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Article

Analysis of Synthetic Cathinones Commonly Found in Bath Salts in Human Performance and Postmortem Toxicology: Method Development, Drug Distribution and Interpretation of Results

Laureen J Marinetti* and Heather M Antonides

Montgomery County Coroner's Office (MCCO)/Miami Valley Regional Crime Laboratory (MVRCL), Dayton, Ohio

*Author to whom correspondence should be addressed. Email: marinettil@mcohio.org

To date, the Toxicology Section of the Montgomery County Coroner's Office/Miami Valley Regional Crime Laboratory has identified six synthetic cathinones, commonly found in bath salt products, in 43 cases. Thirty-two cases will be reviewed here, including all of the postmortem cases, all of the human performance cases that had blood specimens submitted, and one urine-only human performance case. The following compounds have been confirmed: 3,4-methylenedioxypyrovalerone (MDPV), 3,4-methylenedioxymethcathinone (methylone), pyrovalerone, pentylone, alpha-pyrrolidinopentiophenone (alpha-PVP) and methedrone. The method also screens for mephedrone, butylone and 3-fluoromethcathinone. Case demographics show 42 white males and females ranging in age from 19 to 53 years. The remaining case was that of a 34-year-old Hispanic male. The 43 cases represent 17 driving under the influence, two domestic violence, four suicides, 12 overdoses, six accidents, one drug-facilitated assault and one homicide. Data will be presented on the distribution of some of these cathinones in various matrices. After review, blood concentration does not appear to predict outcome regarding fatalities or impairment. The highest MDPV concentration occurred in a suicide by hanging and the highest methylone concentration was in a driver. The confirmation method is a liquid–liquid extraction with detection by liquid chromatography triple quadrupole mass spectrometry using electrospray ionization in multiple reaction monitoring mode.

Introduction

Cathinone is a naturally occurring beta-ketone amphetamine analogue present in the leaves of the *Catha edulis* plant. The synthetic cathinones are derivatives of this compound (Figure 1). Recreational use of synthetic cathinones is relatively new to the United States (US), commencing in approximately 2009. They are marketed as research chemicals, bath salts, jewelry cleaner, ice melter, hookah pipe cleaner or plant food, with the words "not for human consumption" printed on the label. Most of the synthetic cathinone supply originates in China or India; however, where it enters the US, or if it is also manufactured here, is not clear (1). The synthetic cathinones are marketed to drug users as legal alternatives to methamphetamine, cocaine or 3,4-methylenedioxymethamphetamine (MDMA). Synthetic cathinones are usually in the form of white or off-white powder or small candy-like objects that are supplied in small plastic containers or small Ziploc bags. Users generally snort, inject, smoke or orally consume synthetic cathinones.

The desired effects of synthetic cathinones are euphoria, rush, alertness, talkativeness, sexual arousal, focused mind and an overall positive feeling. The effects occur within 30 to 45 min of administration and the desired effects last from 1 to 3 h; however, the undesirable side effects of synthetic cathinone use can last from hours to days. The side effects are neurological and cardiovascular in nature and include the following: anxiety, insomnia, fatigue, mydriasis, agitation, aggression, combative behavior, panic, disorientation, confusion, memory loss, blackouts, myoclonus, excited delirium, paranoia, hallucinations, increased suicidal ideations, chest pain, tachycardia, breathing difficulties, hyperthermia, sweating and hypertension. Local hospital emergency room staff and first responders have reported that synthetic cathinone users are often violent and uncontrollable, exhibiting paranoid behavior and delusions. The synthetic cathinone users have physically attacked the first responders, biting and hitting them (2, 3). Examples of paranoid or odd behavior that have been reported include swimming in a snow bank to get away from snipers and believing people were hiding in walls. One user broke into a home, decorated it with Christmas decorations, then sat down and watched television (3–7). The behavior of synthetic cathinone users is similar to behavior exhibited by users of methamphetamine, cocaine, phencyclidine (PCP) and/or lysergic acid diethylamide (LSD), or in those suffering from excited delirium syndrome. When treating these users, common methods of chemical submission are often not effective, thus making treatment a challenge.

The first Montgomery County Coroner's Office (MCCO)/Miami Valley Regional Crime Laboratory (MVRCL) case in which synthetic cathinones were detected in biological specimens was a driving under the influence (DUI) fatality in March 2011. Since that time, 42 additional cases involving synthetic cathinone use have been confirmed in postmortem and human performance specimens, including data from an embalmed body. The Chemistry Section of the MVRCL also began receiving submissions of products containing synthetic cathinones in early 2011. Working together with the Chemistry Section, authentic standards were obtained for as many of the synthetic cathinones as possible to identify them in both dosage form and in toxicology casework. The most prevalent synthetic cathinones identified by the Chemistry Section were incorporated into a method by the Toxicology Section to screen blood, urine and other biological specimens.

Initially, local hospitals were averaging one admission per day due to synthetic cathinone use. Once some of the synthetic cathinones were controlled in the fall of 2011, the incidents dropped to one per week (2). Ohio state law has scheduled six synthetic cathinones in Schedule I: 3,4-methylenedioxymethcathinone (methylone), mephedrone, 3,4-methylenedioxypyrovalerone (MDPV), methedrone and 3- and 4-fluoromethcathinone.

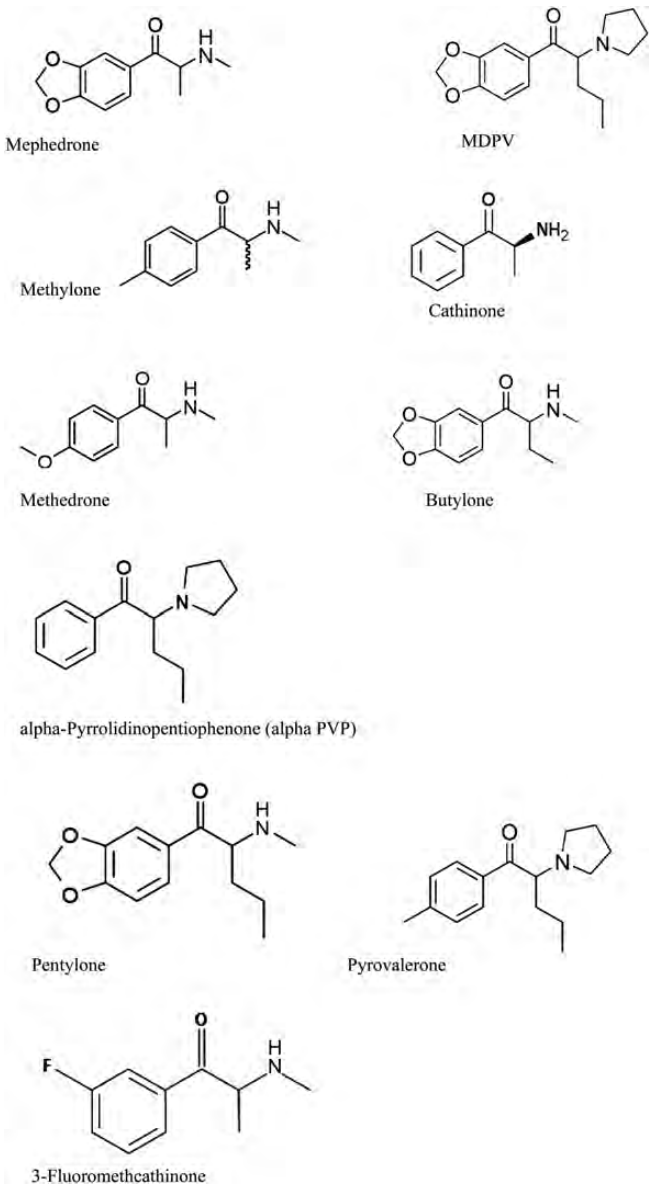

Figure 1. Cathinone and synthetic cathinone structures.

Federal law has scheduled methylone, mephedrone and MDPV in Schedule I and pyrovalerone in Schedule V.

