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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)

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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.

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.

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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 µl of aqueous ammonia. The solution pH was confirmed to be around 9, evaporated to dryness under nitrogen, and reconstituted in 100 µl of *n*-propyl acetate:methanol (1:1).

2.3. Preparation of infusion standards for LC analysis

An aliquot (2 µl) 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/µl.

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 µm film thickness) as the pre-column coupled to a BPX5 (4 m, 0.15 mm i.d., 0.25 µm film thickness) as the separation column connected by a SilTite® µ-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 µl 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 µm; Thermo Scientific, Waltham, MA, USA).

The column temperature was set at 40 °C and the injection volume was 10 µl. 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 µg/µl 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 µl injection volume) and GC-PCI-MS/MS (1 µl 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 µl 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.

Table 1
GC-PCI-MS/MS MRM and LC-ESI-MS/MS SRM conditions for 104 psychotropic compounds.

No.	Compound name	Class	Average molecular weight	Mono-isotopic mass	GC-EI-MS			GC-PCI-MS/MS								LC-ESI-MS/MS							
					Largest peaks (molecular ion in bold)			RT	Parent ion	Product ion 1	CE	Product ion 2	CE	Product ion 3	CE	RT	Parent ion	Product ion 1	CE	Product ion 2	CE	Product ion 3	CE
1	Methylhexanamine	Aliphatic amines	115.2	115.1	Not available											3.48	116.2	57.4	12	41.5	22	43.5	21
2	N,N-Dimethylcathinone	Cathinones	177.2	177.1	72	77	70	1.214	178.0	105.1	21	72.1	27	133.1	12	3.31	178.1	105.2	20	77.3	39	72.3	26
3	Buphedrone	Cathinones	177.2	177.1	72	77	57	1.254	178.0	160.2	9	131.1	21	91.1	24	3.65	178.1	130.1	33	131.1	21	160.1	10
4	3-Fluoromethcathinone	Cathinones	181.2	181.1	58	95	56	1.074	182.0	164.1	12	149.1	21	103.1	24	3.40	182.0	149.1	19	164.1	12	148.1	31
5	4-Fluoromethcathinone	Cathinones	181.2	181.1	58	95	56	1.098	182.0	164.1	12	149.0	18	123.1	21	3.52	182.0	149.1	20	148.1	27	164.1	12
6	N-Ethylbuphedrone	Cathinones	191.3	191.1	86	77	58	1.356	192.0	174.2	12	130.2	27	145.1	18	3.89	192.0	130.1	28	146.1	15	145.1	18
7	Pentedrone	Cathinones	191.3	191.1	86	44	77	1.408	192.0	174.2	12	132.1	18	91.2	18	4.10	192.0	144.1	29	145.1	20	174.1	10
8	4-Methylethcathinone	Cathinones	191.3	191.1	72	44	91	1.456	192.0	174.2	9	145.1	18	119.1	18	4.13	192.0	132.1	16	130.1	31	91.2	28
9	4-Methylbuphedrone	Cathinones	191.3	191.1	72	91	57	1.474	192.0	174.1	9	145.2	21	105.1	24	4.22	192.0	144.1	30	174.1	11	159.1	17
10	3,4-Dimethylmethcathinone	Cathinones	191.3	191.1	58	56	77	1.576	192.0	174.2	9	159.2	18	144.1	27	4.37	192.0	159.1	19	158.1	30	174.1	11
11	Methedrone	Cathinones	193.2	193.1	58	77	92	1.648	194.0	176.2	9	161.2	18	146.1	24	3.72	194.0	161.1	19	146.1	28	176.1	11
12	4-Methyl-N-Methylbuphedrone	Cathinones	205.3	205.1	86	71	91	1.530	206.0	105.1	21	161.1	12	86.1	24	4.30	206.0	105.2	20	161.1	13	91.2	32
13	4-Methoxy-N,N-Dimethylcathinone	Cathinones	207.3	207.1	72	77	92	1.706	208.0	72.2	21	163.2	12	135.1	21	3.89	208.0	72.4	19	135.1	20	163.1	13
14	α-Pyrrolidinobutiophenone (PBP)	Cathinones	217.3	217.1	112	113	70	1.750	218.0	91.1	18	112.3	24	147.1	18	4.00	218.0	91.2	26	147.1	16	112.2	26
15	4-Methyl-α-PPP	Cathinones	217.3	217.1	98	56	91	1.840	218.0	119.2	24	98.2	24	147.2	15	4.27	218.0	119.2	23	147.1	16	98.2	25
16	4-Methyl-α-ethylaminopentiophenone	Cathinones	219.2	219.2	100	91	101	1.682	220.0	202.2	12	144.2	27	105.2	21	4.54	220.1	144.1	26	202.1	11	105.2	23
17	α-Pyrrolidinopentiophenone (PVP)	Cathinones	231.3	231.2	126	77	127	1.858	232.0	91.2	21	126.1	27	105.1	27	4.48	232.1	91.2	23	161.1	16	126.2	25
18	Pentylone	Cathinones	235.3	235.1	86	44	65	2.006	236.0	188.2	15	218.2	9	175.1	18	4.27	236.0	188.1	17	175.0	20	218.1	12
19	α-Pyrrolidinopentiothiophenone (PVT)	Cathinones	237.1	237.1	126	111	127	1.906	238.0	126.2	18	97.1	27	167.1	12	4.00	238.0	126.2	21	97.2	25	111.1	37
20	α-Pyrrolidinohexanophenone (PHP)	Cathinones	245.4	245.2	140	77	105	1.980	246.0	91.1	24	140.3	27	105.1	24	4.84	246.1	91.2	25	140.2	26	105.2	25
