IDENTIFICATION AND STABILITY OF SYNTHETIC CATHINONES IN BIOLOGICAL SAMPLES

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by

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DEDICATION

"It may be hard, it may take time, but stick with it and you'll be fine"

To my advisor, Dr. Sarah Kerrigan, through all the tears and uncertainty, for her unwavering confidence and faith in me, and for reminding me that in ten years from now that it will not matter that figures and analysis was not perfect the first time around.

To my PhD cohort, for meaningful, reassuring conversations and laughter during stressful times.

To my parents, for their continued support during these last few years of schooling.

ABSTRACT

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Synthetic cathinones ("bath salts") are a class of novel psychoactive substances abused for their psychostimulant and euphoric effects. However, these drugs have received international and national attention due to severe and life threatening adverse effects. In order to properly associate pharmacological, impairing, or toxic effects with synthetic cathinone use, toxicologists must be able to detect and reliably interpret results. The detection of these synthetic phenethylamines relies on validated analytical techniques. Quantitative assays determine the concentration of drug present in biological samples at the time of analysis, which may be significantly different from the concentration at the time of collection or time of death. Drug stability must be understood in order to determine the extent to which these changes influence analytical results. This research provides the forensic toxicology community with a comprehensive understanding of the stability of these compounds in biological matrices.

A method for the detection of twenty-two synthetic cathinones, isolated from blood and urine using liquid chromatography quadrupole/time of flight mass spectrometry (LC-Q/TOF MS) was developed and validated. This method was used to assess synthetic cathinone stability in blood (pH 7) and urine (pH 4 and 8) stored at 32°C, 20°C, 4°C, and -20°C for six months. The selected synthetic cathinones were representative of the various structural analogs, including unsubstituted secondary amines (methcathinone, ethcathinone, buphedrone, and pentedrone); ring substituted secondary amines (3-FMC, 4-FMC, 4-MEC, 4-EMC, 3,4-DMMC, mephedrone, and methedrone); methylenedioxy-

substituted secondary amines (methylone, ethylone, butylone, pentylone, eutylone); and tertiary amines (α-PVP, naphyrone, pyrovalerone, MPBP, MDPBP, and MDPV). The significance of analyte, storage temperature, storage time, concentration, and matrix pH were systematically assessed.

Stability was influenced by structure, matrix pH, and storage temperature. Halogenated cathinones (3-FMC, 4-FMC) were the least stable and the tertiary cathinones bearing the methylenedioxy group (MDPBP, MDPV) were the most stable. The analysis of authentic urine samples from cathinone users supported these experimental findings. Matrix pH and cathinone structure had a more profound influence than prolonged storage.

In addition to detecting synthetic cathinones from antemortem specimens to support experimental stability findings, synthetic cathinones were also identified in a series of fifty fatalities to determine postmortem distribution and redistribution. Drugs were identified in central and peripheral blood, urine, liver, vitreous humor, and stomach contents. Central to peripheral blood (C/P) and liver to peripheral blood (L/P) ratios were determined for seven synthetic cathinones to assess postmortem redistribution (PMR). While synthetic cathinones appear to exhibit low to moderate PMR, the highest C/P ratios were observed for cathinones bearing a secondary amine and a methylenedioxy group.

KEY WORDS: Synthetic cathinones, High resolution mass spectrometry, LC-Q/TOF, Stability, Urine, Blood

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ABBREVIATIONS

2C-B	4-bromo-2,5-dimethoxyphenethylamine
2C-C	2,5-dimethoxy-4-chlorophenethylamine
2C-D	2,5-dimethoxy-4methylphenethylamine
2C-E	2,5-dimethoxy-4-ethylphenethylamine
2C-H	2,5-dimethoxyphenethylamine
2C-I	2,5-dimethoxy-4-iodophenethylamine
2C-T-2	2,5-dimethoxy-4-ethylthiophenethylamine
2C-T-4	2,5-dimethoxy-4-isopropylthiophenethylamine
2C-T-7	2,5-dimethoxy-4-propylthiophenethylamine
2-FMC	2-fluoromethcathinone
3,4-DMMC	3,4-dimethylmethcathinone
3-FMC	3-fluoromethcathinone
3-MMC	3-methylmethcathinone
4-EMC	4-ethylmethcathinone
4-FMC	4-fluoromethcathinone
4-F-PVP	4-fluoro-α-pyrrolidinovalerophenone
4-MEC	4-methylethcathinone
4-MeOMC	4-methyoxymethcathinone
4-MeO-PVP	4-methyoxy- α-pyrrolidinopropiophenone
4-MMC	4-methylmethcathinone
4-MTA	4-methylthioamphetamine
4-OH-MET	4-hydroxy-*N*-methyl-*N*-ethyltrptamine
AM	antemortem
BBB	blood brain barrier
C/P	cardiac blood to peripheral blood ratio
CI	chemical ionization
CID	collision-induced dissociation
DAD	diode array detector
DEA	Drug Enforcement Administration
Df	Degrees of freedom
DFSA	drug facilitated sexual assault
DMC	dimethylcathinone
DOB	2,5-dimethoxy-4-bromoamphetamine
DOC	2,5-dimethoxy-4-chloroamphetamine
DOET	2,5-dimethoxy-4-ethylamphetamine
DOI	2,5-dimethoxy-4-iodoamphetamine

DOM	2,5-dimethoxy-4-methylamphetamine
DUID	driving under the influence of drugs
EDTA	ethylenediaminetetraacetic acid
EI	electron impact
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EMIT	enzyme multiplied immunoassay technique
ESI	electrospray ionization
GC	gas chromatography
HLMs	human liver microsomes
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrometry
IA	immunoassay
IV	intravenously
L/P	liver to peripheral blood ratio
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantitation
m/z	mass to charge ratio
MBDB	1,3-benzodixolyl-*N*-methylbutanamine
MD	methylenedioxy
MDA	3,4-methylenedioxyamphetamine
MDEA	*N*-methyl-diethanolamine
MDMA	3,4-methylenedioxymethamphetamine
MDPBP	3,4-methylenedioxy-α-pyrrolidinobutyrophenone
MDPV	3,4-methylenedioxypyrovalerone
MeO	methoxy
mg	milligrams
min	minutes
mL	milliliters
MS/MS	tandem mass spectrometry
MS	mass spectrometer or spectrometry
NaF	sodium fluoride
NFLIS	National Forensic Laboratory Information System
ng	nanograms
OF	oral fluid
PM	postmortem
PMR	postmortem redistribution
Q/TOF	quadrupole/time of flight

S/N	Signal to noise ratio
UHPLC	ultra-high pressure liquid chromatography
Vd	volume of distribution
α-PBP	α-pyrrolidinobutiophenone
α-PHPP	α-pyrrolidinoheptanophenone
α-PPP	α-pyrrolidinopropiophenone
α-PVP	α-pyrrolidinovalerophenone

CHAPTER I

INTRODUCTION

Abuse of novel psychoactive substances (NPS) has escalated worldwide (1-3). These drugs provide alternatives to many illicit substances, including stimulants, opioids, and hallucinogens. Their appeal can be attributed to their ease of purchase, wide availability, low cost, and in some cases, lack of regulatory control (3-7). The research presented here pertains to synthetic cathinones, a class of NPS commonly referred to as "bath salts".

History

Synthetic cathinones are derived from the natural occurring alkaloid cathinone, found in *Catha edulils* (*khat*), a shrub originally cultivated on the Horn of Africa and Arabian Peninsula (8, 9). Historically, the leaves of the *khat* plant were chewed for their stimulant and euphoric effects. More recently however, powerful synthetic analogs of cathinone have become appealing alternatives to methamphetamine and cocaine.