In addition to the scheduled cathinones, the Toxicology Section has encountered cases with the unscheduled synthetic cathinones pentylone and alpha-pyrrolidinopentiophenone (alpha-PVP) (8). The MVRCL Chemistry Section has encountered cases with several more synthetic cathinones than those listed previously, including: 4-methylethcathinone (4-MEC), pentedrone, 4-methyl-alpha-pyrrolidinopropiophenone (MPPP) and 5,6-methylenedioxy-2-aminoindane (MDAI). Purchasing certified standards to confirm and quantify every synthetic cathinone can be difficult and expensive. New chemicals are evolving regularly; the trend seems to indicate that the illicit manufacturers will simply move on to another similar chemical or isomer that is not yet controlled.

Experimental

The MCCO/MVRCL Toxicology Section performs analyses in both human performance and postmortem forensic toxicology. Cases are received from the southern half of the state of Ohio, which includes urban, suburban and rural areas. The majority of the presented cases originated from suburban or rural areas. The toxicology analyses of each case included: volatile analysis to include methanol, ethanol, isopropanol and acetone; an enzyme-linked immunoassay (ELISA) screen comprised of amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoids, carisoprodol, cocaine metabolites and opiates; a basic drug screen by gas chromatography–mass spectrometry (GC–MS) on blood, urine or liver. Cases that had a history indicative of synthetic cathinone use or in which subject behavior suggested synthetic cathinone use were analyzed by the liquid chromatography–mass spectrometry-mass spectrometry (LC–MS-MS) confirmation method, independent of the GC–MS drug screen findings. The synthetic cathinones were not always detected in the GC–MS drug screen due to the limit of detection of this method for these chemicals. Meyer *et al.* also found that to reliably detect some synthetic cathinone metabolites by GC–MS, enzyme digestion and derivatization were required (9). MDPV could be detected by GC–MS down to 10 ng/mL. In addition, GC–MS also detected the high methylone case and the alpha-PVP cases. To avoid missing low concentrations of synthetic cathinones, it is best not to rely solely on a GC–MS drug screen for detection. Mass spectral data from the GC–MS basic drug screen to include the total ion chromatograph (TIC), library matches, and ions from 3 mL of blood and the TIC from 2 mL of urine from Postmortem Case 1 is shown in Figures 2A and 2B, respectively. Figure 3 shows the basic drug screen of 2 mL of urine from Postmortem Case 16, including the TIC and ions and library matches of both pentylone and MDPV. As shown in Figures 2A and 3, the higher concentrations of pentylone and methylone were detected by spectral library match. However, although pyrovalerone was confirmed in Case 16 by LC–MS-MS, it was not detected in the GC–MS basic drug screen in the urine.

Methods

The analytical method was developed by a modification of the Toxicology Section's current sympathomimetic procedure using literature published by Meyer *et al.* and Katagi *et al.* (10–12). Whole blood, plasma, urine, vitreous, cerebrospinal fluid (CSF), bile and tissue homogenates were analyzed in this procedure. MDPV, methylone, methedrone, mephedrone, butylone, pyrovalerone and 3-fluoromethcathinone were extracted into an organic solvent from an alkalinized specimen. The organic solvent was reduced to residue, reconstituted with methanol, and injected onto an Agilent 6410 LC–MS-MS instrument. Detection and quantitation were performed in the multiple reaction monitoring (MRM) mode. Time segments were determined by the specific column and instrument conditions, with a total run time of 12 min. In addition, pentylone and alpha-PVP ions were included in the method to screen all cases for these chemicals. The lab is currently awaiting the availability of a certified standard to quantify pentylone and alpha-PVP. A limited quantity of authentic standard for pentylone and alpha-PVP was obtained from standards purchased by the

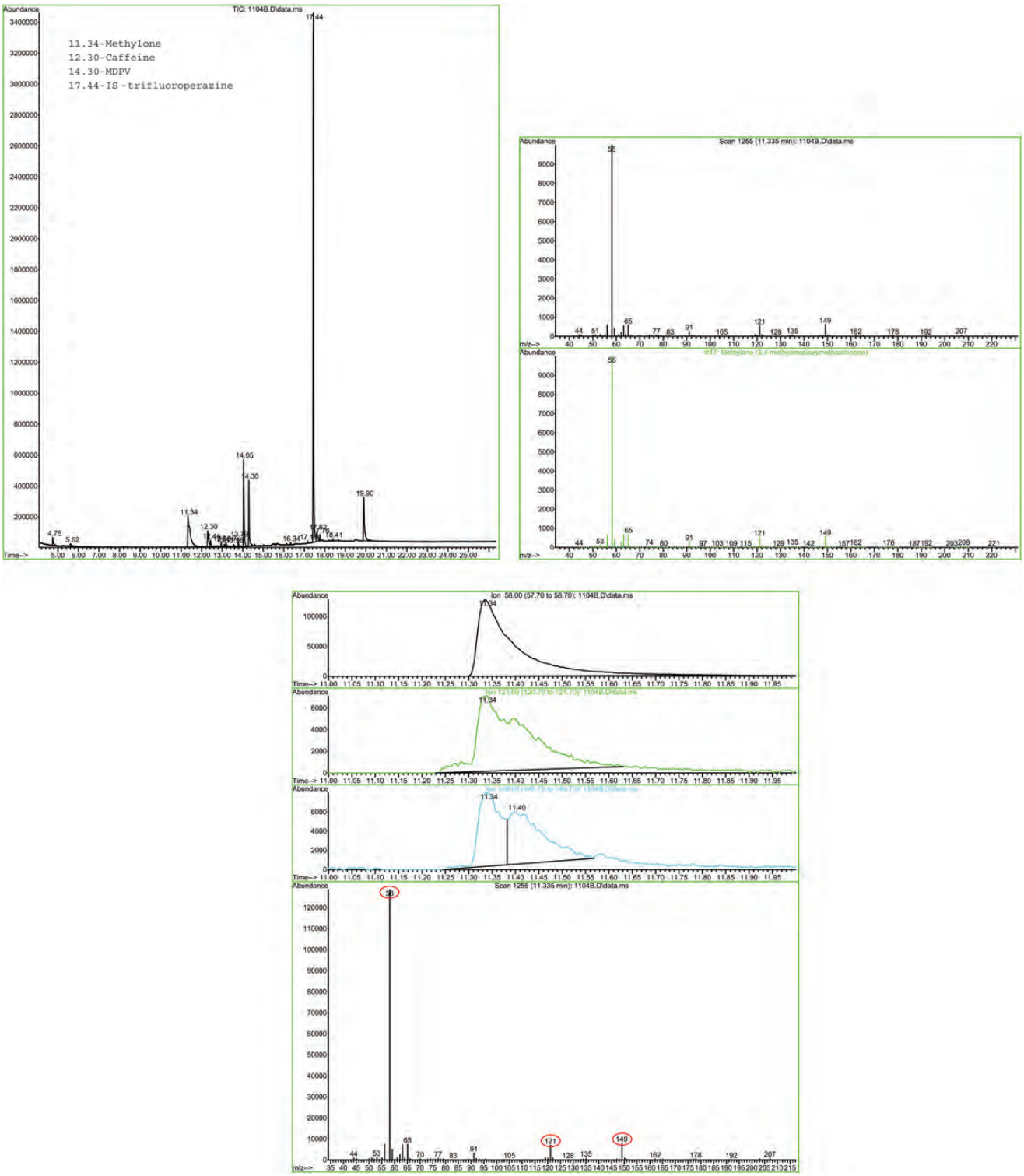

Figure 2. GC–MS data from basic drug screens: 3 mL of blood from PM Case 1 (A); 2 mL of urine from PM Case 1 (B).

MVRCL Chemistry Section. This small amount was enough to identify the ions using the Agilent Optimizer software in combination with the molecular weight; however, not enough was available for quantitation purposes.