21	3,4-Methylenedioxy-α-PPP	Cathinones	247.3	247.1	98	56	99	2.218	248.0	98.2	21	147.1	21	149.1	21	3.98	248.0	147.1	22	98.2	25	177.1	16
22	4-Fluoro-α-Pyrrolidinopentiophenone	Cathinones	249.2	249.2	126	95	127	1.818	250.0	109.2	30	125.9	21	123.1	27	4.51	250.1	109.2	24	126.2	24	179.1	17
23	PV8	Cathinones	259.2	259.2	154	155	77	2.120	260.0	91.2	24	105.1	27	154.2	27	4.99	260.1	105.2	24	189.1	18	91.2	41
24	4′-Methyl-α-pyrrolidinohexanophenone (MPHP)	Cathinones	259.2	259.2	140	141	91	2.150	260.0	105.2	24	140.2	24	189.2	15	4.99	260.1	91.2	24	154.2	25	105.2	27
25	4-Methoxy-α-PVP	Cathinones	261.4	261.2	126	127	77	2.266	262.0	121.1	24	126.2	24	191.1	12	4.52	262.0	121.2	25	126.2	22	191.1	16
26	DL-4662	Cathinones	265.3	265.2	100	58	165	2.186	266.0	248.2	12	188.1	27	151.1	24	4.42	266.1	188.1	24	248.1	12	217.1	15
27	Diphenidine	Cathinones	265.2	265.2	174	91	175	2.246	266.0	181.2	15	166.2	27	86.2	15	4.97	266.1	181.1	18	165.1	37	103.2	33
28	PV9	Cathinones	273.2	273.2	168	169	77	2.256	274.0	91.1	21	105.2	24	168.2	24	5.34	274.1	91.2	27	168.1	27	105.2	26
29	Naphyrone	Cathinones	281.4	281.2	126	127	84	2.658	282.0	141.2	24	126.2	27	211.2	18	5.38	282.0	141.1	24	211.1	17	127.1	46
30	4-Methoxy PV8	Cathinones	289.2	289.2	154	135	77	2.524	290.0	121.2	24	154.3	30	135.1	24	5.14	290.1	121.1	26	219.1	16	154.2	22
31	2-Methoxyamphetamine (2-MA)	Phenethylamines	165.2	165.1	44	91	122	1.436	166.0	149.1	6	121.1	15	91.1	24	3.65	166.1	121.2	14	91.2	30	77.3	28
32	3-Methoxyamphetamine (MMA)	Phenethylamines	165.2	165.1	44	78	91	1.464	166.0	121.1	15	149.1	6	91.1	27	3.71	166.1	121.2	17	78.3	43	91.2	29
33	p-Methoxyamphetamine (PMA)	Phenethylamines	165.2	165.1	44	122	78	1.486	166.0	149.1	9	121.1	15	91.1	24	3.82	166.1	91.2	26	121.2	14	65.4	38
34	7-APB	Phenethylamines	175.2	175.1	44	131	77	1.320	176.0	131.1	24	159.1	9	91.1	27	4.06	176.0	131.1	16	91.2	30	77.3	38
35	4-APB	Phenethylamines	175.2	175.1	44	131	77	1.340	176.0	159.1	9	131.1	21	91.1	24	4.09	176.0	131.1	20	115.2	36	77.3	41
36	5-APB	Phenethylamines	175.2	175.1	44	131	77	1.382	176.0	159.1	9	131.1	24	91.1	24	4.16	176.0	131.1	18	91.2	29	159.1	5
37	* 2-APB	Phenethylamines	175.2	175.1	44	131	77	1.388	176.0	131.1	24	91.1	27	159.1	12	4.20	176.0	115.2	36	131.1	18	77.3	42
38	6-APB	Phenethylamines	175.2	175.1	44	131	77	1.396	176.0	159.1	9	131.1	21	91.2	27	4.26	176.0	115.2	43	91.2	33	77.2	44
39	5-APDB	Phenethylamines	177.2	177.1	44	134	77	1.524	178.0	161.1	9	133.1	21	105.1	27	3.76	178.0	133.0	25	161.0	20	105.0	16
40	6-APDB	Phenethylamines	177.2	177.1	44	134	77	1.548	178.0	161.1	9	133.1	21	105.2	27	3.79	178.0	133.1	19	161.1	5	105.2	26
41	* 4-Methoxymethamphetamine (PMMA)	Phenethylamines	179.3	179.1	58	121	78	1.602	180.0	149.1	9	121.1	21	91.1	27	3.62	180.1	135.1	17	133.1	15	105.2	21
42	* 3,4-Methylenedioxyamphetamine (MDA)	Phenethylamines	179.2	179.1	44	136	77	1.676	180.0	163.1	6	105.1	24	133.1	15	3.85	180.1	91.2	27	121.1	16	78.3	38
43	* 4-Methylthioamphetamine (4-MTA)	Phenethylamines	181.3	181.1	44	138	122	1.816	182.0	165.1	9	117.1	15	137.1	15	4.13	182.1	117.2	17	137.1	18	91.2	44
44	*α-Ethyltryptamine	Phenethylamines	188.3	188.1	131	130	58	2.316	189.0	130.1	21	172.2	9	118.0	15	4.16	189.1	130.1	18	77.3	42	103.2	38
45	7-MAPB	Phenethylamines	189.3	189.1	58	77	131	1.380	190.0	131.1	24	159.1	9	91.1	24	4.25	190.1	131.1	22	159.1	15	77.3	43
46	4-MAPB	Phenethylamines	189.3	189.1	58	131	77	1.420	190.0	159.1	9	131.1	21	91.1	27	4.27	190.0	131.1	21	159.1	11	91.2	32
47	5-MAPB	Phenethylamines	189.3	189.1	58	131	77	1.468	190.0	131.1	24	159.1	9	91.1	27	4.30	190.0	131.1	21	59.1	10	91.2	32
48	6-MAPB	Phenethylamines	189.3	189.1	58	77	131	1.486	190.0	159.2	9	131.1	21	91.2	27	4.30	190.0	131.1	20	91.2	31	77.3	42
49	* 3,4-Methylenedioxymethamphetamine (MDMA)	Phenethylamines	193.3	193.1	58	77	135	1.770	194.0	163.1	9	105.1	24	133.1	18	3.79	194.2	163.1	10	135.1	18	133.1	19
50	* 1,3-Benzodioxolylbutanamine (BDB)	Phenethylamines	193.2	193.1	58	136	77	1.878	194.0	135.1	15	177.2	6	147.2	9	3.96	194.1	135.1	11	77.3	35	147.1	5
51	* 2,5-Dimethoxyamphetamine (DMA)	Phenethylamines	195.3	195.1	44	152	137	1.808	196.0	179.2	9	151.1	15	121.1	27	3.96	196.1	151.1	13	91.3	31	164.1	15
52	* 3,4-Methylenedioxyethylamphetamine (MDEA)	Phenethylamines	207.3	207.1	72	44	135	1.832	208.0	163.1	9	105.1	24	133.1	18	3.99	208.1	135.1	22	105.2	25	133.1	17
53	* 1,3-Benzodioxolyl-N-methylbutanamine (MBDB)	Phenethylamines	207.3	207.1	72	135	77	1.910	208.0	135.1	24	177.1	9	147.1	12	4.10	208.1	135.1	19	77.3	35	147.1	16

