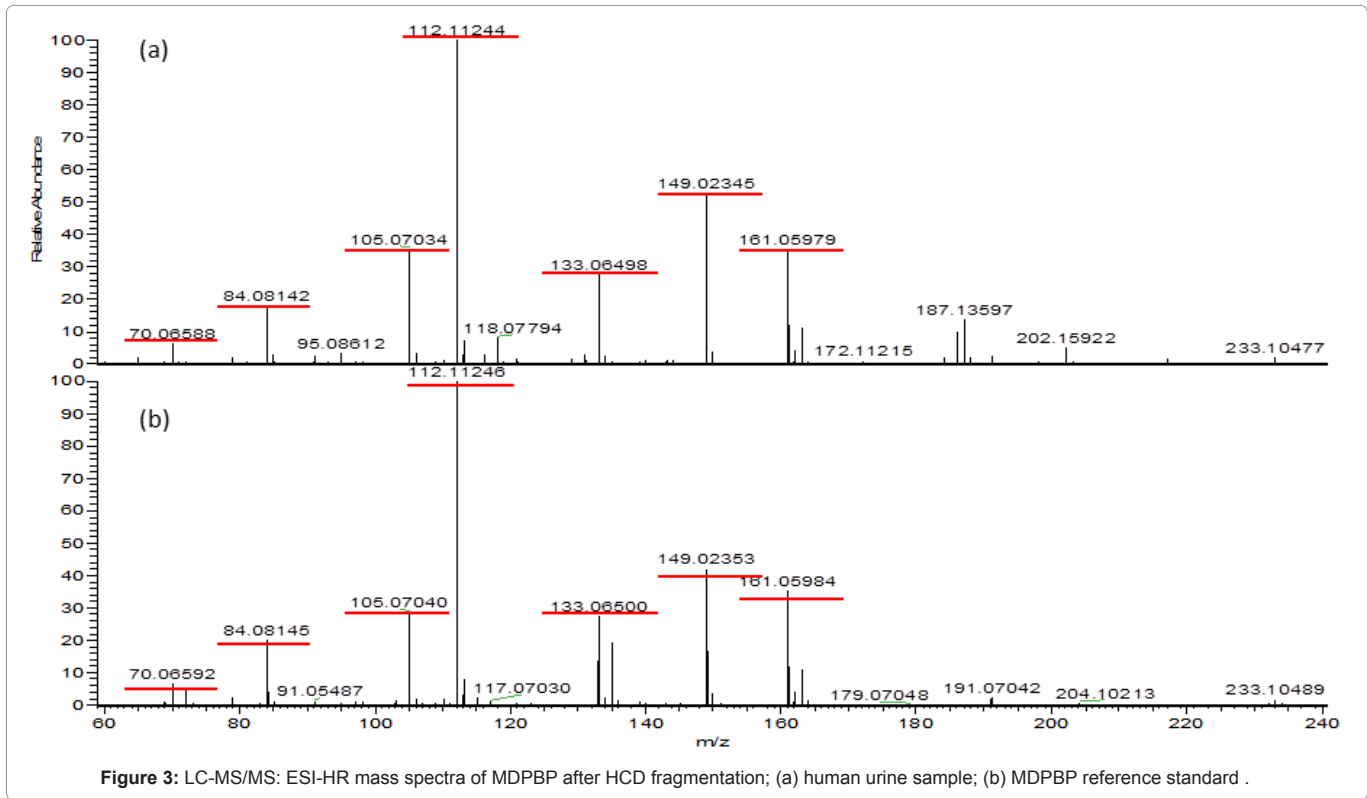
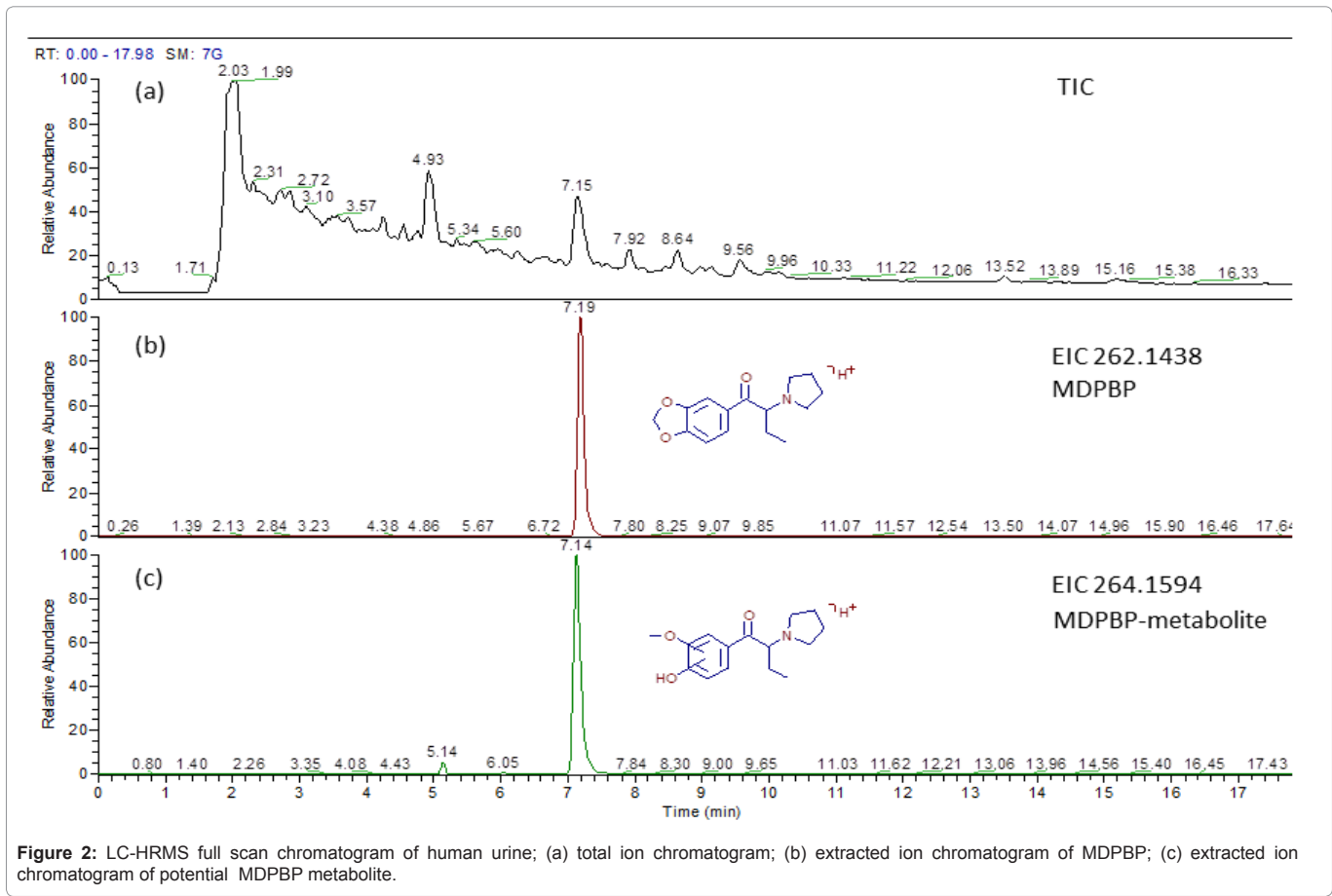