High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile and water were manufactured by Burdick & Jackson and purchased through VWR (West Chester, PA). Sodium chloride, sodium sulfate, ammonium

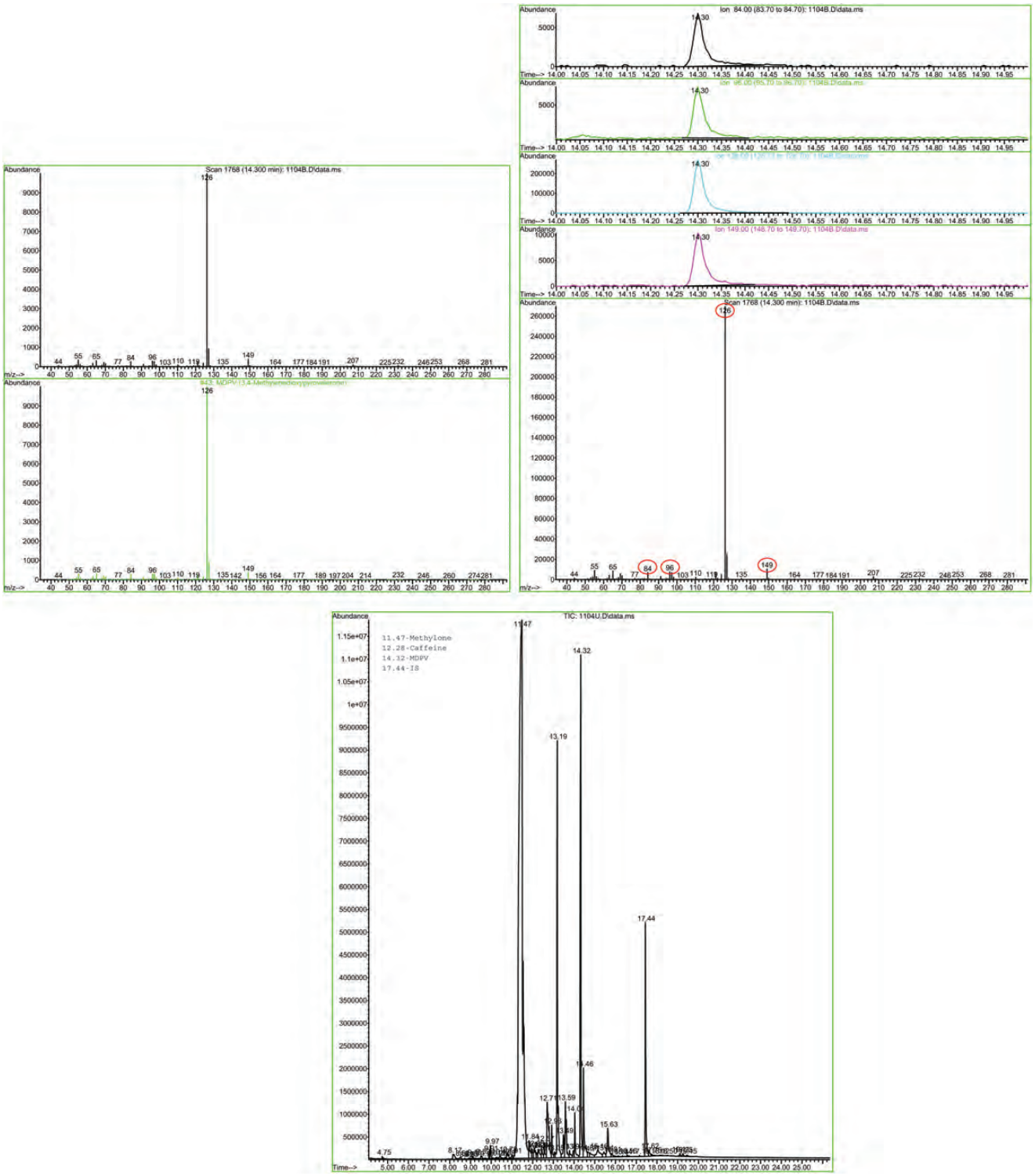

Figure 2. Continued.

hydroxide, ammonium acetate, methylene chloride and *n*-butyl chloride were purchased from Fisher Scientific (Pittsburgh, PA) and were ACS grade. Certified reference standards were purchased from Cerilliant Corporation (Round Rock, TX) and include mephedrone-d_3, methylone-d_3, butylone-d_3, MDPV-d_8, MDPV, methedrone, mephedrone, methylone, butylone, pyrovalerone and 3-fluoromethcathinone. The methylone, mephedrone and MDPV standards that were used for controls were purchased from Lipomed (Cambridge, MA).

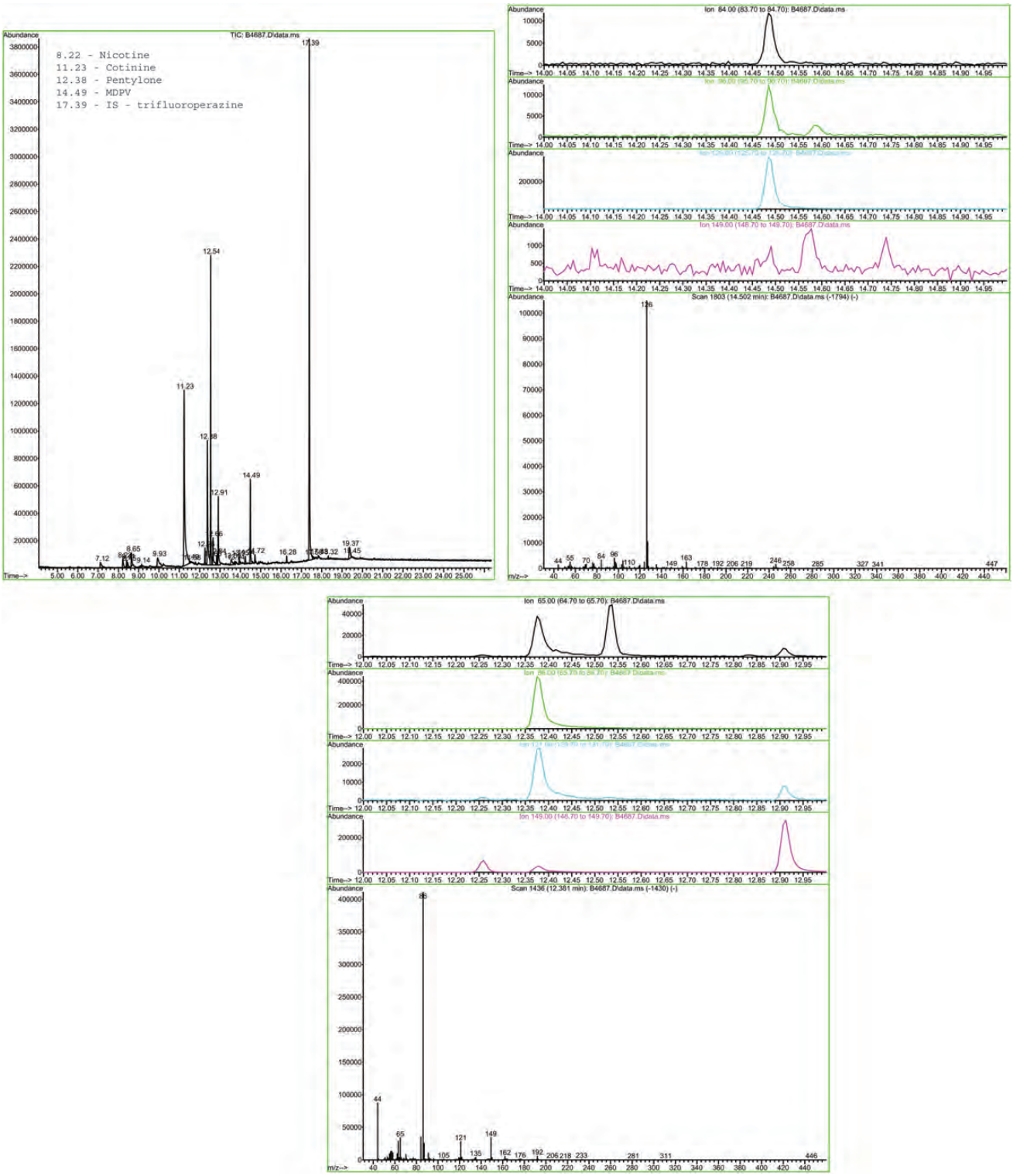

Figure 3. GC–MS data from a basic drug screen of 2 mL of urine from PM Case 16.

