LC-High-resolution MS Analysis of the Designer Drugs Methylenedioxy-Pyrovalerone (MDPV) and Methylenedioxy-alpha-pyrolidinobutyrophenone (MDPBP) and Their Main Metabolites in Human Urine

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In the present work, LC- Q ExactiveOrbitrap high resolution MS techique in both full-scan MS and targeted MS/MS modes was applied to identify psychoactive substances in human urine samples. Methylenedioxy-pyrovalerone (MDPV), methylenedioxy-alpha-pyrrolidino-butyrophenone(MDPBP) and the main metabolites of both compounds were identified. The preliminary full-scan screening was folowed by targeted ion fragmentation (t-MS^2) enabling confirmatory analysis and mass spectral characterization. The presented case is the first confirmation of MDPBP abuse in Romania supported by the toxicological identification of the parent compound and the main metabolites in human urine.

Keywords: designer drugs; MDPV; MDPBP; HR-MS

New psychoactive substances (NSPs) were referred to as *designer drugs*, *new synthetic drugs*, and, in Romania, *ethnobotanical drugs* are the terms used more often. Considered *legal high*, these drugs are sold as *bath salts*, *chemical reagent* or *plant food*, labeled as *not for human consuption* in order to circumvent legislation [1]. They can easily obtained through internet website and local drug suppliers. Synthetic cathinones have recently emerged and become popular drugs of abuse. Their dramatic increase has resulted in part from media attention as well as widespread availability [2].

According to the ESPAD Report 2015, the prevalence of consumption of ethnobotanic products at least once in a lifetime places NSPs among the most consumed drugs among young people in Romania, along with cannabis and inhalants [3].

With regard to the NSPs identification, there is a significant problem in the official reporting of the number of medical emergencies or deaths due to the difficulties represented by toxicological analysis. NSPs detection and identification are real analytical challenges because of the similarity of their chemical structures, the lack of spectrum in analytical libraries, and the limited availability of reference materials [4]. Immunological tests, as well as HPLC (high performance liquid chromatography) or GC-MS (gas chromatography–mass spectrometry) methods, are ineffective in this case. LC-high resolution mass spectrometry (HRMS) screening is one of the most powerful and effective analytical tools in this area [5].

Only a few of laboratories are able to detect and confirm the drug consumption in Romania. At the present, specific information regarding synthetic cathinones are very important to facilitate the detection of these compounds in biological samples.

The case described above concerns an adult young woman (20 years old), victim of a sexual assault and the aggressor, a young man, 32 years old.Both admitted that having snorting during the night an illegal powdered drug that was distributed under the name *Magic*, additionally to alcohol consumption. Urine samples were collected about 10 h after the drug consumption. Both urine samples were subjected to routine immunochemical screening. Test results for common drug groups were negative.

A general post-target screening using LC- high resolution mass spectrometry (LC-HRMS) in full-scan mode was applied. The post target aproach, based on monitoring theoretical exact masses using narrow mass windows usualy 5 ppm, permits a rapid and simple review of the presumptive psychoactive substances. A comprehensive list of about 130 psychoactive compounds, belonging to different drugs classes such as: tryptamines analogues, opioids, cocaine, cannabinoide, synthetic canabinoides, amphetamines, and substituted cathinones was monitored. The list was based on the literature, concerning the prevalence of the drugs.

The preliminary full- scan LC-HRMS analysis of the urine samples suggested the presence of 3,4-methylenedioxy-pyrovalerone (MDPV) and 3,4-methylendioxy-pyrrolidino-butyrophenone (MDPBP). In addition, the main metabolites of both compounds were identified.

Screening of the samples for targeted compounds was followed by an MS/MS analysis as targeted ion fragmentation (t-MS^2) enabling confirmatory analysis and mass spectral characterization. To our knowledge this is the first report of MDPBP abuse in the Romania.

Experimental part

Chemicals, reagents and materials

Organic solvents (methanol, acetonitrile) used were purchased from Merck. Formic acid (98-100%), acetic acid, β-Glucuronidase, amonium acetate, ultrapure water (LC-MS grade) were purchased from Merck. Solid phase extraction (SPE) Strata-X cartridges (100mg/3mL) used for solid phase extraction were purchased from Phenomenex.

Analysed samples

Urine specimens from both victim and aggressor were provided about 10 h after presumptive drug consumption. Blank urine samples were analysed as control samples. All samples were stored at 4°C in refrigerators during 24 h, until they were extracted.