The first synthetic cathinones, methcathinone and mephedrone, were synthesized in the late 1920s to treat depression and serve as appetite suppressants (7, 8). However, due to notable abuse and dependence, these drugs were quickly withdrawn from use. Only two synthetic cathinones are currently approved for therapeutic use in the United States. These include the antidepressant bupropion (Zyban®, Wellbutrin®) and the appetite suppressant diethylpropion (Tenuate®). More recently, cathinone derivatives have re-emerged as powerful 21st century psychostimulants.

When they first resurfaced, synthetic cathinones were thought to be a "legal" alternative to controlled substances. They could be purchased both in "head shops" and

online, labelled with intentionally misleading information such as “not for human consumption,” “bath salts”, and “plant food” to circumvent existing legislation (3, 6, 8). Typically purchased in pill or powder form, their street names include Atomic, Blaze, Cloud 9, Ivory Wave, White Lightening, and Rave (5, 6). Due to a rise in severe adverse effects and documented fatalities, many countries, including the United States, enacted legislation to deter their use.

Scheduling

In the US, drugs are scheduled under the Federal Controlled Substances Act. Among the five scheduled categories, a Schedule I drug is a substance with no medical use and a high potential for abuse and dependence. Under the Controlled Substances Analogue Enforcement Act of 1986, any substance that is structurally similar to a previously controlled drug can be classified as such, given that it is sold with the intent for human consumption. As a result, many novel psychoactive substances are sold with the aforementioned labels. Under the Analogue Act, select synthetic cathinones are already scheduled. One of the early synthetic cathinones, methcathinone, was listed as a Schedule I drug in 1993. Almost two decades later, methylone, mephedrone, and MDPV were added (10). In additional to Federal regulation, thirty states introduced legislation in 2011 to prohibit the use and distribution of designer drugs and novel psychoactive substances (11). At the state level, “general class bans” have been widely used, because this approach prohibits the use of chemically or pharmacologically similar compounds. By 2014, ten additional synthetic cathinones, including 4-MEC, α-PVP, butylone, pentedrone, pentylone, 4-FMC, 3-FMC, and naphyrone, were Federally controlled and forty-eight states had enacted legislation to ban illicit cathinones (12). The Federal government’s

temporary scheduling was extended in 2016, and as of March 2017 all were permanently scheduled. **Table 1.1** lists the common names, IUPAC names, and current scheduling status of the twenty-two cathinones included in this study. At the time of this report, the only cathinones included in this study that are not Federally scheduled are eutylone, MDPBP, methedrone, and MPBP. However eutylone and MPBP are positional isomers of pentylone and α-PVP, respectively, and could be regulated based on this relationship.

Table 1.1. Common name, IUPAC name, and Federal scheduling status for the twenty-two synthetic cathinones included in this research.

Common Name	IUPAC Name	Other Names	Federal Scheduling Status
3,4-DMMC	1-(3,4-dimethylphyenyl)-2-(methylamino)-propan-1-one	3,4-dimethylmethcathinone	Schedule I (2017)*
3-FMC	1-(3-fluorophenyl)-2-(methylamino)-propan-1-one	3-fluoromethcathinone	Schedule I (2017)
4-FMC	1-(4-fluorophenyl)-2-(methylamino)-propan-1-one	4-fluoromethcathinone, flephedrone	Schedule I (2017)
4-EMC	1-(4-ethylphenyl)-2-methylaminopropan-1-one	4-ethylmethcathinone	Schedule I (2017)*
4-MEC	1-(4-methylphenyl)-2-ethylaminopropan-1-one	4-methylethcathinone	Schedule I (2017)
Buphedrone	2-methylamino-1-phenyl-butan-1-one	α-methylamino-butyrophenone	Schedule I (2012)*
Butylone, bk-MBDB	2-methylamino-1-(3,4-methylenedioxy-phenyl)-butan-1-one	β-keto-*N*-methylbenzodioxoyl-butanamine	Schedule I (2017)
Ethcathinone	2-ethylamino-1-phenyl-propan-1-one	*N*-ethylcathinone	Schedule I (2012)*
Ethylone, bk-MDEA	2-ethylamino-1-(3,4-methylenedioxy-phenyl)-propan-1-one	3,4-methylenedioxy-*N*-ethylcathinone	Schedule I (2017)*
Eutylone, bk-EBDB	1-(1,3-benzodioxol-5-yl)-2-(ethylamino)-butan-1-one	β-keto-ethylbenzodioxolylbutanamine	
MDPBP	1-(1,3-benzodioxol-5-yl)-2-(1-pyrrolidinyl)-1-butan-1-one	3,4-methylenedioxy-α-pyrrolidinobutyrophenone	
MDPV	1-(1,3-benzodioxol-5-yl)-2-(pyrrolidinyl)-pentan-1-one	3,4-methylenedioxypyrovalerone	Schedule I (2012)
Mephedrone, 4-MMC	2-methylamino-1-(4-methylphenyl)-propan-1-one	4-methylmethcathinone	Schedule I (2012)

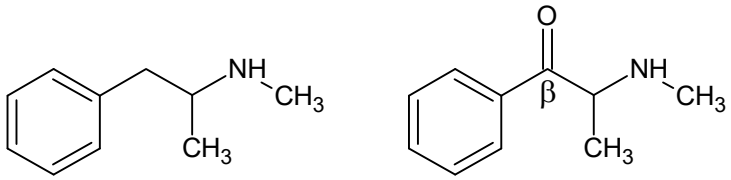
(continued)

Common Name	IUPAC Name	Other Names	Federal Scheduling Status
Methcathinone	2-methylamino-1-phenyl-propan-1-one	ephedrone	Schedule I (1993)
Methedrone, bk-PMMA	1-(4-methoxyphenyl)-2-(methylamino)-propan-1-one	4-methyoxy-methcathinone	
Methylone, bk-MDMA	2-methylamino-1-(3,4-methylenedioxyphenyl)-propan-1-one	3,4-methylenedioxy-*N*-methylcathinone	Schedule I (2012)
MPBP	1-(4-methylphenyl)-2-(1-pyrrolidinyl)-butan-1-one	4-methyl-α-pyrrolidinobutiophenone	
Naphyrone	1-(2-naphthyl)-2-(1-pyrrolidinyl)-pentan-1-one	naphthylpyrovalerone	Schedule I (2017)
Pentedrone	2-methylamino-1-phenyl-pentan-1-one	α-methyalmino-valerophenone	Schedule I (2017)
Pentylone	1-(1,3-benzodioxol-5-yl)-2-(methylamino)-pentan-1-one	β-keto-methylbenzodioxolylpentanamine	Schedule I (2017)
Pyrovalerone	1-(4-methylphenyl)-2-(1-pyrrolidinyl)-pentan-1-one	-	Schedule V (1988)
α-PVP	1-phenyl-2-(1-pyrrolidinyl)-pentan-1-one	α-pyrrolidinovalerophenone	Schedule I (2017)

*Not officially scheduled, but are isomers of Schedule I cathinones.

Chemistry

Synthetic cathinones can be classified as phenethylamines, similar to amphetamine, methamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). The novel psychoactive substances differ from these well-known substances by the addition of a ketone group to the β-carbon **(Figure 1.1)**.



Methamphetamine Methcathinone

Figure 1.1. Chemical structures of methamphetamine and methcathinone which differ only by the addition of the β-ketone.