A combined working standard was prepared as a 1 µg/mL solution in methanol. The internal standards, combined, were also prepared as a 1 µg/mL solution in methanol.

One milliliter of sample was used. Calibrators were at the following concentrations: 5, 10, 50, 75, 100, 150 and 200 ng/mL. Fifty microliters of internal standard and 50 µL of concentrated

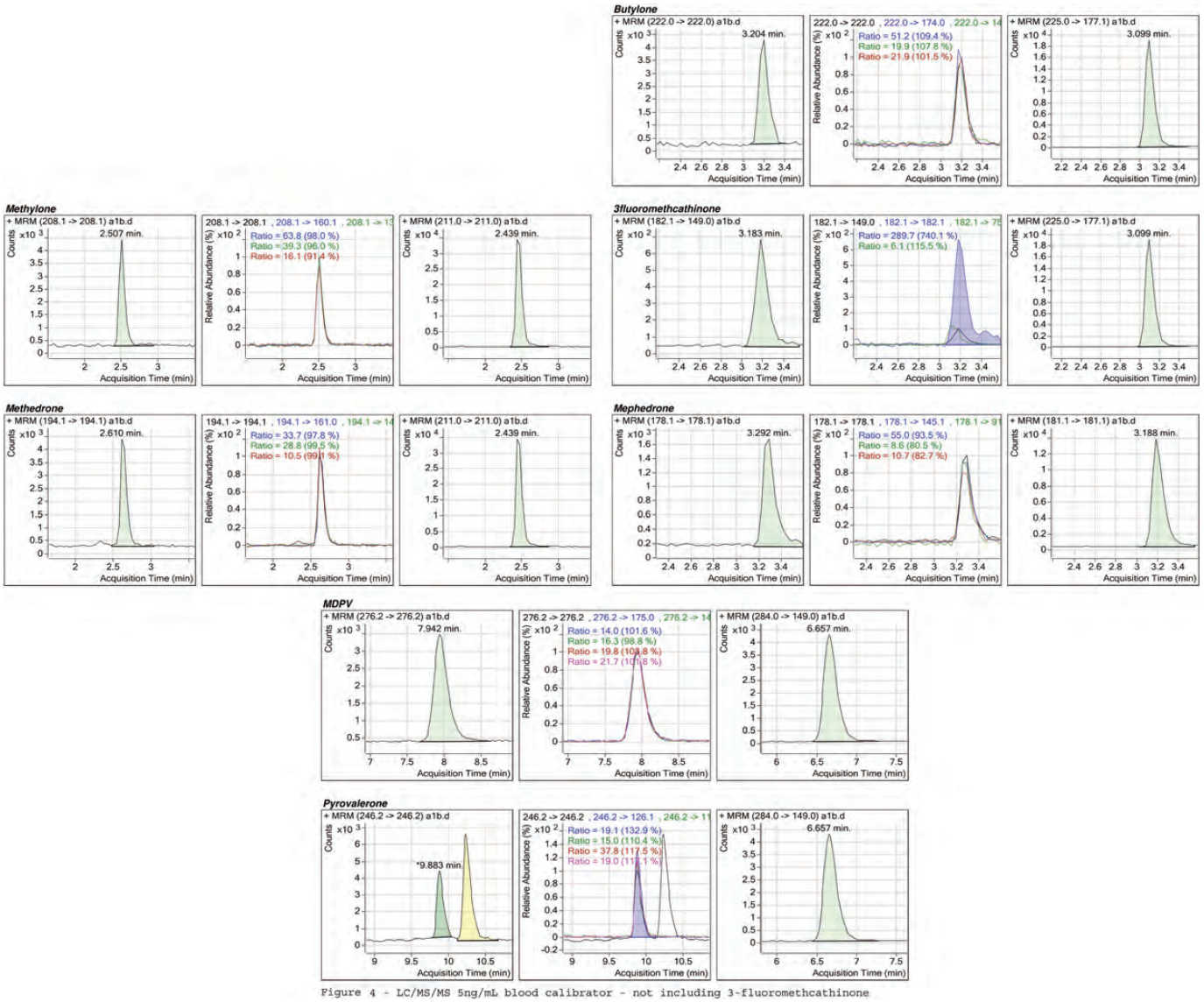

Figure 4. LC–MS-MS spectra from the 5 ng/mL calibrator in 1 mL of blood (3 fluoromethcathinone was not included in this calibrator).

ammonium hydroxide were added to the sample. A few granules of sodium chloride were added to avoid emulsions. After the addition of 3 mL of extraction solvent (methylene chloride–*n*-butyl chloride, 1:4), samples were then rotated and centrifuged. The organic layer was removed, passed through a sodium sulfate filter and then dried at room temperature under nitrogen. The residue was reconstituted with 200 µL of methanol and 10 µL was injected onto the instrument. An Agilent XDB-C18 5 µm, 4.6 × 150 mm column was used for analyte separation. The instrumental conditions were as follows: gas temperature, 350°C; gas flow, 12 L/min, nebulizer pressure, 50 psi; capillary, 3,500 (positive), 4,000 (negative); flow rate, 0.8 mL/min. A mobile phase program was used with Channel A composed of 50/50 methanol–acetonitrile and Channel B composed of 5 mM ammonium acetate buffer. A 50%/50% B mixture was maintained for 6 min; at 6.5 min, the mobile phase was 90% A/10% B and held until 10 min. The mixture then returned to 50% A/50% B at 10.5 min and remained until the end of the 12 min run.

Results

The method was linear throughout the calibration range, with a correlation coefficient for all analytes of 0.990 or greater using a weighted curve of $1/x$. The upper and lower limits of quantitation corresponded to the low and high calibrator concentrations. The selectivity of the method was demonstrated by analyzing three GC–MS drug screen controls containing a variety of commonly encountered acidic, neutral and basic drugs. No interferences were detected with the analytes of interest. Negative matrix-matched controls and the synthetic cathinone high calibrator were also analyzed with no interferences detected, either from the matrix or from elevated concentrations of the synthetic cathinones. The stability was