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Urine enzymatic hydrolysis followed by SPE extraction

A volume of 1000 µL of urine was adjusted to *p*H 5.5 with 500 µL acetate buffer and incubated for 2.5 h at 37°C with 20 µL β-glucuronidase. An SPE procedure was applied in order to clean and concentrate the sample, using Strata X, 100 mg / 3 mL, reverse phase, SPE cartridge.The cartridge was previously preconditioned with 3mL methanol followed by 3 mL water. After the application of the sample, the column was washed with 3 mL water, followed by 3 mL 20% methanol in water, and vacuum dried for 3 min. The analytes were eluted with 3 mL methanol. The eluate was concentrated by evaporation under flow of high purity nitrogen, in a water bath at 42°C (Thermo Scietific, Germany). After evaporation, the residue was dissolved in 50 µL methanol and 450 µL water and filtrated thru 0.2 µm micro-filter.

HR-MS-MS analysis

In the present work, Q Exactive high-performance quadrupole - Orbitrap LC–MS/MS was applied to identify psychoactive substances in the urine samples.

LC parameters

A Thermo Scientific Dionex Ultimate 3000 Series RS pump coupled with a Thermo Scientific Dionex Ultimate 3000 Series TCC-3000RS column compartments and a Thermo Fisher Scientific Ultimate 3000 Series WPS-3000RS autosampler controlled by Chromeleon 7.2 Software (Thermo Fisher Scientific, Waltham, MA and DionexSoftronGMbH Part of Thermo Fisher Scientific, Germany) were used for analysis.

The application of a 15 min gradient over an ultra-performance Accucore U-HPLC Column C18 (150 x 2.1 mm, 2.6 µm), (Thermo Scientific) was used. A flow rate of 0.4 mL min^{-1} was set in the U-HPLC system. The mobile phase consisted of: eluent A, 100% water containing 100 µL formic acid (*p*H 3.5); eluent B, 100% methanol containing 100 µL formic acid. The step gradient was as follows: 0 - 1 min 100% A; 1 - 2.5 min linear increase to 40% B; 2.5 - 10 linear increased to 100% B and hold 3 min; 13 - 13.1 decreasing to 0% B; 13.1 - 15 min 100% A. The column temperature was set at 40°C and the injection volume at 20 µL.

HESI (Heated Electrospray) ion source was used for the ionization. The HESI parameters were optimized as follow: sheath gas flow rate 40 unit; aux. gas unit flow rate 10; capillary temperature 250°C; aux gas heater temperature 300°C; spray voltage 2800 V (-2800 V for ESI-); S lens RF level 50.

MS parameters

Detection of compounds was performed using a Q-Exactive high resolution mass spectrometer. Full scan data in both positive and negative mode was acquired at a resolving power of 70 000 FWHM at m/z 200. For the compounds of interest, a scan range of m/z 130–1000 was used; the automatic gain control (AGC) was set at 3e6 and the injection time was set to 200 ms. Scan-rate was set at 2 scan/sec. External calibration was performed with calibration solution in positive and negative mode.

In addition to the full scan acquisition, a targeted MS/MS analysis was performed using the mass inclusion list and expected retention times of the target analytes, with a 30s time window. The Orbitrap spectrometer was operated in positive mode at 17500 FWHM. The AGC target was set to 2e5, with the maximum injection time of 20 ms.Thequadrupole was operated at an isolation window of *m/z* 2. Collision energy was set at 45 NCE.

Data were evaluated by the Quan/Qual Browser Xcalibur 2.3 (Thermo Fisher). The mass tolerance window was set to 5 ppm for the two analysis modes.

Detection was based on accurate mass measurements and pattern recognition of the product ions compared to MS/MS data generated by HighChem Mass Frontier 7.0 software.

Results and discussion

LC/HRMS full-scan screening of the urine samples allowed for the accurate mass determination of two synthetic cathinones: MDPV (3,4-methylen-dioxi-pyrovalenone) and MDPBP (3',4'-methylen-dioxi-α-pyrrolidinobutirofenone). The main metabolites of both compounds were identified in both urine samples (fig. 1).

