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Short communication

# Identification of substituted cathinones: 3,4-Methylenedioxy derivatives by high performance liquid chromatography–quadrupole time of flight mass spectrometry

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ABSTRACT

The potential of high performance liquid chromatography–high resolution mass spectrometry (HPLC–HRMS) for screening of synthetic cathinones in legal highs was examined. Samples were analysed by liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometer (Q/TOF). Nanoelectrospray ionisation (nanoESI) was employed. MS and MS/MS spectra were acquired. Six 3,4-methylenedioxy derivatives: methylone, butylone, pentylone, MDPBP, MDPV and BMDP were detected and identified. The fragmentation pattern of 3,4-methylenedioxy derivatives in collision induced dissociation (CID) was derived and described, which will facilitate future screenings and identifications of new synthetic cathinones. For 3,4-methylenodioxy derivative cathinones the loss of neutral groups $CH_4O_2$, $H_2O$, amines and imines is observed. The loss of water and the methylenedioxy group does not occur when cyclic amino group – pyrrolidynyl is present in the molecule. Phenyloxazole cations are formed when $CH_4O_2$ is lost. The formation of the metylenedioxybenzoyloxonium and allyldioxybenzoyloxonium ions is typical for 3,4-methylenodioxy derivatives, however, the formation of the former appears to be inhibited by the presence in the molecule of the group of atoms able to form very stable tropylium carbocation.



## 1. Introduction

Cathinones is a common name of synthetic designer drugs that are cathinone derivatives [1]. The parent compound cathinone ((*S*)-2-amino-1-phenyl-1-propanone) is an alkaloid responsible for stimulant effect of khat (*Catha edulis*). They form a large family of compounds produced by substitutions at three locations of the cathinone molecule: phenyl ring, amino group and propanone terminus. Alkyl, alkoxy, alkylenodioxy, haloalkyl, cyclic groups and halides may be introduced to the molecule. Phenyl ring may be exchanged by any monocyclic or fused-polycyclic ring, which also can be substituted to any extent with various groups and substituents. Substituted cathinones are reported to have central nervous system stimulant properties and to produce amphetamine-like effects [1–7].

Like synthetic cannabinoids, cathinones belong to the group of compounds that are being most often developed nowadays with the intent to overcome bans on drugs. New designer drugs including stimulant and entactogen cathinones are sold in head-shops and via web-site often as products by different names, brands and purposes of use and frequently marked as not-for-consumption. Synthetic cathinones seem to be gaining stronger market position [8–14]. The lists of scheduled drugs have been revised and updated in many countries recently, including United Kingdom and Poland, to prevent the flood of newly developed cathinones. However, new derivatives have continued to appear and derivatives that are already illegal continue to be identified and reported.

There is the increasing demand for fast and reliable methods for screening and confirmation of substituted cathinones to help control the illegal and not yet illegal drug market. In drug testing, infrared spectroscopy, mass spectrometry, nuclear magnetic resonance spectroscopy, Raman spectroscopy and X-ray diffractometry are techniques of high discriminating power [15–28]. However, legal highs and illicit narcotics are often mixtures of compounds and for IR, Raman spectroscopy, NMR it is known that a mixture sample that produces a spectrum combining more than one chemical entity may not provide definitive drug identification.

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Identification of psychoactive substances in complex mixtures is of no concern for LC–MS or GC–MS analyses. Although structural information obtained from MS/MS spectra is limited (e.g. the position of substituent on the ring, stereoisomeric configuration cannot be obtained) unless the sophisticated techniques such as H/D exchange or the combination of different fragmentation methods and various types of mass spectrometers are employed, this drawback of the LC–MS and GC–MS methods is, however, balanced out by method advantages such as high speed, versatility and sensitivity. In the case of LC–MS, the results of analysis may be known and expert reports can be prepared within circa 5–10 min from the delivery of a product to the lab. Very small amount of product (5–10 μg or even less) is sufficient to detect and identify psychoactive ingredients. Methods based on LC–MS/MS [26,28–31] have been published for the screening and quantification in serum, whole blood and pharmaceutical formulations of several cathinone derivatives. They were based on targeted MRM and SRM monitorings. High-resolution tandem mass spectrometry coupled to high performance liquid chromatography (HPLC–HRMS/MS) is a powerful analytical tool which delivers appropriate sensitivity and selectivity for simultaneous multicomponent detections and identifications [32–35] and is likely to succeed also in non-targeted screening of synthetic cathinones. The accurate mass of a molecular ion obtained from MS scan enables the molecular formula generation and subsequent targeted MS/MS scans deliver confirmatory information.