(*continued on next page*)

Table 1 (*continued*)

No.	Compound name	Class	Average molecular weight	Mono-isotopic mass	GC-EI-MS			GC-PCI-MS/MS								LC-ESI-MS/MS							
					Largest peaks (molecular ion in bold)			RT	Parent ion	Product ion 1	CE	Product ion 2	CE	Product ion 3	CE	RT	Parent ion	Product ion 1	CE	Product ion 2	CE	Product ion 3	CE
54	*Mescaline	Phenethylamines	211.3	211.1	182	167	181	2.038	212.0	195.2	9	168.2	12	197.2	6	3.55	212.1	165.1	22	133.1	22	135.2	5
55	Butylone	Phenethylamines	221.3	221.1	72	149	65	1.888	222.0	174.1	15	204.2	9	146.2	21	3.90	222.0	174.1	17	131.1	33	146.1	23
56	* 2,3,4-Trimethoxyamphetamine (TMA-3)	Phenethylamines	225.3	225.1	44	182	167	1.918	226.0	209.1	9	194.1	18	181.1	15	3.72	226.1	209.1	11	181.1	20	91.3	46
57	* 3,4,5-Trimethoxyamphetamine (TMA)	Phenethylamines	225.3	225.1	44	182	167	2.046	226.0	209.1	9	168.1	9	181.2	15	3.86	226.1	209.1	5	179.1	27	181.1	15
58	* 2,4,5-Trimethoxyamphetamine (TMA-2)	Phenethylamines	225.3	225.1	182	44	167	2.080	226.0	209.1	9	194.2	21	179.2	21	3.90	226.1	209.1	10	194.1	18	91.2	37
59	* 2,4,6-Trimethoxyamphetamine (TMA-6)	Phenethylamines	225.3	225.1	182	44	181	2.136	226.0	209.2	9	181.1	18	121.1	27	4.34	226.2	209.1	11	181.1	17	121.2	30
60	* 3,4,5-Trimethoxymethamphetamine	Phenethylamines	239.3	239.2	58	182	167	2.108	240.0	209.1	12	58.1	12	168.1	6	3.83	240.1	209.1	13	178.1	20	181.1	18
61	* 2,4,5-Trimethoxymethamphetamine	Phenethylamines	239.3	239.2	58	182	167	2.126	240.0	209.1	12	194.2	18	181.1	18	3.96	240.1	209.1	10	179.0	29	151.1	5
62	* 2,4,5-Trimethoxy-N,N-dimethylamphetamine	Phenethylamines	253.3	253.2	181	208	151	2.586	254.0	209.2	9	194.1	18	181.2	18	4.55	254.1	209.1	9	179.1	25	181.1	23
63	* 2,5-Dimethoxy-4-propylthiophenethylamine (2C-T-7)	Phenethylamines	255.4	255.1	226	183	225	2.520	256.0	239.1	9	197.1	12	182.1	21	4.79	256.0	239.1	9	197.0	20	224.0	19
64	* 4-Bromo-2,5-dimethoxyphenethylamine (2C-B)	Phenethylamines	259.2	259.0	230	232	77	2.202	260.0	243.0	9	228.0	21	212.9	27	4.38	261.1	229.2	20	244.1	11	214.3	29
65	*Brolamfetamine (DOB)	Phenethylamines	273.3	273.0	44	230	232	2.214	274.0	257.0	9	229.0	18	178.1	21	4.52	275.0	229.6	19	228.0	34	179.1	23
66	* 2,5-Dimethoxy-4-Iodophenethylamine (2C-I)	Phenethylamines	307.1	307.0	278	263	77	2.366	308.0	291.0	9	276.0	21	260.9	30	4.54	307.9	290.9	12	275.9	22	164.1	16
67	*m-Chlorophenylpiperazine (mCPP)	Piperazines	196.7	196.1	154	156	**196**	2.128	197.0	154.1	21	119.1	24	140.2	24	4.27	197.0	154.1	18	119.2	23	118.2	28
68	1,4-Dibenzylpiperazine (DBZP)	Piperazines	266.4	266.2	91	175	120	2.432	267.0	91.2	24	175.2	15	134.2	24	4.42	267.0	91.2	33	175.1	18	134.1	23
69	Mepirapim	Synthetic cannabinoids	313.4	313.2	214	83	144	3.334	314.0	214.2	12	144.1	30	271.2	9	5.12	314.1	214.1	18	144.1	34	116.2	42