The analytes detected in the full-scan HRMS screening analysis were considered as preliminary positive results and subjected to an MS-MS analysis for confirmation. The exact masses of the *suspect* compounds were added to

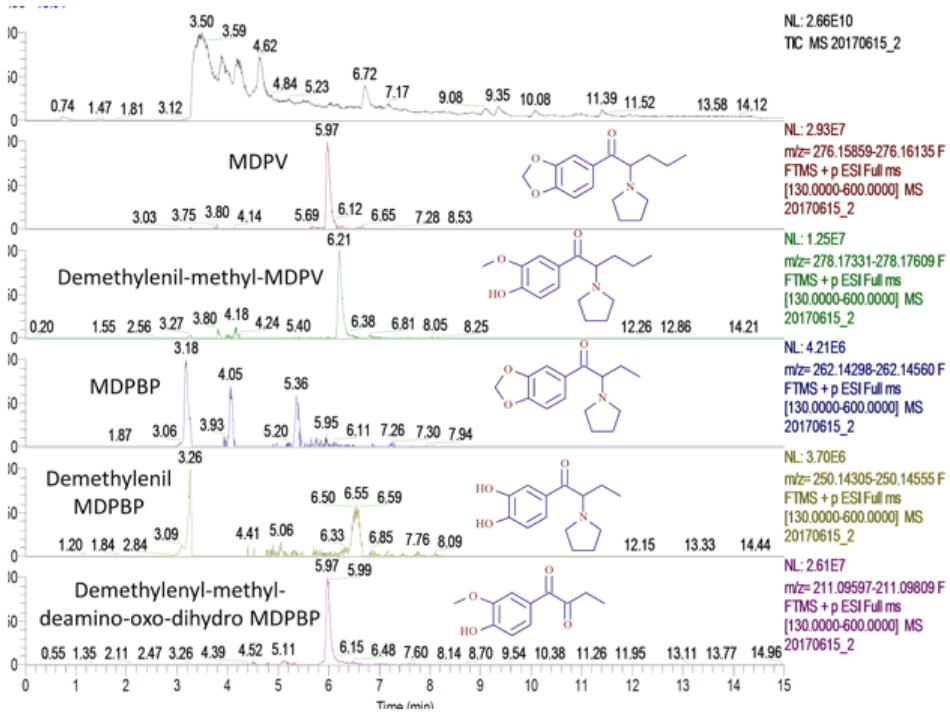

Fig.1 LC-HRMS full scan chromatogram of human urine; (a) total ion chromatogram; (b) extracted ion chromatogram of MDPV; (c) extracted ion chromatogram of MDPV metabolite. (d) extracted ion chromatogram of MDPBP; (e), (f) extracted ion chromatogram of MDPBP metabolites

Table 1

DETECTED COMPOUNDS, RETENTION TIME, MASS ERROR AND THE MAIN PRODUCT IONS AFTER CID

Detected compounds	RT	Mass error Δppm	Precursor (m/z)	Product ions (m/z)
MDPV	6.02	0.15	276.1594[M +H]$^+$	230.15; 163.07; 154.12; 149.05; 123.04; 112.07
MDPBP	3.17	-0.27	262.1438[M +H]$^+$	244.13; 202.11; 145.08; 84.08

the inclusion list and time windows were set on the basis of expected retention time for each compound. The transitions of at least four product ions were monitored (table 1).

The fragment ions obtained was compared to the fragmentation pathway generated by MassFrontier software. Besides literature sources, software tool as MassFrontier was very helpful, as it allows justification of the fragment ions observed (fig. 2, 3).

