



## Short Communication

# GC-PCI-MS/MS and LC-ESI-MS/MS databases for the detection of 104 psychotropic compounds (synthetic cannabinoids, synthetic cathinones, phenethylamine derivatives)



Brian Waters<sup>1</sup>, Natsuki Ikematsu<sup>1</sup>, Kenji Hara, Hiroshi Fujii, Tomoko Tokuyasu, Mio Takayama, Aya Matsusue, Masayuki Kashiwagi, Shin-ichi Kubo\*

Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, Japan

## ARTICLE INFO

## Article history:

Received 7 December 2015  
 Received in revised form 24 February 2016  
 Accepted 24 February 2016  
 Available online 26 February 2016

## Keywords:

Psychotropic compounds  
 Chemical ionization  
 Tandem mass spectrometry  
 Mass spectral database  
 Forensic toxicology

## ABSTRACT

Designer psychotropic compounds continue to be a major problem in Japan and all around the world. Electron impact mass spectrometry (GC-EI-MS) and liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) data on these compounds have been widely reported. In this report, we present a detection method that has been rarely utilized to analyze these types of compounds, gas chromatography with positive chemical ionization and tandem mass spectrometry (GC-PCI-MS/MS). We report on the development of GC-PCI-MS/MS and LC-ESI-MS/MS databases of 104 psychotropic compounds, including 32 cannabinoid derivatives, 29 cathinone derivatives, 34 phenethylamine derivatives, and several other designer compounds. Using this database, we were able to detect 5 psychotropic compounds in an actual forensic autopsy case. If GC-PCI-MS/MS is used together with the more established methods of GC-EI-MS and LC-ESI-MS/MS, we believe the forensic toxicology community could be better prepared to deal with the challenges of these ever-changing compounds.

© 2016 Elsevier Ireland Ltd. All rights reserved.

## 1. Introduction

In recent years, psychoactive compounds used as designer or recreational drugs have increased in scope and use all over the world [1]. These drugs present a problem for law enforcement because it is difficult to keep pace with their quickly changing structures and target them to be scheduled and controlled. This is also a challenge for the toxicology community, since the use and abuse of these drugs typically precedes the ability to isolate and identify the harm they can do to the public. To this end it is increasingly important to be able to quickly and effectively identify and quantitate these compounds from blood and urine.

GC-EI-MS spectral data and LC-ESI-MS/MS data have been reported in several places and can be easily accessed [2–5]. One major advantage of GC-PCI-MS is the elucidation of the molecular ion [6,7]. This ability coupled with the information determined using tandem mass spectroscopy significantly increases the amount of structural information gained.

We report on the development of GC-PCI-MS/MS and LC-ESI-MS/MS databases of 104 psychotropic compounds, including 32 cannabinoid derivatives, 29 cathinone derivatives, 34 phenethylamine derivatives, and several other designer compounds.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The compounds without any designation were purchased from Cayman Chemical (Ann Arbor, Michigan, USA). Compounds marked with (\*) were synthesized in our laboratory. All other reagents were of analytical grade.

### 2.2. Preparation of shooting standards for GC analysis

Compounds received in powder form were dissolved in methanol or 1 M hydrochloric acid to make stock solutions at concentrations of 1.0 mg/ml. An aliquot (1 µl) of stock solution was pipetted into a clean glass test tube, evaporated to dryness under a stream of nitrogen gas, and reconstituted in 100 µl of *n*-propyl acetate:methanol (1:1) to prepare a shooting standard at a concentration of 10 ng/µl.

\* Corresponding author at: 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.

E-mail address: [kuboshin@fukuoka-u.ac.jp](mailto:kuboshin@fukuoka-u.ac.jp) (S.-i. Kubo).

<sup>1</sup> B. Waters and N. Ikematsu contributed equally to this manuscript.

For GC analysis, compounds received as the hydrochloride salt required conversion to the free base. In these cases, the stock solution was pipetted into a glass test tube along with 3 ml of dichloromethane and 50  $\mu$ l of aqueous ammonia. The solution pH was confirmed to be around 9, evaporated to dryness under nitrogen, and reconstituted in 100  $\mu$ l of *n*-propyl acetate:methanol (1:1).

### 2.3. Preparation of infusion standards for LC analysis

An aliquot (2  $\mu$ l) of stock solution was added to a clean glass test tube, evaporated to dryness under nitrogen, and reconstituted in 1 ml of the mobile phase of the LC method (0.1% formic acid in water) to prepare an infusion standard at a concentration of 2 ng/ $\mu$ l.

### 2.4. GC-PCI-MS/MS equipment and conditions

The GC-PCI-MS/MS system was a GCMS-TQ8030 (Shimadzu, Kyoto, Japan). The column used was a tandem column consisting of a BPX5 (2 m, 0.25 mm i.d., 0.5  $\mu$ m film thickness) as the pre-column coupled to a BPX5 (4 m, 0.15 mm i.d., 0.25  $\mu$ m film thickness) as the separation column connected by a SilTite<sup>®</sup>  $\mu$ -Union connector (SGE Analytical Science Pty. Ltd, Melbourne, Australia) [8].