More than 40 different synthetic cathinones have been reported to the National Forensic Laboratory Information System (NFLIS) over the past few years, and they can be differentiated by substituents to either the α-carbon, the aromatic ring (most often at position 3 or 4), or to the nitrogen terminus (8, 13, 14) **(Figure 1.2)**. Substituents on the cathinone framework commonly include halogens (R_1 and R_2), alkyl (R_1-R_5), methyoxy (R_2) and methylenedioxy groups (R_1 and R_2). The addition of a pyrrolidine group on the nitrogen (R_4 and R_5) creates a sub-population of tertiary amines with distinct chemical and pharmacological properties.

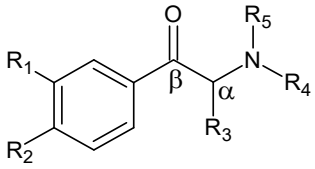


Figure 1.2. General structure of synthetic cathinones indicating locations of substituents (R_1-R_5).

Twenty-two synthetic cathinones were studied in the research presented here. The synthetic cathinones chosen are representative of secondary and tertiary amine cathinones with and without ring substituents, including halogens, alkyl groups, methyoxy groups, and methylenedioxy groups **(Figure 1.3, Table 1.2). Figure 1.3** displays synthetic cathinones (color-coded) by sub-population**.**

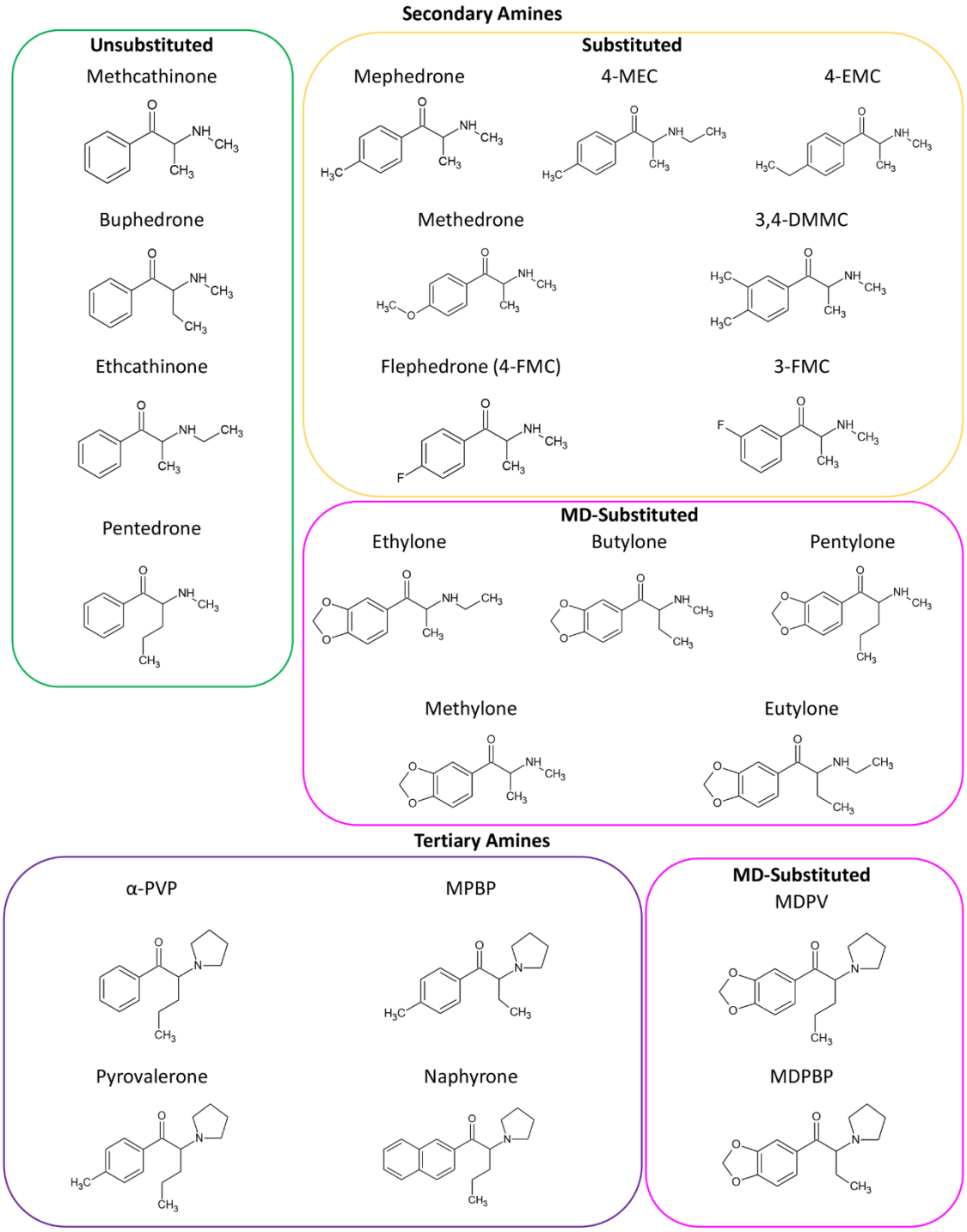


Figure 1.3. Chemical structures of twenty-two synthetic cathinones included in this study. Green indicates a secondary amine cathinone bearing no ring substituents; yellow indicates a ring-substituted secondary amine cathinone; magenta indicates a methylenedioxy-substituted cathinone; and purple indicates a tertiary amine (pyrrolidine-type) cathinone.

Table 1.2. Substituents at R_1-R_5 for the twenty-two synthetic cathinones included in this study and the nominal molecular weight.

Name	R_1	R_2	R_3	R_4	R_5	MW
3,4-DMMC	CH_3	CH_3	**CH_3**	**H**	**CH_3**	191
3-FMC	F	CH_3	**CH_3**	**H**	**CH_3**	181
4-EMC	H	C_2H_5	**CH_3**	**H**	**CH_3**	191
4-MEC	H	CH_3	**CH_3**	**H**	**C_2H_5**	191
Buphedrone	H	H	**C_2H_5**	**H**	**CH_3**	177
Butylone	3,4-Methylenedioxy		**C_2H_5**	**H**	**CH_3**	221
Ethcathinone	H	H	**CH_3**	**H**	**C_2H_5**	177
Ethylone	3,4-Methylenedioxy		**CH_3**	**H**	**C_2H_5**	221
Eutylone	3,4-Methylenedioxy		**C_2H_5**	**H**	**C_2H_5**	235
Flephedrone (4-FMC)	H	F	**CH_3**	**H**	**CH_3**	181
MDPBP	3,4-Methylenedioxy		**C_2H_5**	**Pyrrolidinyl**		261
MDPV	3,4-Methylenedioxy		**C_3H_7**	**Pyrrolidinyl**		275
Mephedrone	H	CH_3	**CH_3**	**H**	**CH_3**	177
Methcathinone	H	H	**CH_3**	**H**	**CH_3**	163
Methedrone	H	OCH_3	**CH_3**	**H**	**CH_3**	193
Methylone	3,4-Methylenedioxy		**CH_3**	**H**	**CH_3**	207
MPBP	H	CH_3	**C_2H_5**	**Pyrrolidinyl**		231
Naphyrone	Naphthyl		**C_3H_7**	**Pyrrolidinyl**		281
Pentedrone	H	H	**C_3H_7**	**H**	**CH_3**	191
Pentylone	3,4-Methylenedioxy		**C_3H_7**	**H**	**CH_3**	235
α-PVP	H	H	**C_3H_7**	**Pyrrolidinyl**		231
Pyrovalerone	H	CH_3	**C_3H_7**	**Pyrrolidinyl**		245

The chemical structure of the cathinones greatly influence their pharmacological activity. Just like their non-β-ketone counterparts, the presence of the secondary or tertiary amine defines their basic nature. While the pKa values are not known for all of the analogs, values in the range 7.2 to 8.9 have been reported (15-17). The physico-chemical properties of the drug greatly influence its pharmacology. The addition of halogens, pyrrolidine group and properties of the alkyl side chain, can influence the lipophilicity of these drugs. A more lipophilic drug will readily transverse the blood-brain barrier (BBB), potentially enhancing physiological effects in the central nervous system (13, 18). *N*-alkylation will also maintain the desired stimulant effects of phenethylamines (13, 19, 20). Phenethylamines contain a

chiral center (α-carbon) producing two enantiomeric forms. The S-(-)-enantiomer has been proven to be more potent than the R-(+)-enantiomer in synthetic cathinones, as with cathinone and methamphetamine (8, 21-23).