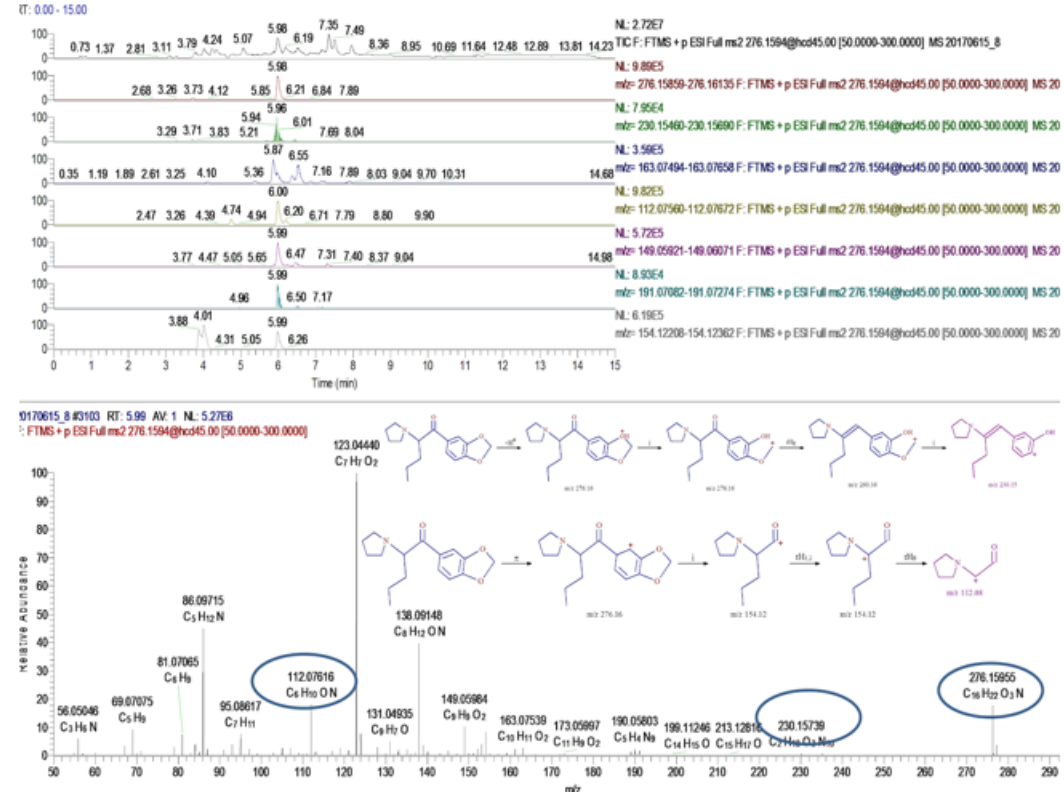

Fig. 2 LC-MS/MS: ESI-HR mass spectra of MDPV after HCD fragmentation and proposed fragmentation pathways by MassFrontier 7.0. Software

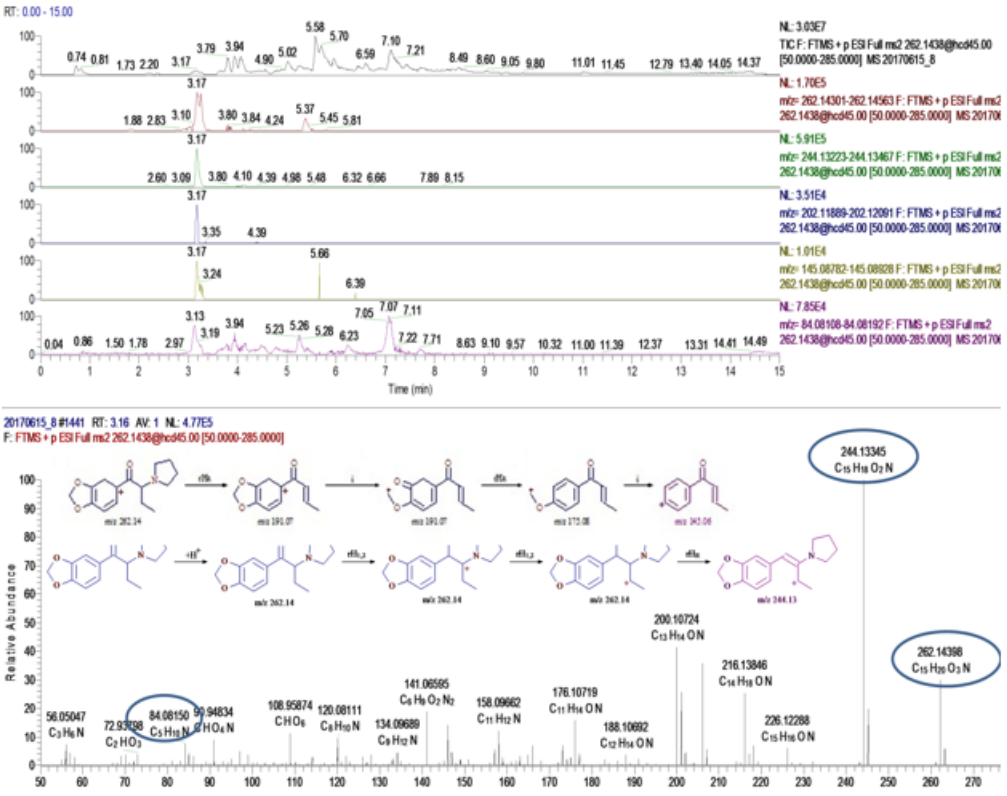

Fig.3 LC-MS/MS: ESI-HR mass spectra of MDPBP after HCD fragmentation and proposed fragmentation pathways by MassFrontier 7.0. Software

The identification of the compounds was performed without having the reference standards available. The overall information given by Q-ExactiveOrbitrap HRMS (full-spectrum acquisition, accurate mass of protonated molecule and relevant fragment ions) and the comparison fragmentation pathway generated by MassFrontierallows the preliminare confirmation of the detected compounds [6]. The interpretation of fragmentation patterns of HR-MS/MS spectra is an option that can lead to successful structure elucidationeven in the absence of reference standards [7]. As MDPBP's popularity has increased only in the recent years, and analytical standards was not initially available, such approach has been used in other studies to confirm the identity of this compound [8].