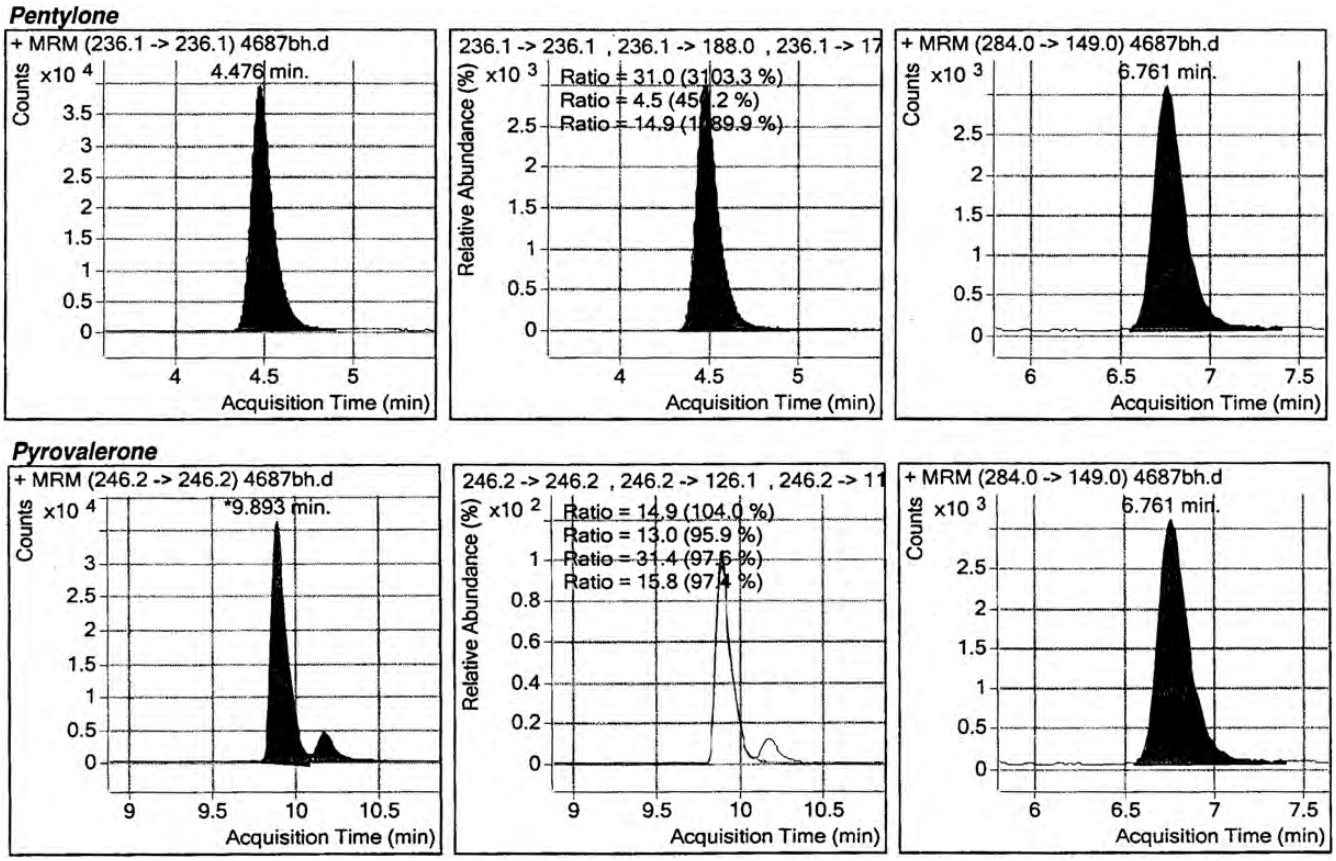

Figure 5. LC–MS-MS spectra from 1 mL of blood from PM Case 16.

evaluated by analyzing positive cases over the course of two months, with consistent results obtained over that time period. The results were also consistent when comparing frozen and refrigerated specimens. Accuracy was established by sending three cases to a reference laboratory for MDPV quantitation. The reference laboratory results agreed with the Toxicology Section's results within ± 20%. LC–MS-MS data from the 5 ng/mL calibrator in blood are shown in Figure 4; LC–MS-MS data from heart blood and liver specimens from Postmortem Case 16 are shown in Figures 5 and 6. The 3-fluoromethcathinone was not calibrated in these runs. Positive and negative controls made in whole blood were analyzed in each analytical run.

Controls were made by using standards purchased from a different manufacturer from those standards used to make the calibrators. Low and high controls in vitreous fluid fortified with MDPV, methedrone and methylone were within 20% of the target value. Low and high controls fortified in liver and brain for MDPV were also within 20% of the target value. However, low controls for methylone and methedrone in brain and liver were not reproducible. High controls for brain were within 20% for methedrone and methylone, but only the liver high control was within 20% for methedrone, not methylone. This inconsistency may be related to the fact that these two chemicals have previously been described by Sorensen (13) as less stable than MDPV in alkaline conditions. Therefore, these data suggest that tissue samples may be subject to matrix effects, depending on both the synthetic cathinone and tissue type, and should not be used to screen for synthetic cathinones. The low and high control target values and the between-run and within-run precision for MDPV, methylone and methedrone in blood are listed in Table I.

Table II contains information from nine human performance cases, including history and corresponding synthetic cathinone concentration in blood. Human performance (HP) Case 9, a urine specimen only, was included to demonstrate the presence of the adulterant benzocaine. Benzocaine was also documented in HP Case 7. HP Case 3 contained lidocaine as an adulterant. The concentration range of MDPV for blood specimens in HP cases was 6 to 368 ng/mL, with an average of 100 ng/mL. One methylone case is included with a concentration of 7 ng/mL (HP Case 4). Apparently, those subjects with higher concentrations of MDPV showed the most marked impairment. However, some of the individuals with low concentrations of synthetic cathinones showed behavior consistent with the crash commonly experienced after a prolonged or intense stimulant high, (HP Cases 4 and 5).

Table III contains data from 23 postmortem (PM) cases. When available, other matrices in addition to blood were analyzed for synthetic cathinones. The concentration range of MDPV for PM blood specimens was 10 to 640 ng/mL, with an average of 109 ng/mL. These data show that the synthetic

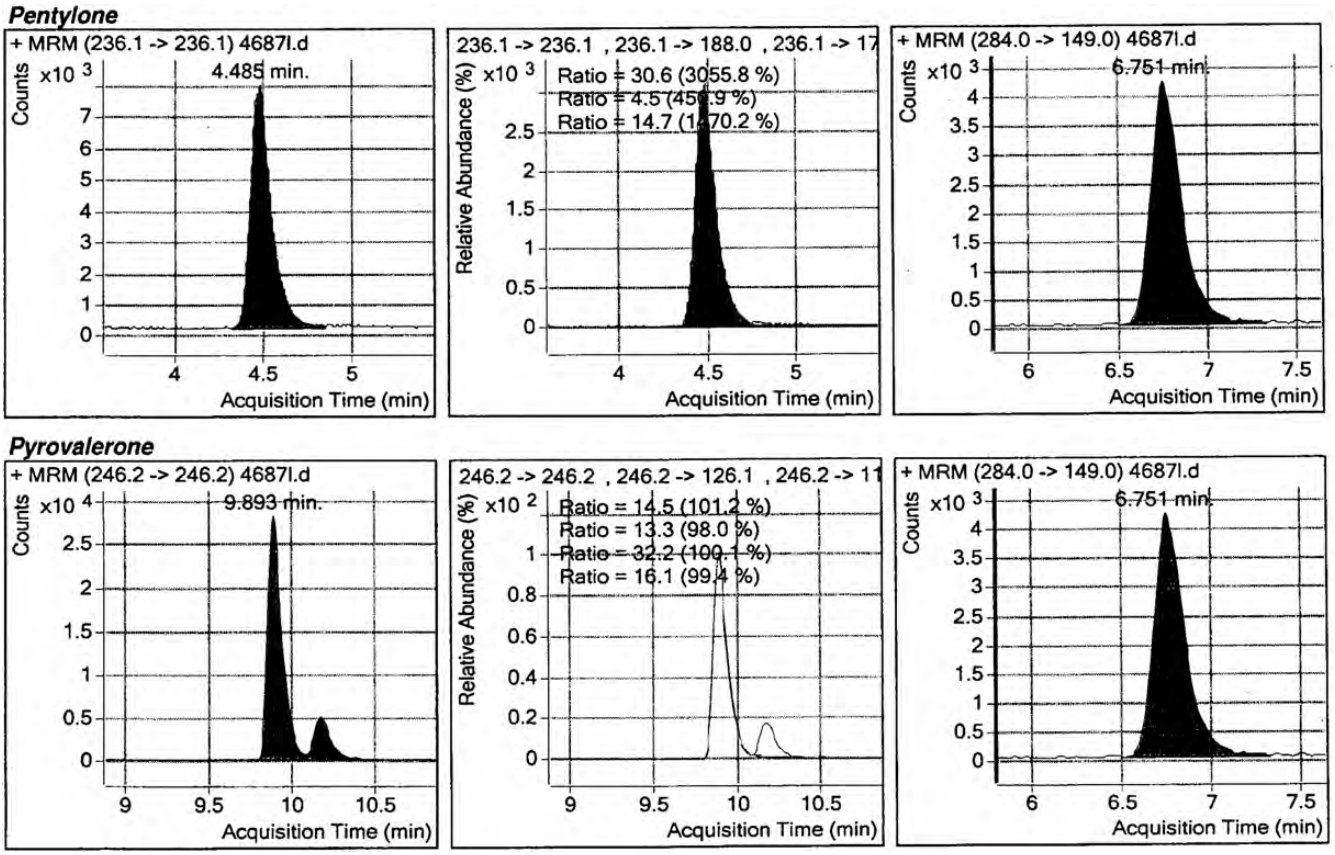

Figure 6. LC–MS-MS spectra from 1 mL of liver homogenate (1:3 dilution) from PM case 16