Cathinones also exist as tautomers in equilibrium. **(Figure 1.4)**. The keto tautomer has been shown to be more stable than the enol isomer (24). The enol tautomer, or enamine form, may be less stable due to its ability to further tautomerize into an imine. However, increased stability may be achieved through intramolecular hydrogen bonds (24, 25). During fragmentation, the formation of the enol tautomer is most likely an intermediate step for the loss of water (26, 27). Fragmentation of bupropion through collision-induced dissociation and electron impact ionization was determined to be the result of the keto tautomer (28).

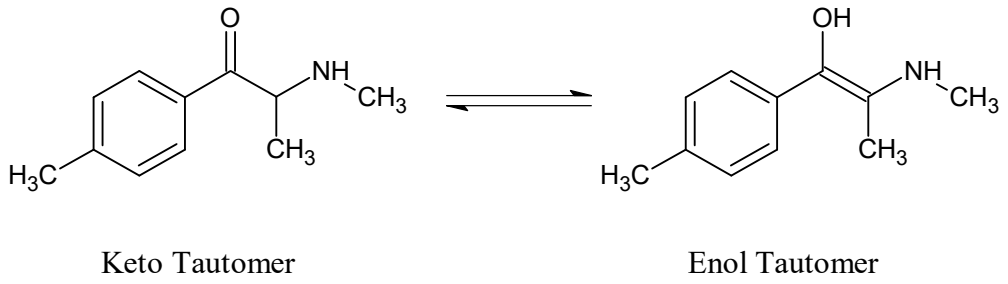


Figure 1.4. Keto-enol equilibrium of β-keto amphetamines (shown for mephedrone).

Detection Methods

In forensic toxicology laboratories, biological evidence suspected of containing an unknown drug will be analyzed first by a screening method followed by a confirmatory method. Although antibody-based immunoassay (IA) techniques are widely used, they are often limited in scope. Chromatographic and mass spectrometry-based screening

techniques are often more labor intensive, but can potentially identify a much larger number of drugs simultaneously.

Confirmatory analyses can be completed using an array of different techniques, most commonly gas chromatography (GC) or liquid chromatography (LC)-based mass spectrometry (MS). These techniques are used for both qualitative and quantitative analysis. Although this approach enjoys widespread use, the detection of synthetic cathinones is challenging due to the number of different derivatives and the frequency with which new derivatives appear in forensic investigations (9, 29).

Immunoassay Techniques

Immunoassay screening tests designed for amphetamine and methamphetamine have been used in attempt to detect synthetic cathinones. Unfortunately, many studies have shown that many of these immunoassays exhibit poor cross-reactivity towards the cathinones (14, 30-38). If a positive IA screen was achieved, concentrations exceeded the typical average concentration identified in biological samples. For example, in a study completed by DeRuiter *et. al.* using an enzyme multiplied immunoassay technique (EMIT) designed for methamphetamine and amphetamine, methcathinone and cathinone concentrations greater than 200,000 ng/mL were necessary to produce a positive result, compared with 1,000 ng/mL of methamphetamine or amphetamine (23).

Swortwood *et. al.* investigated the cross reactivity of eight synthetic cathinones on sixteen commercial immunoassay platforms. Target analytes for the sixteen kits included amphetamine, methamphetamine, benzylpiperazine, ketamine methyphenidate, mephentermine, MDPV, mephedrone, phencyclidine, and cotinine. Only two of the sixteen kits resulted in positive results at synthetic cathinone concentrations <1,250 ng/mL; all

other kits required concentrations greater than 10,000 ng/mL (36). Synthetic cathinone screening methods using biochip array technology have been developed, but these techniques are limited to the synthetic cathinones that were popular at the time of development (36). These antibody-based assays cannot compete with the rapidly changing landscape of novel psychoactive substances. In order to effectively screen for novel synthetic cathinones, more specialized chromatographic or mass spectrometry-based techniques may be required. To that end, chromatographic screening techniques for novel psychoactive substances, including synthetic cathinones, have been developed for GC and LC instrumentation (25, 39-46). However, chromatographic-based screens require similar sample preparation necessary for confirmatory analysis, and are therefore more resource-intensive than conventional immunoassays.

Chromatographic Methods

Drug confirmation in biological evidence requires highly specialized analytical instrumentation and methodology. These techniques require sample preparation (to isolate analytes of interest), are more costly, and require advanced training to interpret data. Gas chromatography-mass spectrometry (GC-MS) is still one of the most widely used confirmatory techniques in forensic toxicology laboratories. However, GC-MS analysis is limited to molecules that are non-polar, volatile, and thermally stable. Chemical derivatization can improve these properties, but further increases sample preparation time.

Liquid chromatography-mass spectrometry (LC-MS) can be used to analyze a wide variety of compounds and is more amenable to polar species. LC methods are also advantageous due to their increased selectivity, specificity, and sensitivity compared to GC-MS methods (35, 45, 47-49). Increased sensitivity and selectivity can decrease limits

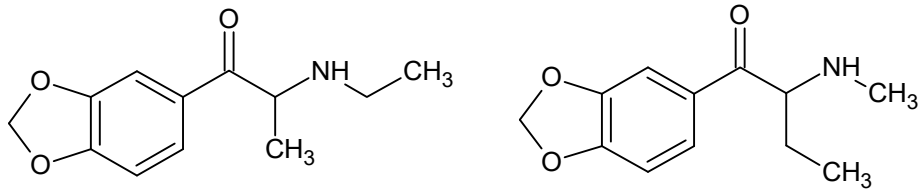
of detection (LOD) and quantitation (LOQ). GC-MS methods for cathinones often reported LOQs over 20 ng/mL (50-59), whereas LC methods report LOQs <20 ng/mL (33, 44, 47, 48, 60-67).

For the detection of synthetic cathinones and other novel psychoactive substances, LC separation is most effectively coupled with tandem mass spectrometry (MS/MS) or high resolution mass spectrometry (HRMS) using quadrupole/time of flight (Q/TOF), Orbitrap™ or QExactive™ detectors. There is widespread use of MS/MS detectors in forensic toxicology laboratories and HRMS methods are growing in popularity. HRMS in particular has accurate mass resolution, allowing compounds with similar nominal masses but different accurate masses (due to elemental composition) to be differentiated. These instruments also collect and store data for all ions detected, allowing for retrospective data interrogation, which is of particular importance for emerging drugs or drugs that do not currently have available reference standards (47, 48, 61, 64, 68). In drug metabolism studies, HRMS may not require the use of reference standards because the structures of biotransformed products can be structurally elucidated through the combination of accurate mass and detailed mass spectra fragmentation patterns (69, 70).

Detection Challenges of Synthetic Cathinones

The proliferation of synthetic cathinones has resulted in the synthesis of multiple constitutional isomers. From a chemical standpoint, two, three, or four of these drugs may share the same molecular formula, but are structurally different. Two examples are ethylone and butylone; both have the chemical formula of $C_{12}H_{15}NO_3$ and differ by the location of an ethyl group. In ethylone, the ethyl group is located on the nitrogen, and in

butylone it is located at the α-carbon **(Figure 1.5).** These constitutional isomers pose challenges for a chromatographic separation and detection.