70	CP-47,497	Synthetic cannabinoids	318.5	318.3	215	233	**318**	3.056	*301.0*	233.2	9	121.2	24	148.3	21	0.83	316.8	113.3	17	181.1	16	45.0	49
71	JWH-251	Synthetic cannabinoids	319.4	319.2	214	144	215	3.416	320.0	105.1	24	214.2	21	144.1	27	6.56	320.0	214.3	16	105.3	23	144.2	32
72	UR-144 N-(5-hydroxypentyl) metabolite	Synthetic cannabinoids	327.5	327.2	230	144	312	3.370	328.0	125.2	18	144.4	27	97.2	27	6.09	328.1	125.2	16	230.0	19	144.1	34
73	JWH-015	Synthetic cannabinoids	327.4	327.2	**327**	127	326	3.700	328.0	155.2	24	127.2	33	200.1	24	6.36	328.0	155.0	25	127.0	25	200.0	25
74	AB-PINACA	Synthetic cannabinoids	330.4	330.2	215	286	145	3.326	331.0	215.2	21	286.1	12	314.2	6	5.65	331.0	215.2	21	286.2	10	145.1	31
75	JWH-022	Synthetic cannabinoids	339.4	339.2	155	**339**	127	3.886	340.0	155.1	21	127.2	30	212.1	24	6.57	340.0	155.2	23	127.1	50	145.1	33
76	UR-144 N-pentanoic acid metabolite	Synthetic cannabinoids	341.5	341.2	Not Available											6.04	342.1	125.1	16	244.1	17	324.1	18
77	JWH-073 N-(3-hydroxybutyl) metabolite	Synthetic cannabinoids	343.4	343.2	127	270	155	4.062	344.0	155.3	27	127.2	39	284.0	27	5.92	344.0	155.1	26	127.2	39	145.0	42
78	UR-144 N-(5-chloropentyl) analog	Synthetic cannabinoids	345.9	345.2	248	144	330	3.368	346.0	125.2	27	247.9	21	97.3	27	6.79	370.0	248.0	25	125.0	25	N/A	
79	AB-PINACA N-(4-fluoropentyl) isomer	Synthetic cannabinoids	348.4	348.2	233	304	145	3.384	349.0	233.1	24	304.2	12	332.2	6	5.38	349.1	233.1	25	213.2	3	145.1	37
80	5-Fluoro AB-PINACA	Synthetic cannabinoids	348.4	348.2	233	304	145	3.490	349.0	233.1	21	304.1	15	332.1	3	5.40	349.0	303.9	12	233.0	25	145.0	40
81	JWH-250 N-(5-hydroxypentyl) metabolite	Synthetic cannabinoids	351.4	351.2	230	144	91	3.978	352.0	121.2	24	91.3	27	149.2	15	5.67	352.0	121.2	20	91.3	43	186.3	5
82	A-796260	Synthetic cannabinoids	354.5	354.2	100	114	56	3.474	355.0	125.1	15	114.2	24	97.1	27	5.62	355.1	125.2	16	114.1	27	97.2	26
83	NNEI	Synthetic cannabinoids	356.2	356.2	244	144	115	4.334	357.0	214.2	15	144.3	30	158.2	30	6.30	357.0	214.1	25	144.1	38	279.0	16
84	MN-18	Synthetic cannabinoids	357.5	357.2	215	145	**357**	4.100	358.0	215.1	21	145.2	30	250.9	27	5.93	358.0	155.0	25	127.0	25	N/A	
85	JWH-018 N-(4-hydroxypentyl) metabolite	Synthetic cannabinoids	357.5	357.2	127	270	155	4.186	358.0	155.0	24	340.1	6	127.0	45	6.69	358.0	215.2	16	145.0	32	117.1	40
86	5-Fluoro AMB	Synthetic cannabinoids	363.4	363.2	233	304	145	3.180	364.0	233.1	18	304.1	12	145.1	30	6.07	364.0	233.1	20	304.1	25	213.0	25
87	JWH-210	Synthetic cannabinoids	369.5	369.2	214	**369**	312	4.120	370.0	183.2	24	214.1	24	155.3	30	6.87	371.0	183.0	25	214.0	15	155.0	25
88	JWH-018 N-pentanoic acid metabolite	Synthetic cannabinoids	371.4	371.2	Not Available											5.86	372.0	155.0	25	127.0	25	244.0	25