cathinone concentration alone does not determine the toxicity of the chemical. Underlying disease and age also appear to play roles, similar to other stimulant drugs. The highest blood MDPV concentration, 640 ng/mL, was in a suicide by hanging (PM Case 17). In contrast, a fatality by MDPV intoxication exhibited a blood concentration of only 91 ng/mL (PM Case 3). PM Case 3 also shows the consumption of a bath salt product adulterated with lidocaine. Brandt *et al.* analyzed various bath salt products and found caffeine, benzocaine, lidocaine and procaine in the product, in addition to the various cathinones (17). In PM Case 1, the death was caused by injuries sustained in an auto accident; however, this decedent had a blood methylone concentration of 729 ng/mL. There were four suicide by hanging cases (PM Cases 4, 12, 17 and 22) with synthetic cathinones as the only significant drug findings. A reported side effect of bath salt use has been an increase in suicidal tendencies (2, 3).

In PM Cases 8, 10, 12, 13 and 16, both peripheral and heart blood were available for analysis. The average heart to peripheral blood ratio was 1.48, with a range of 1.3 to 1.7. The distribution data from PM Cases 1, 8, 12, 14, 16 and 17 suggest that bile may be a good specimen to screen for synthetic cathinone use in the absence of urine. Table IV shows the distribution of MDPV and pyrovalerone in other biological matrices. The specimens from PM Case 14 represent data from an embalmed body. The cavity blood/fluid was not suitable for quantitation because values from multiple analyses were inconsistent, but in other specimens, quantitation data were reproducible.

PM Case 16 is the first MCCO/MVRCL case that contained pyrovalerone and pentylone; the urine GC–MS drug screen also showed MDPV. This suggests historical use of different bath salt type products and is consistent with the reported history of chronic bath salt use. The other drugs identified in combination with the synthetic cathinoines were varied. The most common drugs were opiates, analgesics other than opiates, benzodiazepines and antidepressants. There were only two cases with ethanol, and those concentrations were low (PM Cases 9 and 15). PM Cases 18, 19, 20 and 21 are some of the most recent cases and are the first cases containing alpha-PVP. This is the most common synthetic cathinone the Toxicology Section is currently detecting.

Discussion and Conclusion

Both postmortem and human performance forensic toxicology laboratories should be aware of the increased use of synthetic cathinones and their implications in impairment and death cases. The most recent casework consists of alpha-PVP or pentylone and reflects the move from the synthetic cathinones which are controlled, to those that are currently not controlled. Kasick *et al.* (18) followed two patients admitted to a

hospital in central Ohio after the consumption of bath salts. Both their psychological and physiological symptoms are documented and classified as an intense stimulant toxidrome. The findings in these two individuals are consistent with the histories that have been encountered in the casework presented here. The authors assumed that the patients had ingested mephedrone; however, there was no toxicological confirmation that this was the specific synthetic cathinone used. Although mephedrone was screened for in our cases, only one case was positive. The product used by the patients in the Kasick study probably did not contain mephedrone, but more likely one of the other synthetic cathinones that are common around the central part of Ohio. Spiller *et al.* (19) analyzed blood and/or urine from 19 patients; their results were consistent with those reported here, and they did not detect any mephedrone. López-Arnau *et al.* (20) conducted receptor studies with butylone, mephedrone and methylone, which showed that these synthetic cathinones had effects similar to MDMA, with inhibition of both 5-hydroxytryptamine (5-HT) and dopamine uptake. Methylone was found to be the most potent, with effects that persisted after drug withdrawal. In addition, the authors studied the psychomotor effects in rats, which showed hyperlocomotion by the activation of 5-HT (2A) receptors and an increase in extracellular dopamine. Prosser and Nelson report that cardiovascular complications following synthetic cathinone use were very common, and as a group were the most common complications seen in users that presented for medical care (21).

Considering that the major effects of the synthetic cathinones are on the neurological and cardiovascular systems and are serious in nature, a screening protocol should be employed to allow for the detection of these dangerous chemicals. It is a constant challenge for the laboratory to keep up with clandestine manufacturers as they attempt to stay one step ahead of law enforcement by changing the chemicals just enough to elude prosecution.

Table I
Analytical precision results for MDPV, Methedrone and Methylone

Analyte	Within-run			Between-run		
	Number (*n*)	Target (ng/mL)	Within-run (%)	Number (*n*)	Target (ng/mL)	Between-run (%)
MDPV	5	13	7.7	9	13	5.1
MDPV	6	104	1.0	9	104	5.0
Methedrone	5	15	4.0	9	15	5.1
Methedrone	5	130	6.1	9	130	4.0
Methylone	5	13	9.2	9	13	8.5
Methylone	6	120	4.5	9	120	2.5

Table II
Human Performance Cases*

Case	Age/gender/race	Synopsis	Toxicology (ng/mL)
1	32/F/W	This MVA driver hit three parked cars, and did not know where she was or that she hit any cars.	Blood MDPV: 200 Fluoxetine: 180 Norfluoxetine: 290 Historic cocaine use
2	23/M/W	This was an MVA driver at fault. Due to the severity of the intoxication, he required admission to a hospital. There was some delay in specimen collection. He admitted to smoking/inhaling bath salts, cocaine and THC all night.	Blood MDPV < 10 THC: ND THC-COOH: 18 No cocaine or metabolite
3	32/M/W	A DUI driver was found with pills and white powder. The powder contained MDPV and lidocaine.	Blood MDPV: 29 Lidocaine < 50 Oxycodone < 20
4	30/M/W	The subject was passed out in a vehicle with two syringes located near him. The syringes were tested and contained methylphenidate.	Blood MDPV: 24 Methylone: 7 Clonazepam: 11 7-AMC: 14 Alprazolam: 95 Citalopram: 110 Methylphenidate: ND
5	NA/M/W	This was a victim of a robbery facilitated by drugging. Once the victim became unresponsive, the suspects stole the victim's car. Ativan and Zanaflex were suspected by police as the possible drugs used.	Victim's blood MDPV: 52 Lorazepam and temazepam: both less than 10 Zanaflex: ND
6	25/M/W	In this vehicular homicide/assault, the subject is a known bath salt user. The subject stated they would never prove he used bath salts. There was a delay in specimen collection. The specimen was analyzed for bath salts only.	Blood MDPV: 6
7	25/M/W	The subject was fleeing and eluding police. Three needles and one vial of white powder were located. The white powder contained MDPV and benzocaine.	Blood MDPV: 368 Tramadol: 360 Benzocaine: positive
8	44/M/W	The subject was involved in a domestic dispute with his girlfriend, who stabbed him in the stomach with a knife while he was driving. The subject admitted to bath salt use.	Blood MDPV: 21
9	32/F/W	This was a DUI driver with drugs found in the vehicle. The drugs from the vehicle contained benzocaine and MDPV.	Urine MDPV: positive Benzocaine: positive

*Note: white (W), male (M), female (F), motor vehicle accident (MVA), not available (NA), 7-aminoclonazepam (7-AMC), not detected (ND).