Ethylone Butylone

Figure 1.5. Chemical structures of constitutional isomers ethylone and butylone.

In order to differentiate between regioisomers, complete chromatographic resolution may be necessary (15, 71-74). Isomeric separation has been achieved for many synthetic cathinones; however, the fluorinated cathinones (2-, 3-, and 4-FMC) have presented the greatest challenge (71, 75-77). The separation of ethylone and butylone has also presented a problem using reversed-phase (RP) liquid chromatography (25). While RP chromatography is most common for LC analysis, alternative approaches, including hydrophilic interaction liquid chromatography (HILIC), have been implemented (60, 78). Separation of cathinones using reversed-phase chromatography can be achieved, but may involve moderate to long run times and considerable skill.

Another challenge is the specificity of cathinone mass spectra produced by GC-MS analysis. Isomeric substances can produce very similar mass spectra using electron impact (EI) ionization techniques (49, 72, 75, 79, 80). Cathinones fragment readily, often producing no molecular ion. EI fragmentation is dominated by the dissociation of the α-β carbon bond (27). The major ion produced by this dissociation is an iminium ion, comprised of the structural formula $C_nH_{2n+2}N^+$, containing a double bond between the carbon and nitrogen for aliphatic amine cathinones, and $C_nH_{2n}H^+$ for pyrrolidine cathinones

(23, 27). The mass to charge ratio (*m/z*) observed from this dissociation in secondary amine cathinones is *m/z* 44, 58, 72, 86, and 100 derived from the equation 16 + 14*n,* where *n* is the number of carbons (27). For tertiary amine cathinones the observed *m/z* from this dissociation are *m/z* 126, 70, 55, 42, and 41 (27). As seen in **Figure 1.6**, the iminium ion (*m/z* 72) is the base peak for both ethylone and butylone. The formation of *m/z* 44 by ethylone can help differentiate these two constitutional isomers, but these differences are visible only in the fine spectra. Softer ionization techniques, including chemical ionization (CI) in GC analysis, and electrospray ionization (ESI) in LC analysis, help to preserve the molecular ion and reduce fragmentation, which assists with structural elucidation and drug identification.

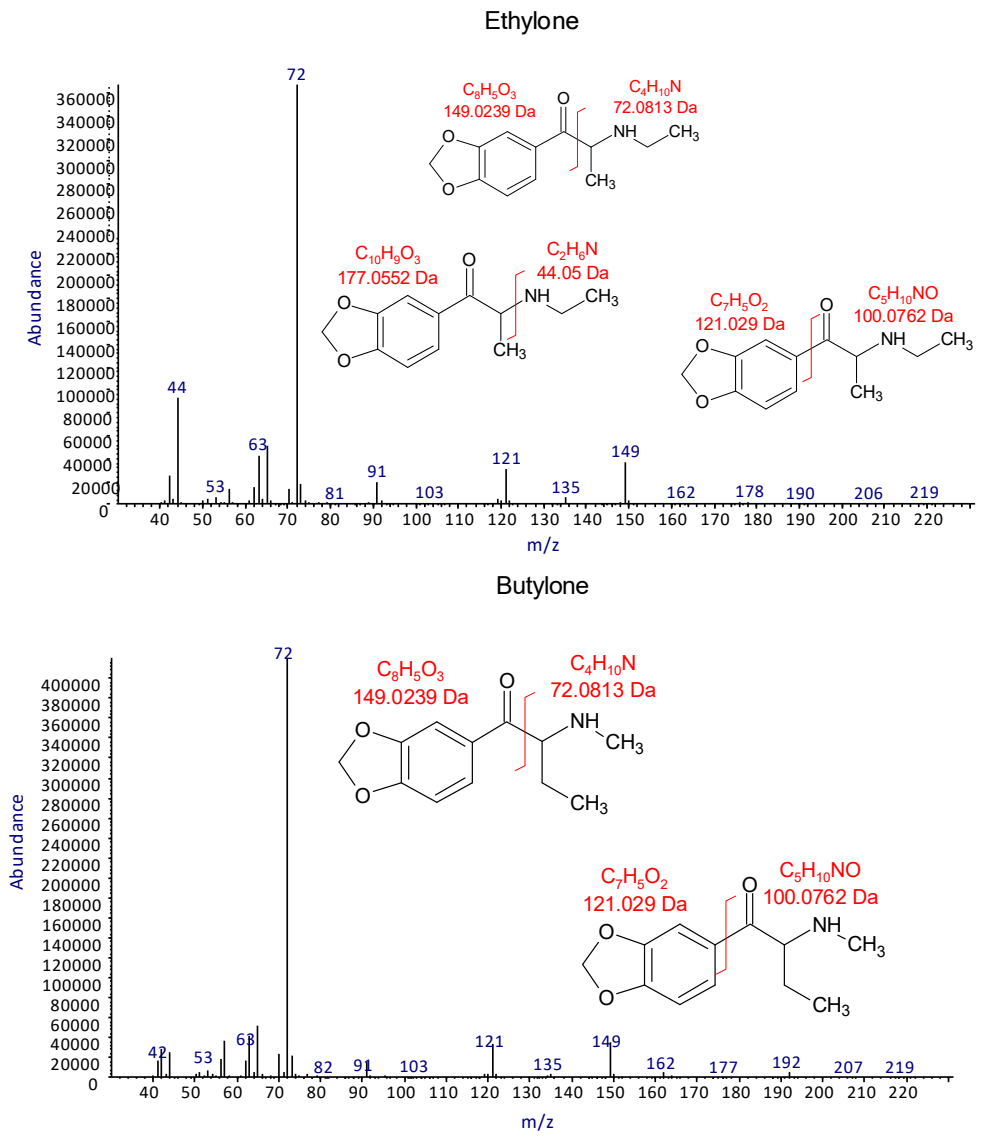


Figure 1.6. In-house GC-EI-MS spectra for ethylone (top) and butylone (bottom) to demonstrate the abundant iminium ion (*m/z* 72) resulting from α-β cleavage that occurs during EI fragmentation.

By LC-ESI analysis, synthetic cathinones are fragmented by collision-induced dissociation (CID) (81). By studying the methylenedioxy-type cathinones (ethylone, butylone) using HRMS, Fornal proposed CID fragmentation pathways for all cathinone types (81, 82). While only one characteristic ion, the iminium ion, is produced by GC-EI analysis, Fornal concluded that methylenedioxy (MD)-substituted cathinones under ESI

CID fragmentation produce four characteristic ions resulting from the loss of a water molecule, the loss of a neutral molecule CH_4O_2 (the methylenedioxy group) to form phenyloxazole, the loss of an amine, and the loss of an iminium ion. In non-MD synthetic cathinones, water loss was observed in secondary amine cathinones only. The loss of the amine, which formed a $[M\text{-}NR_3R_4+H]^+$ cation, was observed by all synthetic cathinones. However, the abundance of this ion varied depending on different structural features. If the aromatic ring was 4-methyl substituted (*e.g.* mephedrone, 4-MEC) the abundance of this ion increased. In contrast, a decrease was observed in 4-methoxy (4-MeO, methedrone) substituted cathinones.

Just as in GC-EI analysis, α-β cleavage occurred under CID fragmentation producing the iminium ion, particularly if the CID voltage was greater than 20 eV (81). The iminium cation abundance was higher if the synthetic cathinone was 4-MeO substituted or contained both a pyrrolidine and methylenedioxy group. The alpha-beta cleavage could also produce an oxonium cation; however, it was significantly less abundant than the iminium ion (81).