Figure 1: GC-MS screening of human urine, EIC m/z 112.



with the increase of molecular ion by two mass units in LC-HRMS in relation to parent MDPBP allowed for deductions to be made concerning structural modification during biotransformation. We can expect to find in MDPBP a metabolic scheme very similar to the structurally-related drug methylenedioxypropylvalerone (MDPV) which has a metabolism that has already been elucidated in controlled experimental studies [12,13]. Zaitso et al. [14] summarized in their review covering metabolism of 3,4-methylenedioxyphenylalkylamines that the main urinary metabolites of these drugs were the 4-hydroxy-3-methoxy-metabolites produced by demethylenation followed by the *O*-methylation of the 3-hydroxy groups which were excreted partly in free and partly in sulphated and glucuronated forms. The mass spectra concerning MDPBP and its dominant metabolite gained from GC-MS and LC-HRMS analysis of the human urine sample reported in this paper are in accordance with this conclusion about biotransformation [14]. To our knowledge, this is the first report regarding a human metabolite of MDPBP.

MDPBP, a novel cathinone, is not commonly found in “legal highs”. The pharmacology of MDPBP is not known but due to its structural similarity to MDPV, it may be assumed to have a similar pharmacological potency. MDPV is a very potent dopamine and no adrenaline uptake inhibitor associated with possible addiction and significant cardiovascular, neurological, psychopathological toxicity [15,16]. The predominant action on dopamine transporter may be responsible for the risk of addiction [15-17]. Usage of drugs with effects on noradrenergic neurotransmission may lead to cardiovascular toxicity over time [17]. The dangerous toxic effects seen after consumption of illegal products of questionable and/or unspecified composition can also appear due to additive interaction with different compounds used as additives in the mixture. The recognition of intoxication by synthetic cathinones may be hampered by lack of adequate toxicological methods of detecting and identifying these drugs and/or their metabolites in biological samples. The information on biotransformation and appropriate analytical data related both to parent compounds and metabolites are critical for successful diagnosis. Our report may be considered as a partial contribution to this effort.

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