Table III
Postmortem Cases*

Case	Age/gender/race/COD	Synopsis	Toxicology (ng/mL)	Distribution (ng/mL or ng/g)
1	37/M/W MVA Accident	The decedent was involved in an MVA as the at-fault driver.	Heart blood MDPV: 56 Methylone: 729 THC: 15 THC-COOH: 118	MDPV Bile: 412 Vitreous: 33 Methylone Bile > 400 Vitreous > 200
2	20/M/W MVA Accident	The decedent was involved in an MVA as the at-fault driver. The vehicle drove off the roadway. A small plastic bottle with white residue was found in his pocket.	Cavity blood MDPV: 31	Urine: positive
3	39/M/W MDPV intoxication Accident Autopsy findings: bitten tongue, cerebral edema, mild cardiomegaly	The decedent was a known user of bath salts. His product of choice was called Posh. He was known to become manic and uncontrollable when he used. He was found dead at home, with overturned furniture and broken lamps.	Femoral blood MDPV: 91 Lidocaine: 100 Lidocaine was also in the Posh product.	Vitreous: 132 Urine > 200
4	29/M/W Hanging Suicide	The decedent had recently reconciled with his wife and discovered she was snorting bath salts. He tried to dissuade her by snorting bath salts himself to show her how she acted while under the influence. He was later found hanging.	Heart blood MDPV: 129 Historic THC use	Vitreous: 20
5	34/M/W Multiple drug intoxication Accident Autopsy findings: cerebral and pulmonary edema and frothy material in the airway	The decedent was a known drug user who was using heroin with a friend. When the friend regained consciousness, he found the decedent unresponsive.	Femoral blood MDPV: 185 Morphine: 24 Fluoxetine: 760 Norfluoxetine: 1,600 Trazodone: 70 Mirtazepine < 50	Vitreous: 195 Urine > 200
6	46/M/W Natural Autopsy findings: Sepsis, acute lobar pneumonia w/chronic intravenous drug abuse, hypertensive, ASCVD	The decedent's mental status became altered at home. While at the hospital he was hypotensive and then coded.	Heart blood MDPV: 10 Hospital blood MDPV < 5 DPH: 230 Alprazolam: 15 Tramadol < 50 Historic heroin use	Brain: 16 Liver: 12 Vitreous: 17
7	33/F/W Multiple drug intoxication Accident	The decedent was found dead in a hotel room with bath salt containers.	Femoral blood MDPV: 46 Morphine: 390 Hydrocodone: 179 Citalopram: 320 Cocaine < 50 BE > 700 Alprazolam: 50 6-MAM was ND in blood, vitreous and urine	Vitreous: 48 Urine > 200
8	47/M/W Multiple drug intoxication Accident Autopsy findings: PE and hypertensive ASCVD	The decedent had a history of illicit and prescription drug abuse, HTN and chronic pain. He was found unresponsive.	Peripheral blood MDPV: 162 Oxymorphone: 43 Diazepam: 313 Nordiazepam: 494 Temazepam: 33 DPH: 80	Heart blood: 280 Liver: 3,720 Bile > 750 Urine: 13,900 CSF: 105 Brain: 168 Vitreous: 159
9	33/M/W MDPV intoxication Accident Autopsy findings: prominent decomposition and no injuries	The decedent was a drug abuser, found dead after 2 days with straws and White Horse brand bath salts. He was likely dead 2 days in a hot apartment, then refrigerated for 3 days before autopsy.	Liver (only specimen submitted) MDPV > 4,800 ng/g Ethanol: 0.044 g/100 mL Trazodone: presumptive ID beta-phenethylamine: positive	NA
10	43/F/W Multiple drug intoxication Accident Autopsy findings: PE and cardiomegaly	The decedent had a history of asthma, seizures and depression. She had a previous suicide attempt by setting herself on fire. She was on numerous medications and was found unresponsive in bed.	Peripheral blood MDPV: 18 Fentanyl: 8 Norfentanyl < 1 Trazodone: 540 Gabapentin: 6,800 Norvenlafaxine: 220 Tramadol < 50 Diazepam: 301 Nordiazepam: 281	Heart blood: 28 Brain < 20 CSF: 14 Liver: 52 Vitreous: 14
11	51/M/W Multiple drug intoxication Accident Autopsy findings: emphysema and one coronary severely obstructed	The decedent suffered severe depression since his wife's death 6 months ago. He was found inside a motorcycle trailer with a mason jar having minimal red liquid residue in it. All of his medication bottles were inside the house. Meds: valproic acid, buproprion, Pristiq, diazepam, glimepiride, hydrocodone, Nuvigil (r-modafinil).	Femoral blood MDPV: 129 Bupropion/Metab: 24/216 Morphine: 40 Oxycodone < 20 Diazepam: 303 Nordiazepam: 229 Ethylene glycol: ND Modafinil/valproic Acid: not tested	Liver: 388 Vitreous: 191

(*continued*)

Table III Continued

Case	Age/gender/race/COD	Synopsis	Toxicology (ng/mL)	Distribution (ng/mL or ng/g)
12	32/M/W Hanging Suicide Autopsy findings: cervical pressure furrow and pulmonary congestion	The decedent was found dead, hanging from an electrical cord on a fence in a field. He had a recent falling out with his girlfriend and his brother. He was a heavy bath salt user and has had multiple interactions with local police.	Peripheral blood MDPV: 102 Chlorpheniramine < 50 Dextromethorphan: 60	Heart blood: 133 Brain: 136 Liver: 256 CSF > 200 Vitreous: 205 Bile > 200 Urine: 6100
13	32/M/W Natural Autopsy findings: Perforated esophageal ulcer	The decedent was found lying unresponsive and nude in an empty bathtub. There was no sign of trauma. A jar of K-2 spice herbal incense was present. He had overdosed on K-2 three weeks ago. He had a history of drug abuse and had recently been released from a drug rehabilitation center.	Peripheral blood MDPV: 36 Citalopram: 200 JWH-122: positive JWH-210: positive Trazodone: 50	Heart blood: 56 Brain: 148 Liver: 668 Bile: UNS Vitreous: 130 CSF: 52
14	53/F/W Multiple drug intoxication Accident	The decedent had a history of CHF, COPD, diabetes, hypertension, was oxygen dependent, and had bipolar disorder. She was found unresponsive. She was initially released as a natural death until adult children and a sister alleged the decedent's husband had caused her death by choking her or by an overdose of medication. The body was embalmed.	Cavity blood MDPV: positive BE > 1,000 Morphine: 212 Alprazolam: 15 Promethazine: 200 Citalopram: 1,100 DPH: 330 Dextromethorphan: 140 Trazodone: 140 Cyclobenzaprine: 380	Brain: 20 Liver: 432 Bile: 140 Vitreous: 48 Spleen: 80 CSF: 33
15	19/M/W Natural Autopsy findings: several large B/L PEs and chest wall contusions	The decedent was involved in a minor car crash. He did not seek medical help for 2 days. He then went to ER for headache and chest pain. He was given Vicodin and sent home. Five days later he has increasing SOB, went to the ER and died after intubation in the ER. He was known to use marijuana and prescription medications recreationally.	Femoral blood MDPV: 63 Cyclobenzaprine: 60 Dextromethorphan < 50 Midazolam < 10 Hydromorphone < 5 Ethanol: 0.012 g/100 mL	Urine > 200
16	33/M/W Autoerotic asphyxia Accident	The decedent was last known alive when he went upstairs to his room. The next morning he was found suspended from a pole attached to his bed by a rope. It was reported by his brother that he had been using bath salts and there was evidence of cross-dressing.	Femoral blood Pyrovalerone: 42 Pentylone: positive Amphetamine < 50 Urine also contained MDPV	Pyrovalerone Heart blood: 59 Brain: 48 Liver: 124 Bile: 70 Vitreous: 24 All positive for pentylone: waiting for standard to be able to quantify
17	24/M/W Hanging Suicide	The decedent was discovered suspended by a braided rope from a tree. He reportedly abused Vicodin, Percocet, cocaine and bath salts. Several suicide notes were discovered in his bedroom.	Femoral blood MDPV: 640 MDPV was the only drug detected	Brain: 896 Liver: 6,080 Bile: 1,880 Vitreous: 940
18	44/F/W Multiple drug intoxication Accident	The decedent had a history of bipolar disorder, previous attempted suicide, abuse of Oxycontin and was on numerous medications. There was also a past history of domestic violence. Her boyfriend admitted that he had been abusing bath salts.	Femoral blood Pentylone: positive alpha-PVP: positive Hydrocodone : 29 Oxycodone: ND DPH: 50 Promethazine: 90 Cyclobenzaprine: 64 Amitriptyline: 1,530 Nortriptyline: 1,060 Quetiapine: 438 Gabapentin: positive Clonazepam < 10	Urine Pentylone: positive alpha-PVP: positive
19	35/F/W Multiple drug intoxication Accident	The decedent was a known abuser of multiple drugs including intravenous drug abuse. She had earlier stated that she wanted to "party." She was found unresponsive in bed. A spoon with residue and a syringe with what appeared to be blood were found in her vehicle at the scene.	Heart blood Morphine: 309 Codeine: 27 Citalopram: 120 alpha-PVP: positive Vitreous 6-MAM: 57 Morphine: 163 Codeine: 35	Urine alpha-PVP: positive
20	50/F/W Multiple drug intoxication Accident	The decedent was found unresponsive on a sofa in her living room. She had a history of heroin abuse.	Heart blood alpha-PVP: positive Morphine: 284 Codeine < 20 Vitreous Morphine: 171 Codeine: 30 6-MAM: 28	Urine alpha-PVP Morphine Codeine 6-MAM DPH Venlafaxine Norvenlafaxine Lidocaine All were positive