The aim of the study was to demonstrate the applicability of LC–ESI-Q/TOF for screening of samples for cathinones as well as to determine the fragmentation patterns caused by collision induced dissociations (CID) of 3,4-methylenodioxy derivative cathinones to facilitate future fast screenings and identifications of newly developed cathinones. The knowledge of the fragmentation pattern may help in the screenings for newly developed cathinones.

## 2. Materials and methods

### 2.1. Materials

Samples of material seized from head shops were analysed. Analytical standards of examined designer drugs were purchased from Cayman Chemicals, USA and LGC Standards, Poland. Acetonitrile of LC–MS purity grade was acquired from Merck (Warsaw, Poland). Formic acid of analytical purity grade was purchased from SigmaAldrich (Poznan, Poland).

### 2.2. Equipment

Agilent Technologies liquid chromatograph 1200 series composed of a nano pump (G2226A), a capillary pump (G137A), a thermostat (FC/ALS G1330B) and a microautosampler MicroWPS G1733A) coupled to a tandem mass spectrometer Agilent Technologies 6538 UHD Accurate Mass Q-TOF LC/MS equipped with a HPLC-chip-cube (G4240A) were used. Instrument control was performed by Agilent Mass Hunter Acquisition module, version B.02.

### 2.3. Sample preparation

Pills, capsules and powders of samples were crushed in a mortar and dissolved in methanol. The extracts and solutions were filtered through 0.25 μm filters and analyzed by LC–MS.

### 2.4. LC–MS method

Chromatographic separations were carried out on a large capacity chip 150 mm C18, 5 μm Chip II using a 9-min linear gradient of aqueous 0.1% formic acid (A) and 0.1% formic acid in acetonitrile (B), from 3 to 98% B with a 1 min hold at 98%B and a 1.5 min postrun at 3% B, mobile phase flow 0.5 μl $min^{-1}$. Analytes were ionized in the nanoelectrospray of a chip-cube in positive ion polarity mode. MS and targeted MS/MS acquisitions were carried out. For MS/MS experiments collision energies were set at 10, 20 and 40 eV. MS/MS fragmentation reproducibility expressed as the average standard deviation of normalized percent ion abundances was found not to exceed 0.75. Retention time reproducibility < 0.1%. Data analysis was carried out using Agilent Mass Hunter Qualitative software, v. B.04. More detailed information on LC–MS method conditions and data analysis are presented in [35].

## 3. Results and discussion

To demonstrate the applicability of LC-Q/TOF to screening of legal high samples for cathinones and to determine the fragmentation patterns of 3,4-methylenodioxy derivatives caused by collision induced dissociations (CID), the LC–MS screenings of samples suspected of containing cathinones were performed and followed by targeted LC–MS/MS analyses.

### 3.1. LC–MS screening

Full scan HRLC-MS spectra were acquired in the positive ion mode. Data mining algorithm "find by molecular feature" (FMF) was applied to extract compounds. FMF is an algorithm that finds co-eluting ions that are related i.e. pseudo-molecular, adduct, dimer, single and multiple charged ions, and all their isotopic ions. Extracted compound chromatograms (ECC) and compound mass spectra were generated. Then "molecular formula generator" (MFG) algorithm was employed and formulas of compounds were derived. The list of compounds was searched against an in-house developed database of psychoactive substances. Six compounds were detected as potential 3,4-methylenodioxy derivative cathinones. Table 1 list the $m/z$ of measured ions, generated formulas, calculated monoisotopic masses of pseudomolecular ions $[M+H]^+$ and retention times and accurate mass database hits.

The relative differences between measured and calculated exact masses were within ± 1 ppm for all compounds with isotopic abundance and isotope distance scores greater than 90. Fig. 1 presents structures of methylone, butylone, pentylone, MDPBP, MDPV and BMDP.

### 3.2. LC–MS/MS experiments

To identify compounds targeted MS/MS were performed. Masses and retention times for MS/MS experiments were set according to values indicated in Table 1. To obtain high quality MS/MS data rich in structural information, fragmentation were carried out at three collision energies: 10, 20 and 40 eV separately for each pseudo-molecular ion, no overlaps of retentions times of targeted ions were allowed in method acquisition table. Acquired MS/MS spectra were subjected to data mining using a "find by targeted MS/MS" algorithm, which search compounds basing on compound MS features and fragment ions that explain parent molecule. Extracted compound chromatograms and product ion spectra were derived and analysed. Fig. 2 shows Q/TOF product spectra of compounds **1–6**; Supplementary Table S1 lists product ions of intensities higher than 1%. MFG algorithm was applied on extracted compounds to generate molecular formulas of their parent and daughter ions.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2013.03.016.

The example list of MS/MS formula details derived from spectra for compound **3** is presented in Table 2. The fragment ions elucidate the structure of compound **3** well. Besides cations there are also