Despite being even-electron molecules, synthetic cathinones, excluding pyrrolidine types, form odd-electron radical ions under ESI analysis (83). The most abundant fragmentation product for primary amine ketones is the radical cation iminium ion. The most common iminium ions are $[C_9H_9N]^{+\cdot}$ with a *m/z* of 131.0730, $[C_{10}H_{11}NO]^{+\cdot}$ with a *m/z* of 145.0886, $[C_{10}H_{11}NO]^{+\cdot}$ with a *m/z* of 161.0835, and $[C_{10}H_9NO_2]^{+\cdot}$ with a *m/z* of 159.1042 (83). Cathinones that are 4-alkyl substituted produced the 131, 145, and 161 radical cations, and methylenedioxy cathinones produced either the 131 or 145 radical cation (83). These reported *m/z* ratios are important ion transitions to monitor when

producing synthetic cathinone mass spectra under ESI conditions and can help classify novel cathinones that have yet to be identified. These characteristic fragments are observed in the in-house HRMS mass spectra for ethylone and butylone (**Figure 1.7**).

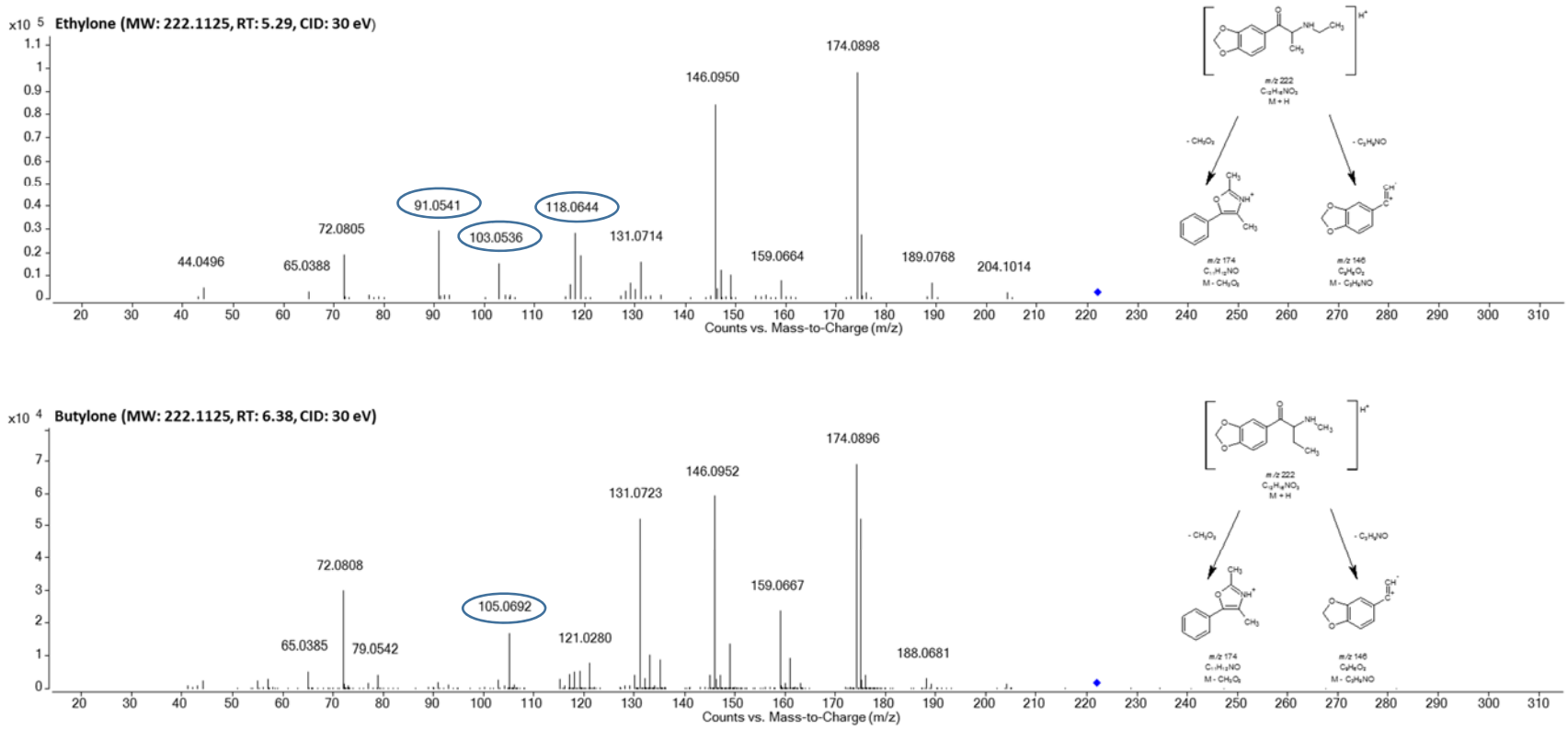


Figure 1.7. In house MS/MS spectra of ethylone (top) and butylone (bottom) using ESI. Identified ion (circled) discriminate ethylone and butylone.

Synthetic cathinones are challenging to detect because of multiple constitutional isomer pairings. Furthermore, they are thermally unstable, which limits the detection ability using GC-MS without derivatization. Studies have shown that cathinone derivatives undergo *in situ* degradation during this common analytical technique (23, 72, 75, 84). This was first established by Noggle and DeRuiter in the mid-1990s with methcathinone, followed by ethcathinone and dimethcathinone. A second, later-eluting peak with a base peak and molecular ion of 2 mass units less (M-2) than the parent was observed. For methcathinone, with a molecular ion of *m/z* 163 and base peak of *m/z* 58 (formed by the imine fragment), the second peak had a molecular ion of *m/z* 161 and base peak of *m/z* 56. It was concluded that the minor peak was a 2,3-enamine, an oxidative (loss of H_2) decomposition product formed during GC analysis (23).

The previous study used secondary amine cathinones to identify the 2,3-enamine decomposition product formed during GC analysis. Additional studies further identified at which step in GC analysis the degradation may have occurred. Tsujikawa *et. al.* investigated thermal degradation of synthetic cathinones by GC-MS using α-PVP and assessed the effect of the injector method, injector temperature, and activity on the liner surface (84). The decomposition product was confirmed for α-PVP. After evaluating splitless, 1:5 split, and 1:10 split injections, the split injections reduced the amount of thermal degradation that occurred. Lower injector temperatures reduced the formation of the thermal degradation product. Liner comparisons included a used deactivated liner, a new deactivated liner, and a new non-deactivated liner. The formation of the thermal degradation product was eliminated by using a new deactivated liner, indicating

decomposition was the result of the formation of active sites on the liner. The non-deactivated liner resulted in the return of the decomposition product (84).

The influence of injector temperature and age of deactivated liner on the thermal degradation of synthetic cathinones was also confirmed by Kerrigan *et. al.* using 18 synthetic cathinones comprised of thirteen secondary and five tertiary amines. The M-2 decomposition product was identified for all 18 cathinones (72).

Gas chromatography analysis requires high temperatures in order to completely vaporize the sample upon injection and maintain a gaseous state throughout separation and analysis. These extremely high temperatures may result in *in situ* thermal degradation of synthetic cathinones. In contrast, thermal degradation does not take place during LC separation, making it a more suitable technique for cathinones. Both GC and LC approaches have been utilized and these are summarized in **Tables 1.3 and 1.4**.

Table 1.3. Gas chromatography mass spectrometry methods for the detection of synthetic cathinones from biological matrices.