(*continued*)

Table III Continued

Case	Age/gender/race/COD	Synopsis	Toxicology (ng/mL)	Distribution (ng/mL or ng/g)
21	51/M/W Multiple GSWs Homicide Autopsy findings: 3 GSWs to the torso and left hand	The decedent had a past prison history. He stole a police car, was chased, escaped and when found was shot by officers.	Cavity blood alpha-PVP: positive Cocaine: ND BE < 50	Urine alpha-PVP: positive BE: positive
22	34/M/H Hanging Suicide	The decedent resided with his girlfriend and was last known to be alive when they argued. He was found in the basement hanging from his neck by a rope through the rafters.	Femoral blood alpha-PVP: positive Pentylone: positive	Bile alpha-PVP: positive Pentylone: positive
23	32/F/W Multiple drug intoxication Accident Autopsy findings: cardiomegaly and findings consistent with an overdose	The decedent was a morbidly obese, known drug user who was found unresponsive on the floor at home. She was taken to the ER and pronounced. Drugs and paraphernalia were found in her pockets to include heroin and bath salts.	Femoral blood MDPV: 47 Nordiazepam: 12 ng/mL Free morphine: 37 ng/mL Dextromethorphan not detected 6-MAM: 7 ng/mL in vitreous	Urine MDPV: positive alpha-PVP: positive methedrone: positive

*Note: white (W), Hispanic (H), male (M), female (F), atherosclerotic cardiovascular disease (ASCVD), chronic obstructive pulmonary disease (COPD), chronic heart failure (CHF), bilateral (B/L), not available (NA), pulmonary emboli (PE), diphenhydramine (DPH), benzoylecgonine (BE), not detected (ND), 6 monoacetyl morphine (6-MAM), gunshot wounds (GSWs), unsuitable specimen for analysis (UNS), shortness of breath (SOB), Hypertension (HTN).

Table IV
Synthetic Cathinone Distribution (ng/mL or ng/g)

Synthetic cathinone	Vitreous	Liver	Brain	Bile	CSF	Spleen
MDPV	$n = 13$	$n = 8$	$n = 6$	$n = 3$	$n = 4$	$n = 1$
Range	14 to 940	12 to 6,080	16 to 896	140 to 1,880	14 to 105	80
Average	164	1,451	230	810	51	
Pyrovalerone	$n = 1$	$n = 1$	$n = 1$	$n = 1$	$n = 0$	$n = 0$
Range	24	124	48	70		

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References

1. Microgram Bulletin. (2011); **44**(9).
2. Meetings of: The Designer Drugs Task Force, Greater Dayton Area Hospital Association, Fall 2011 and Jan 2012.
3. Decker, T. (2011) Docs glad "bath salts" are banned. *Columbus Dispatch*, Oct 18.
4. Caudill, M. (2011) Police deal with new bath salt drug threat. *News Journal*, Jan 30.
5. Caulfield, P. (2011) Burglar high on bath salts breaks into home, puts up Christmas decorations. *New York Daily News*, Nov 14.
6. Smith-Hebson, B., Frolik, C., Larsen, D. (2011) Bath salt, spice injuries steady as Ohio ban nears. *Springfield News-Sun*, Sept 8.
7. Marinetti, L., Antonides, H., Watson, J. (2011) More on bath salts. *Tox Talk*, **36**, 11–13.
8. Maurer, H.H., Kraemer, T., Springer, D., Staack, R.F. (2004) Chemistry, pharmacology, toxicology, and hepatic metabolism of designer drugs of the amphetamine (ecstasy), piperazine, and pyrrolidinophenone types—A synopsis. *Therapeutic Drug Monitoring*, **26**, 127–131.
9. Meyer, M.R., Peng, D., Schuster, F., Maurer, H.H. (2010) Studies on the metabolism of the alpha-pyrrolidinophenone designer drug methylenedioxy-pyrovalerone (MDPV) in rat and human urine and human liver microsomes using GC-MS and LC-high-resolution MS and its detectability in urine by GC-MS. *Journal of Mass Spectrometry*, **45**, 1426–1442.
10. Kiely, E., Lee, C.J., Marinetti, L. (2009) A fatality from an oral ingestion of methamphetamine. *Journal of Analytical Toxicology*, **33**, 557–560.
11. Katagi, M., Zaitsu, K., Shima, H., Kamata, T., Kamata, K., Nakanishi, H. *et al.* (2010) Metabolism and forensic toxicological analyses of the extensively abused designer drug methylone. *TIAFT Bulletin*, **40**, 30–35.
12. Meyer, M., Peters, F., Maurer, H. (2010) Detection of beta-keto amphetamines in urine within the systematic toxicological analysis (STA) using GC-MS. *TIAFT Bulletin*, **40**, 36–38.
13. Sørensen, L.K. (2011) Determination of cathinones and related ephedrines in forensic whole-blood samples by liquid-chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography B*, **879**, 27–36.
14. Logan, B.K., Couper, F.J. (2003) 3,4-Methylenedioxymethamphetamine—Effects on human performance and behavior. *Forensic Science Review*, **15**, 18–19.
15. Isenschmid, D.S. (2002) Cocaine—Effects on human performance and behavior. *Forensic Science Review*, **14**, 91–92.
16. Logan, B.K. (2002) Methamphetamine—Effects on human performance and behavior. *Forensic Science Review*, **14**, 141–143.
17. Brandt, S.D., Sumnall, H.R., Measham, F., Cole, J. (2010) Analyses of second-generation "legal highs" in the UK: Initial findings. *Drug Testing and Analysis*, **2**, 377–382.
18. Kasick, D.P., McKnight, C.A., Klisovic, E. (2012) "Bath salt" ingestion leading to severe intoxication delirium: Two cases and a brief review of the emergence of mephedrone use. *American Journal of Drug and Alcohol Abuse*, **38**, 176–180.
19. Spiller, H.A., Ryan, M.L., Weston, R.G., Jansen, J. (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. *Clinical Toxicology (Philadelphia)*, **49**, 499–505.
20. López-Arnau, R., Martínez-Clemente, J., Pubill, D., Escubedo, E., Camarasa, J. Comparative neuropharmacology of three psychostimulant cathinone derivatives: Butylone, mephedrone and methylone. *British Journal of Pharmacology*, April 18, 2012.
21. Prosser, J.M., Nelson, L.S. (2012) The toxicology of bath salts: A review of synthetic cathinones. *Journal of Medical Toxicology*, **8**, 33–42.