Concerning the product involved in the reported case, distributed under the name *Magic*, the studies indicate the existence of three products on the Romanian market under the names *Pure by Magic*, *Flower Magic* and *Magic*, as the most known and implicitly consumed [9]. All of these products are synthetic stimulent drugs and have different compositions. Various websites suggest an approximate composition of these product: *Magic* is associated with mephedrone and *Magic Flower* with piperidine.A study conducted at the University of Medicine and Pharmacy Iasi, Romania, by M. Corlade (2015) [9] on *ethno-botanical* products available on-line, reveals the same combination of cathynones (MDPV and MDPBP) in the product called *Pure by Magic*.

Regarding the compounds identified in the presented case, MDPV and MDPBP are members of the class of designer drugs known as á-pyrrolidinophenones. The effect of these drugs are pronounced stimulation of central nervous system throw the release of dopamine and norepinephrine [10], and the inhibition of dopamine and norepinephrine transporters [2].

Although the drug has appeared relatively recently [11], due to the unusually intense effects and rapidity with which it has spread among consumers, numerous reports of psychoses produced by its consumption are published [12,13]. Although the desired effect is euphoric, intellectual stimulation and increased levels of energy, most often addicts describe terrifying experiences [13]. The substance may cause prolonged panic attacks, confusion, long-term cognitive impairment, irritability and aggression, paranoid psychosis and / or hallucinations, bizarre behaviour, suicide attempts, or extreme violence [2, 13].

3', 4'-methylenedioxy-α-pyrrolidinobutyrophenone (MDPBP) is a recent identified synthetic substance of the pyrrolidinophenone type (PP) [14]. There are very few reports regarding the new compound MDPBP. It was presumed that metabolic scheme of MDPBP is similar to the structurally-related drug MDPV, which has already been elucidated in experimental studies [15]. Metabolic pathways of MDPBP were proposed following an *in vivo* and *in vitro* recent study [16]. A number of 6 metabolites (the most abundant of which was the compound of formula $C_{11}H_{14}O_4$ withexactmass 211. 09) and the unchanged active substance were identified were detected in human urine by GC-MS / MS in the mentioned study [16].

In the present work, two of the main metabolites: demethylenil MDPBP, $C_{14}H_{19}NO_3$ with exact mass 249.1365, and demethylenil-methyl-deamino-oxo-dihydro MDPBP, $C_{11}H_{14}O_4$ with exact mass 211.09703, were identified with an mass error of -0.038 Δppm and 0.47 Δppm respectively which was in agreement with previous published data [8, 16] and with similar drugs such as MDPV [5, 15]. The main metabolite of MDPV ($C_{16}H_{24}NO_3$ with exact mass 278.1750) [5, 15] was also identified in both urine samples.

Conclusions

MDPV and MDPBP were successfully identified in urine samples by LC-HRMS. Spectral characterization of the compounds and the identification of two urinary metabolites was done bz (t-MS^2) analsys. MDPBP, a novel synthetic cathinone, is not commonly found in *ethnobotanical drugs*. From our knowledge, the present work is the first report of MDPBP abuse in Romania.

Because of the potential toxic effects of the consumption of NSPs of questionable and / or unspecified composition are unknown, the information and the analytical data related to the identification in biological samples of the parent compounds and the metabolites are essential for the recognition of intoxication with synthetic catinones.

High-resolution mass spectrometry (HRMS) prouve to be a valuable analytical tool in toxicological testing, as providing sensitive, full-spectrum MS data with high mass accuracy and MS-MS data.

References

1. KRIIKKU P, WILHELM L, SCHWARZ O, RINTATALO J (2011) New designer drug of abuse: 3,4-methylenedioxypyrovalerone (MDPV). Findings from apprehended drivers in Finland Forensic Forensic SciInt 210(1–3):195–200.