Cathinone	**Analytical Technique**	**Matrix**	**Reference**
Cathinone Methcathinone	GC-MS	U	(30)
Ethylone	GC-MS	U, Bi	(56)
MDPBP	GC-MS	B	(85)
MDPV Pentedrone	GC-MS	B, U	(86)
MDPV	GC-MS	B	(87)
MDPV	GC-MSC	B, U	(88)
MDPV	GC-MS	U, S	(89)
MDPV	GC-MS	U	(50)
Mephedrone	GC-MS	B, U, SC, Bi	(57)
Mephedrone	GC-MS	B, U	(55)
Mephedrone	GC-MS	B, U, SC	(54)

(continued)

Cathinone	Analytical Technique	Matrix	Reference
Mephedrone	GC-MS	U	(90)
Mephedrone	GC-MS	P, U	(51)
Mephedrone	GC-MS	B	(91)
Mephedrone	GC-MS	B, U	(92)
Methcathinone Buphedrone Mephedrone Methylone FMC	GC-MS	U	(93)
Methedrone	GC-MS	B	(94)
Methylone	GC-MS	B, U	(95)
Methylone	GC-MS	B, VH, SC, L	(52)
Methylone	GC-MS	B, U, L	(58)
α-PVP	GC-MS	B, SC	(96)
16 Cathinones	GC-MS	U	(97)

Matrices include blood (B), urine (U), liver (L), plasma (P), serum (S), stomach contents (SC), bile (Bi), and vitreous humor (VH)

Table 1.4. Liquid chromatography mass spectrometry methods for the detection of synthetic cathinones from biological matrices.

Cathinone	Analytical Technique	Matrix	Reference
3,4-DMMC α-PVP	LC-HRMS	U	(98)
3,4-DMMC	LC-MS	B	(99)
3,4-DMMC	LC-MS/MS	B, U	(66)
3,4-DMMC	LC-MS/MS	U	(100)
4-MEC MDPV	LC-MS/MS	H	(101)
4-MEC	LC-MS/MS	B, U	(102)
4-MEC Mephedrone Methcathinone	LC-MS/MS	B	(103)
4-MEC	LC-MS/MS & HRMS	B, P	(104)

(continued)

Cathinone	Analytical Technique	Matrix	Reference
Butylone	LC-MS/MS	B, L	(105)
MDPBP	LC-MS/MS	U	(106)
MDPV	LC-HRMS	U	(107)
MDPV	LC-HRMS	P	(108)
MDPV	LC-MS/MS	B	(109)
MDPV	LC-MS/MS	U	(110)
MDPV	LC-MS/MS	U	(111)
MDPV	LC-MS/MS	B	(112)
MDPV	LC-MS/MS	S	(29)
MDPV	LC-MS/MS	B, U, L, SC	(113)
MDPV Mephedrone Methylone	LC-MS/MS	T	(60)
Mephedrone MDPV	LC-MS/MS	S	(62)
Mephedrone	LC-MS/MS	S	(114)
Mephedrone	LC-MS/MS	B, VH	(115)
Mephedrone	LC-MS/MS	B, U, SC	(116)
Mephedrone MDPV	LC-MS/MS	B, U, S	(117)
Methcathinone Mephedrone	LC-MS/MS	U	(35)
Methylone	LC-MS/MS	B, U, Bi, SC, VH	(118)
Methylone	LC-MS/MS	P	(119)
Pentedrone α-PVP OH-α-PVP	LC-MS	B, L, T, SC	(63)
α-PVP	LC-HRMS	B, U, L, T	(67)
α-PVP	LC-MS/MS	S	(120)
α-PVP OH-α-PVP	LC-MS/MS	B, U, SC, T, L	(121)
α-PVP	LC-MS/MS	U	(122)
6 cathinones	LC-MS/MS	OF	(33)
7 cathinones	LC-MS/MS	B, P, U, VH	(123)

(continued)

Cathinone	Analytical Technique	Matrix	Reference
7 cathinones	LC-MS/MS	B	(77)
8 cathinones	LC-HRMS	U	(61)
8 cathinones	LC-MS/MS	B	(71)
9 cathinones	LC-MS/MS	S	(64)
10 cathinone	LC-MS/MS	OF	(65)
11 Cathinones	LC-MS/MS	U	(76)
11 cathinones	LC-MS/MS	U	(25)
19 Cathinones	LC-MS/MS	B	(45)
23 cathinones	LC-HRMS	B	(68)
32 Cathinones	LC-HRMS	U	(47)

Matrices include blood (B), urine (U), liver (L), plasma (P), serum (S), oral fluid (OF), stomach contents (SC), bile (Bi), vitreous humor (VH), hair (H), and a variety of tissue (T) including brain, kidney, spleen.

Pharmacology

The leaves of the *khat* plant have been historically chewed for their psychostimulant and euphoric effects. The primary psychoactive component in the shrub, cathinone, has been chemically modified to produce over 40 synthetic cathinone analytes, each producing psychostimulant and euphoric effects. These synthetic analogs have provided an alternative option to methamphetamine, MDMA, and cocaine users.

Through data from emergency department intakes, poison control centers, and self-reported forums, synthetic cathinone users world-wide are typically males in their mid to late 20s (5, 124-131). Common routes of administration include insufflation (snorting), inhalation or smoking, oral, rectal, and intravenous (IV) (3, 124, 125, 127, 129, 131-133). The route of administration influences the intensity of effects and the onset of action. Products containing synthetic cathinones are typically sold as a powder or pill, with packages weighing from as low as 25 mg to upward of 1,000 mg (134, 135). When taken

orally or by insufflation, reported doses are 5 to 250 mg and 5 to 125 mg, respectively (8, 132, 134, 136). In contrast, intravenous administration typically involve much lower doses (2 to 20 mg), due to the increased bioavailability of the drug (132). Onset of action also occurs earlier with IV or insufflation (ranging from 10 to 15 minutes), compared with orally administered drug (15 and 45 minutes) (3, 132-134). Desired effects typically last between 30 minutes to 4 hours depending on route of administration, with adverse effects lasting a minimum of eight hours and upwards of two days (3, 8, 132-134, 136). These drugs can be administered in small doses over a short period of time (bingeing) to prolong the desired effects, resulting in the administration of 500 to 1,000 mg in a single session (8, 137, 138).

The sought-after effects of cathinones include psychostimulation and euphoria. Reported effects include increased alertness, energy, motivation, concentration, libido, sociability and talkativeness, and empathy (6, 133, 139). However, numerous adverse effects are associated with synthetic cathinones, from a minor headache and nausea to hallucinations, delusions, and suicidal thoughts (5, 6, 124, 127, 131, 140). Adverse symptoms can be classified as cardiovascular, cognitive, psychiatric, neurological, and perceptual (6). A summary of desired and adverse effects associated with cathinone use is shown in **Table 1.5**.

Table 1.5. Desired and adverse effects associated with synthetic cathinone use.

Desired Effects	**Adverse Effects**
Euphoria	Cardiovascular:
Alertness	Hypertension
Psychomotor Hyperactivity	Myocardial Infarction
Increased Energy	Tachycardia
Empathy	Hyperthermia
Openness	Cognitive:
Sexual Arousal	Confusion
Talkativeness	Impaired memory

(continued)

Desired Effects	Adverse Effects
Increased Concentration	Impaired Coordination
Positive Feeling	Psychiatric:
Sociability	Aggression
Increased Motivation	Agitation
	Violence
	Suicidal Thoughts
	Depression
	Neurological:
	Seizures
	Insomnia
	Bruxism
	Muscle Spasms
	Perceptual:
	Paranoia
	Psychosis
	Other:
	Liver Toxicity
	Rhabdomyolysis
	Organ Failure

References: (4-6, 8, 9, 124, 127, 131, 133, 139, 141-145)

Through exposures reported to poison control centers across the United States, the most common adverse effects were tachycardia, agitation, hypertension, hallucinations, and violent behavior (5, 124, 127). While these studies surveyed and reported symptoms associated with synthetic cathinones as a generality, others have documented specific cathinone symptoms through web surveys or case studies.