89	5-Fluoro-NNEI	Synthetic cannabinoids	374.2	374.2	232	144	115	4.468	375.0	232.1	18	144.1	30	206.1	18	6.10	375.0	232.0	25	144.0	34	116.1	51
90	Cannabipiperidiethanone	Synthetic cannabinoids	376.5	376.2	98	70	129	4.038	377.0	112.3	24	98.3	24	121.1	18	5.34	377.1	112.2	25	121.1	23	98.2	32
91	CP-55,940	Synthetic cannabinoids	376.6	376.3	273	147	121	3.592	*359.0*	127.3	9	121.1	18	215.2	15	5.93	377.6	233.0	25	121.1	25	93.1	25
92	AM1220	Synthetic cannabinoids	382.5	382.2	98	70	127	4.342	383.0	98.2	27	112.1	21	155.3	24	5.68	383.1	112.2	20	98.2	46	155.2	40
93	AKB48 N-(5-fluoropentyl) analog	Synthetic cannabinoids	383.2	383.2	233	145	294	3.962	384.0	135.2	21	107.2	27	93.1	27	6.44	384.1	135.1	17	93.2	32	107.2	37
94	JWH-200	Synthetic cannabinoids	384.5	384.2	100	56	127	4.398	385.0	155.2	27	114.2	24	127.1	30	5.51	385.0	155.0	20	127.1	47	114.2	25
95	BB-22	Synthetic cannabinoids	384.2	384.2	240	144	116	4.498	385.0	240.1	18	144.0	27	97.1	27	6.36	385.1	240.1	16	144.2	45	116.2	53
96	EAM2201	Synthetic cannabinoids	387.2	387.2	232	**387**	312	4.254	388.0	183.2	21	232.1	18	155.2	39	6.55	388.0	183.2	25	152.9	47	129.3	5
97	FDU-PB-22	Synthetic cannabinoids	395.1	395.1	109	252	**115**	4.426	396.0	252.1	15	109.2	30	224.2	21	6.67	396.0	252.0	25	109.0	25	N/A	
98	FUB-PB-22	Synthetic cannabinoids	396.1	396.1	109	252	253	4.486	397.0	252.1	9	109.0	30	224.1	24	6.15	397.0	252.0	12	109.2	30	240.6	5
99	HU-308	Synthetic cannabinoids	414.6	414.3	277	318	353	3.242	*397.0*	71.0	24	106.8	24	57.0	30	0.86	414.9	142.8	21	75.1	38	N/A	
100	WIN-55212-2	Synthetic cannabinoids	426.5	426.2	100	127	56	4.720	427.0	155.1	24	100.3	30	127.2	30	5.77	427.0	155.2	32	127.1	49	100.3	33
101	AM2233	Synthetic cannabinoids	458.3	458.1	98	70	99	4.102	459.0	98.2	27	112.2	21	230.9	30	5.42	458.9	98.2	34	112.2	22	36.9	16
102	*α-Methyltryptamine (AMT)	Tryptamines	174.2	174.1	131	130	44	2.158	175.0	158.2	9	143.1	24	130.1	24	3.82	175.1	143.1	24	130.1	25	117.1	25
103	* 5-Methoxy-diisopropyltryptamine (5-MeO-DiPT)	Tryptamines	274.4	274.2	114	72	160	2.950	275.0	114.2	12	174.2	15	159.2	27	4.62	275.1	114.2	21	130.2	53	174.1	25
104	Mitragynine	Tryptamines	398.5	398.2	214	397	**398**	4.064	399.0	174.0	27	238.1	9	226.1	18	5.43	399.1	174.1	32	238.0	25	226.0	23