2. ZAWILSKA JB, WOJCIESZAK J. Designer cathinones - an emerging class of novel recreational drugs. Forensic SciInt (2013) 231:42–53.

3.*** ESPAD Report 2015 Results from the European School Survey Project on Alcohol and Other Drugs. Luxembourg: Publications Office of the European Union, 2016

4.M. IBANEZ, J. V. SANCHO, L. BIJLSMA, A. L.N. VAN NUIJS, A. COVACI, F. HERNANDEZ. Comprehensive analytical strategies based on high-resolution time-of-flight mass spectrometry to IDENTIFY NEW PSYCHOACTIVE SUBSTANCES. TRENDS IN ANALYTICAL CHEMISTRY 57 (2014) 107–117

5. M. IBANEZ, O. J. POZO, J. V. SANCHO, T. ORENGO, G. HARO, F. HERNANDEZAnalytical strategy to investigate 3,4-methylenedioxy-pyrovalerone (MDPV) metabolites in consumers' urine by high-resolution mass spectrometry. Anal BioanalChem, 2016. DOI 10.1007/s00216-015-9088-1.

6.KERN, S., FENNER, K., SINGER, H. P., SCHWARZENBACH R,P., HOLLENDER,J., Identification of Transformation Products of Organic Contaminants in Natural Waters by Computer-Aided Prediction and High-Resolution Mass Spectrometry. Environ. Sci. Technol., **2009**, 43 (18), pp 7039–7046

7. IBANEZ M.; SANCHO, J. V.; POZO, O. J.; NIESSEN, W.; HERNANDEZ, F. Use of quadrupole time-of-flight mass spectrometry in the elucidation of unknown compounds present in environmental water. Rapid Commun.Mass Spectrom. 2005, 19, 169–178.

8. BALIKOVA M, ZIDKOVA M, OKTABEC Z, MARESOVA V, LINHART I, et al. (2013) The Abuse of 3,4-Methylenedioxypyrrolidinobutyrophenone (MDPBP): A Case Report. J Forensic Toxicol Pharmacol 2:2.doi:10.4172/2325-9841.1000108

9. CORLADE, M., Studii clinico si chimico- toxicologice ale unor produse etnobotanice Rezulamul tezei de Doctorat, Iasi, 2015. Diponibil la: http:// www. umfiasi. ro/ ScoalaDoctorala /TezeDoctorat/Teze%20Doctorat/rezumat_teza_ro_corlade_mihaela.pdf. Accesat, iunie 2017.

10.KELLY,J.P., Cathinone derivatives: a review of their chemistry, pharmacology and

11.WINDER G.S., STERN N., HOSANAGAR A. Are Bath Salts the next generation of stimulant abuse? Journal of Substance Abuse Treatment. 44, 2013, 42–45

12. ANTONOWICZ J.L; METZGER A. K; RAMANUJAM, S. L. Paranoid psychosis induced by consumption of methylenedioxypyrovalerone: Two cases General Hospital Psychiatry, 2011; 33(6): :640.e5-6

13. THORNTON, STEPHEN L; GERONA, ROY R; TOMASZEWSKI, CHRISTIAN A. Psychosis from a Bath Salt Product Containing Flephedrone and MDPV with Serum, Urine, and Product Quantification Journal of Medical Toxicology, 09/2012, Volume 8, Issue 3.

14. WESTPHAL G., T. JUNGE, P. ROSNER, F. SONNICHSEN, F. SCHUSTER. Mass and NMR spectroscopic characterization of 3,4-methylenedioxypyrov alerone: A designer drug with alpha-PYRROLIDINOPHENONE STRUCTURE. FORENSIC SCI. INT. 2009, 190, 1.
15. M.R. MEYER, P. DU, F. SCHUSTER, H.H. MAURER. Studies on the metabolism of the alpha pyrrolidinophenone designer drug methylenedioxy-pyrovalerone (MDPV) in rat urine and human liver microsomes using GC-MS and LC-high-resolution-MS and its detectability in urine by GC-MS. J. Mass Spectrom. 2010, 45, 1426
16. MEYER MR, MAUER S, MEYER GM, DINGER J, KLEIN B, WESTPHAL F, MAURER HH. The in vivo and in vitro metabolism and the detectability in urine of 3',4'-methylenedioxy-alpha-pyrrolidinobutyrophenone (MDPBP), a new pyrrolidinophenone-type designer drug, studied by GC-MS and LC-MS(n.). Drug Test Anal. 2014 6(7-8):746-56.

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