Chemical ionization (CI) with isobutane gas [9] was employed in the positive ion mode at a voltage of 70 eV. The carrier gas was helium delivered at a constant flow of 5.12 ml/min. The oven temperature program was initially 80 °C for 0.5 min, ramped to 200 °C at 70 °C/min, and then increased to 320 °C at 50 °C/min. The injection temperature was 290 °C, the interface temperature was 320 °C, and the ion source temperature was 230 °C.

Multiple Reaction Monitoring (MRM) transitions and collision energies were determined through consecutive 1  $\mu$ l injections of the individual standards.

### 2.5. LC-MS/MS equipment and conditions

The LC system was a Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) with a binary pump and integrated degasser, autosampler, and heated column compartment. The detection system was a TSQ Quantum Access MAX tandem mass spectrometer (Thermo Scientific, Waltham, MA, USA). The column utilized was a Hypersil GOLD PFP column (50 mm x 2.1 mm i.d., 0.5  $\mu$ m; Thermo Scientific, Waltham, MA, USA).

The column temperature was set at 40 °C and the injection volume was 10  $\mu$ l. The mobile phase consisted of a gradient between 0.1% formic acid in water (mobile phase A) and 0.2% formic acid in acetonitrile (mobile phase B). The gradient was 5% B for 5.5 min, linear gradient to 100% B to 8.5 min, and held at 100% B for 4 min. The flow was set at 0.2 ml/min and the eluate was delivered to the MS/MS by electrospray ionization in the positive mode.

Selected Reaction Monitoring (SRM) transitions and collision energies were determined through syringe infusion of the individual standards dissolved in mobile phase A.

## 3. Application to an actual autopsy case

### 3.1. Case report

A man in his early thirties was found nude on his back on a hotel room floor. A plastic case with several medications and two plastic bags containing dried plant material were found in the room. There was no trauma and the autopsy findings were inconclusive.

### 3.2. Sample preparation

Postmortem autopsy right heart whole blood (0.5 g) was added to a 5 ml plastic tube with 0.5 ml of distilled water and 0.01 ml of an internal standard (IS) solution containing 0.05  $\mu$ g/ $\mu$ l of caffeine- $d_3$  dissolved in acetonitrile. Acetonitrile (2.5 ml) was added to the tube along with 0.05 ml of formic acid and 0.01 ml of 5 M hydrochloric acid. The mixture was vortexed well and centrifuged, and the supernatant passed through a Captiva ND Lipids cartridge (3 ml, Agilent). The filtrate was combined with 2 ml of acetonitrile and the pH adjusted to 7 with ammonia water. The mixture was vortexed and centrifuged into 2 fractions. The upper fraction was passed through a SUPELCO PSA (200 mg/3 ml) cartridge. The extract was evaporated to dryness, dissolved in 0.1 ml of a solution of *n*-propyl acetate:methanol (1:1), and analyzed by GC-EI-MS (1  $\mu$ l injection volume) and GC-PCI-MS/MS (1  $\mu$ l injection volume). After GC analysis, the sample was evaporated to dryness again and reconstituted in 0.1 ml of 0.1% formic acid in water for analysis on the LC-ESI-MS/MS (10  $\mu$ l injection volume).

## 4. Results and discussion

The selected ion transitions, optimized collision energies and retention times (RTs) for the GC-PCI-MS/MS and LC-ESI-MS/MS analysis of the 104 compounds are displayed in Table 1. The ion transitions were selected based on their relative intensities, with product ion 1 having the highest intensity and so forth. The three most prominent fragments (highest intensity, left to right) from the GC-EI-MS spectra are also included in Table 1. The GC-EI-MS mass spectral data was in agreement with other reported data [2,3].

MRM instruments can measure multiple transitions in the same experiment by rapidly toggling between the precursor/fragment pairs. SRM instruments monitor a single mass fragment from a single precursor ion [10]. The results are often the same. In this study, for most of the 104 psychotropic compounds, the MRM product ions from the GC-PCI-MS/MS analysis matched the product ions from the SRM transitions of LC-ESI-MS/MS, although occasionally differing in the order of intensity.

In standard GC-EI-MS, particularly with these new psychoactive compounds, many of which have structures that differ by only a few functional groups, mass spectra that are difficult to distinguish are often encountered. In these cases, the ability to differentiate between several similar compounds is made easier with the determination of their molecular weights. The molecular weight can easily be determined using positive chemical ionization, since the pseudo-molecular ion [M+H] is often formed. The pseudo-molecular ion alone cannot distinguish between two compounds with the same molecular weight; however, the coupling of CI with tandem mass spectroscopy produces fragmentation that aids in structural elucidation and differentiation between two species with the same molecular weight. Also, the [M+H] ion is often the most prominent ion, making it the logical choice for the precursor or parent ion in MRM analysis.

LC-ESI-MS/MS has become the method of choice of many forensic toxicology labs because of its high sensitivity and selectivity; however, effective use of LC-ESI-MS/MS as a screening technique requires a highly developed and comprehensive database that is optimized for the particular instrument being used. In this study, reference standards for 104 compounds were able to be gathered and tested, but there are many more compounds that were unable to be collected. This limitation is detrimental to screening by LC-ESI-MS/MS because optimized SRM conditions for a particular compound are required for effective detection and analysis. The scanning capability of GC-MS makes it a better candidate for screening.

Download English Version:

<https://daneshyari.com/en/article/103402>

Download Persian Version:

<https://daneshyari.com/article/103402>

[Daneshyari.com](https://daneshyari.com)