The Parent ion in *italics* were [M−H_2O] rather than [M+H].

* These compounds were synthesized in our laboratory.

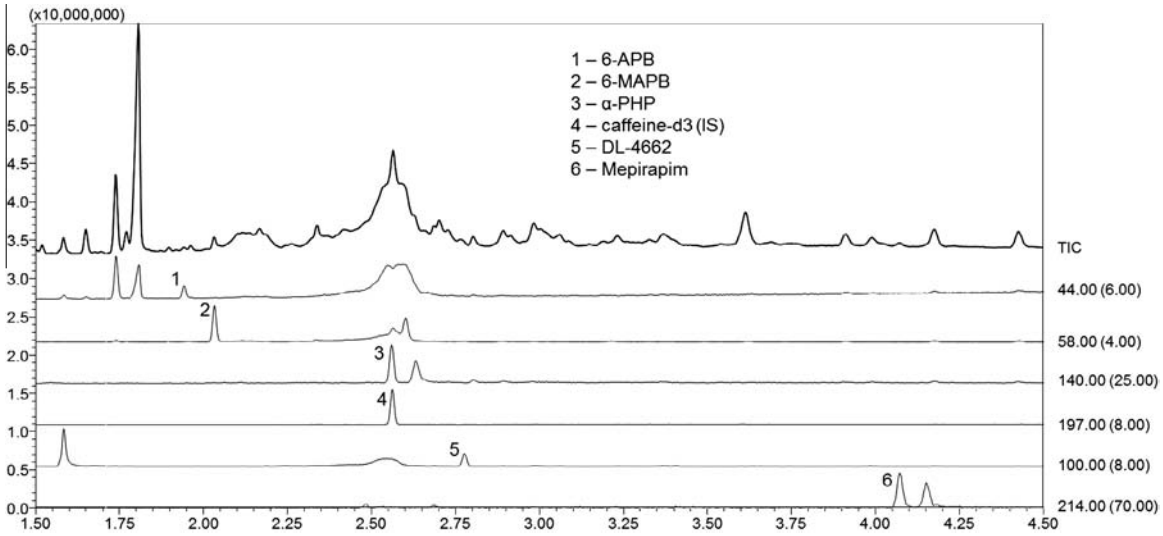

Fig. 1. GC-EI-MS chromatogram of autopsy right heart whole blood.

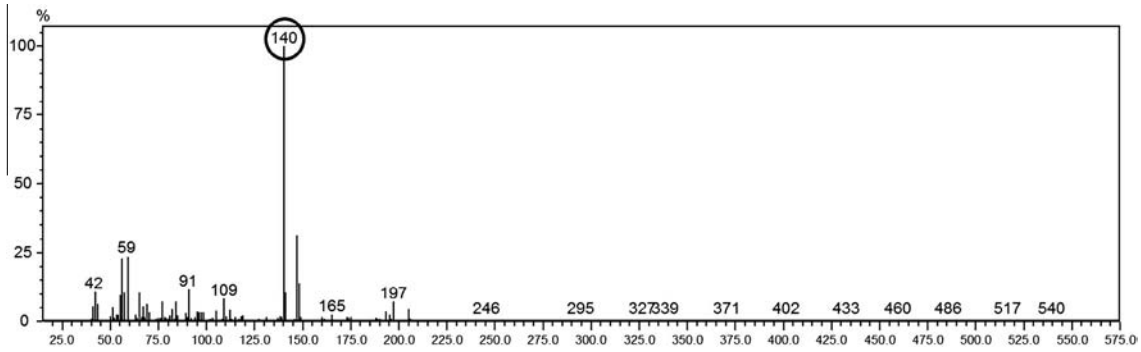

Fig. 2. GC-EI-MS spectrum of peak 3.

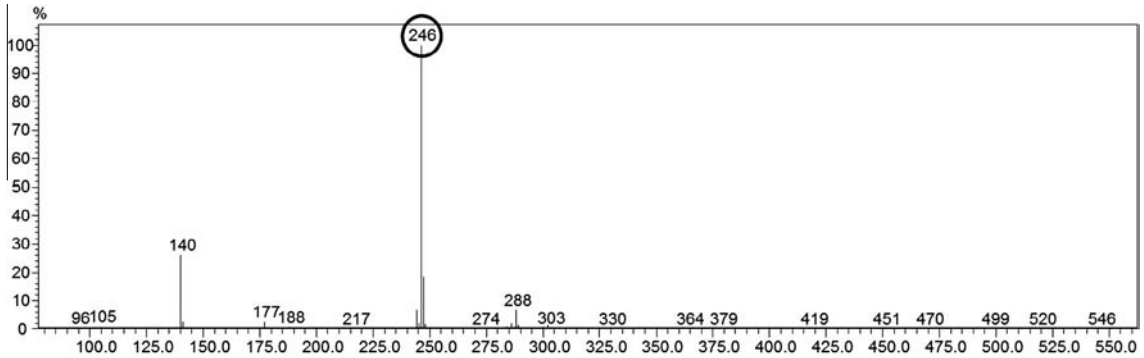

Fig. 3. GC-PCI-MS spectrum of peak 3.

Positive chemical ionization GC–MS/MS using isobutane as the reagent gas was applied to the mass spectrometric detection of 104 psychotropic compounds. Isobutane was selected as the reagent gas due to its tendency to form the pseudo-molecular ion [M+H], which allows for the determination of the compound's molecular weight [9]. CI spectra often yield fewer fragment ions than their EI counterparts, and the major fragment is often the [M+H] ion, confirming the molecular weight and aiding in structure elucidation, particularly when paired with tandem mass spectroscopy.

The employment of GC-PCI-MS/MS allowed for the detection of 101 of 104 psychotropic compounds and the development of a database that could easily be applied to screening or quantitation of these compounds. Along with the utilization of widely used detection techniques such as GC-EI-MS and LC-ESI-MS/MS, the identification and analysis of these compounds can be quickly and effectively accomplished.

We applied this database to an actual autopsy case and were able to detect 5 psychotropic compounds. Fig. 1 shows the GC-EI-MS chromatogram of the autopsy right heart whole blood sample. Fig. 2 shows the GC-EI-MS spectrum for Peak 3. The *m*/*z* 140 ion was observed and MPHP and α-PHP were 2 of the possibilities. Fig. 3 shows the GC-PCI-MS spectrum for the peak in question. The presence of the prominent *m*/*z* 246 ion indicates that the compound is likely α-PHP, which has a molecular weight of 245.

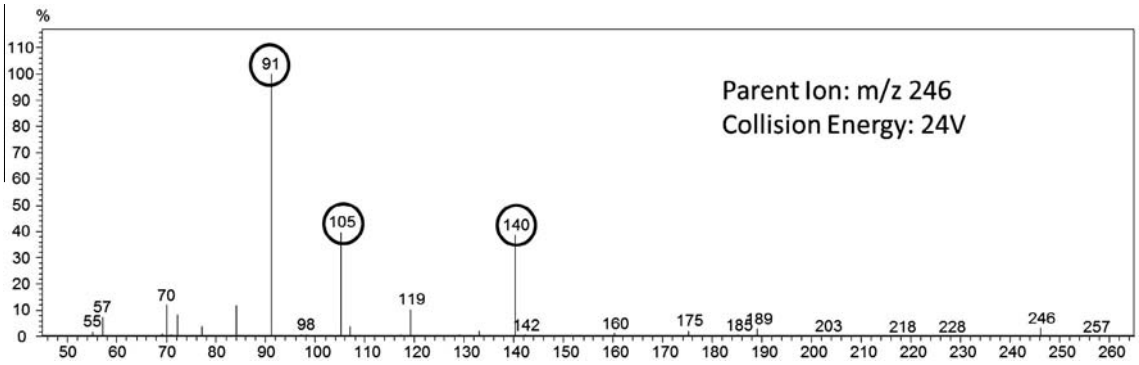

Fig. 4. GC-PCI-MS/MS product ion scan of α-PHP.

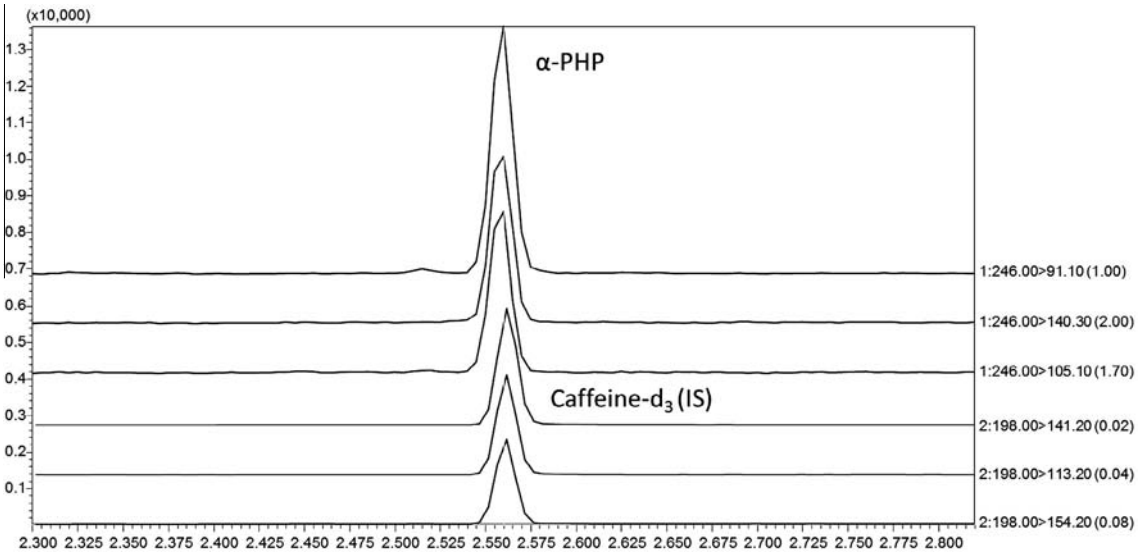

Fig. 5. GC-PCI-MS/MS MRM chromatogram of α-PHP in autopsy right heart whole blood.

Utilizing the MS/MS functionality of the instrument, we were able to further confirm the presence and identity of α-PHP. After identifying *m*/*z* 246 as the precursor ion, we performed a product ion (PI) scan to determine the appropriate transitions and optimal collision energies for a multiple reaction monitoring (MRM) method. Fig. 4 shows the PI scan spectrum. Fig. 5 shows the MRM mode chromatogram. The peaks for α-PHP and caffeine-d_3 (IS) were isolated for effect.

5. Conclusions

New drugs are occasionally not present in mass spectral libraries, especially if the libraries are outdated. Reference standards for these compounds are often not available for purchase, are cost prohibitive, or may not arrive in a timely manner.

The possible determination of an unknown compound's molecular weight and the elucidation of its structure through GC-PCI-MS/MS analysis are powerful tools in forensic toxicology [6]. Thus, we developed GC-PCI-MS/MS and LC-ESI-MS/MS databases of 104 psychotropic compounds that can aid in the identification and analysis of these problematic drugs.

Conflicts of interest

We declare that there are no conflicts of interest in this research.

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