Surveys of mephedrone users provide information about the most common adverse effects associated with the use of this particular cathinone. However, this self-reported data is complicated by poly-drug use and the potential for the drug to be misrepresented to the user (131). The most common adverse effects associated with mephedrone products were headache, nausea, tremors, anxiety, and paranoia (129, 144). Individuals also experienced depression, anxiety, irritability, and memory loss (144). Despite these negative effects,

users found the mephedrone to be addictive and indicated they would use the drug again (4, 129, 131, 144).

Antemortem case reports in a clinical, rather than forensic setting, are typically the result of severe adverse side effects that require emergency services. Although these reports are of interest, they can rarely be used to distinguish lethal from non-lethal blood concentrations, due to tolerance. Cathinone concentrations in antemortem forensic casework is discussed later.

In a case study presented by Thornton *et. al.* a patient with prior psychiatric history was brought to the emergency department following bizarre behavior, suicidal thoughts, and auditory hallucinations one hour after insufflating a white powder. Analysis revealed this powder contained MDPV and flephedrone (4-FMC). The patient claimed he had used bath salts before and never had this reaction. Blood and urine analysis revealed MDPV and its metabolites, flephedrone, and caffeine, ruling out contribution to these symptoms from other drugs (146). Truscott *et. al*. presented a case study involving a patient that had a sudden outburst of violence. After admittance to the emergency department, it was discovered he suffered from rhabdomyolysis and acute renal failure, which have been linked to synthetic cathinone use. MDPV was identified in urine and serum, directly linking these symptoms to the use of MDPV (29). In another case, a driver had insufflated α-PVP within hours of a vehicular accident. The driver was agitated, but felt full of energy. At the time of the accident, he experienced visual hallucinations and a feeling of unconsciousness, which caused the accident and the death of two passengers (147). These are a few of the numerous published case reports documenting synthetic cathinone involvement in emergency room admissions and impaired driving. These cases highlight the unpredictable,

severe, and dangerous adverse effects of synthetic cathinones use resulting from changes in the concentration of neurotransmitters.

Three catecholamine neurotransmitters – dopamine, serotonin, and norepinephrine – are linked to various psychological and physiological effects. Understanding a drug's affinity for each neurotransmitter can determine its pharmacological activity. Drugs with a high affinity for dopamine, the reward system, have a tendency to be more addictive and have a higher risk for dependence. Drugs with a higher affinity for serotonin, linked to mood, will more likely produce effects to enhance emotions, sociability, and libido. Drugs with a higher affinity for norepinephrine, linked to flight or fight reactions, will stimulate the sympathetic nervous system and have stimulant-like effects.

Pharmacodynamic *in vivo* and *in vitro* studies are performed to determine how drugs interact with the catecholamines. Pharmacokinetic *in vivo* and *in vitro* experiments are used to determine how the body eliminates these foreign xenobiotics. *In vivo* experiments for pharmacodynamic studies involve controlled dosing studies, combined with behavioral monitoring and neurotransmitter evaluation. Pharmacokinetics are typically determined *in vivo* using animal models. These studies can provide valuable information regarding the abuse potential and reinforcing behavior related to these drugs, and can also investigate the long-term effects. Given the adverse consequences associated with cathinone use, *in vitro* methods have also been used to examine the pharmacodynamics and pharmacokinetics of this drug class.

Pharmacodynamics

As with other stimulants, including methamphetamine and amphetamine, synthetic cathinones affect the release and reuptake of the catecholamines norepinephrine,

dopamine, and serotonin, and the enzymes involved in catecholamine synthesis, including tryptophan hydroxylase and tyrosine hydroxylase (21, 137, 148, 149). *In vivo* and *in vitro* studies have been performed to better understand how synthetic cathinones interact with neurotransmitters. Dopamine interaction was first documented using the natural precursor, cathinone.

An early *in vitro* study done by Kalix using rabbit striatum, confirmed the mechanism of action for (-)cathinone was similar to (+)amphetamine. Both of these drugs release dopamine from intercellular vesicles. Moreover, there is a direct relationship between dopamine release and dose (21). Nearly twenty years later, Gygi *et. al.* investigated the effect of methcathinone on dopamine *in vivo* using rats. In their first study, rats were either administered a single injection of methcathinone or received multiple injections over the course of sixteen hours. After a single dose, it was concluded that methcathinone decreased the activity of tryptophan hydroxylase. After multiple injections, the concentration of dopamine and serotonin had decreased in the striatum, which controls many cognitive functions (150). In an additional study, to assess long-term effects of these drugs, rats were administered methcathinone over sixteen hours and sacrificed 30 days later. The concentration of dopamine, serotonin, and their metabolites significantly decreased following the administration of methcathinone, relative to the control group (151). These initial studies indicate that cathinones significantly impact neurotransmitter concentrations and cause deficits within the brain for weeks after drug cessation. These deficits provide an explanation for withdrawal effects, including depression, and the long-term consequences of cathinone use.

Investigations into the pharmacology of synthetic cathinone use reappeared in the late 2000s following an increase in synthetic cathinone use worldwide and the accompanying severe cardiovascular, neurological, and psychopathological effects. Many *in vivo* studies revolved around mephedrone, one of the earliest synthetic cathinones. One study performed by Hadlock *et. al.* investigated the effects of mephedrone in rats following several administrations within an hour to mimic a "binge". Within one hour of drug cessation, there was a significant decrease in dopamine and serotonin transporter function and the body temperature of the rats had increased compared to the placebo group. Seven days after the last mephedrone dose there was significant decrease in serotonin reuptake and concentration, possibly indicating that mephedrone has a long-term effect on serotonin transporter function. Mephedrone is also a potent dopamine releaser, evidenced by eager self-administration of the drug in animal studies. It should be noted that rats self-administered mephedrone more times than methamphetamine in a seven-day period, highlighting that these novel drugs may be more addictive and have a greater abuse liability than methamphetamine or MDMA (138). Additional rat studies involving mephedrone revealed that the enantiomers, *R*-mephedrone and *S*-mephedrone, do not share the same affinity for neurotransmitters. The *R* enantiomer was found to be a preferential dopamine releaser, 50 times greater than the *S* enantiomer, and the *S* enantiomer results in a greater release of serotonin (22). Synthesizing mephedrone that contains predominantly the *R*-enantiomer would greatly increase its abuse potential.

A limited number of *in vivo* studies have been performed on other synthetic cathinones. Dose dependence on the abuse and reinforcement potential of MDPV and α-PVP was confirmed through self-readministration and locomotor activity in rats (152, 153).

In vivo studies using methylone also revealed a dose dependent effect on locomotor activity and that its use can result in long-term changes in cognitive function through persisting deficits in neurotransmitter concentrations throughout the brain (18, 154). It should be noted that that these drugs inhibit vesicle monoamine transporter (VMAT2), which prevents monoamine neurotransmitters from returning to vesicles (155). This decreases extracellular storage of neurotransmitters and increases the likelihood of neurotransmitters of entering the synapse.

While *in vivo* studies investigate a single compound at a time, *in vitro* studies allow for the simultaneous investigation of multiple drugs. Simmler *et. al.* used HEK 293 cells to investigate how ten cathinones (cathinone, methcathinone, mephedrone, flephedrone, methylone, ethylone, butylone, pyrovalerone, MDPV, and pyrovalerone) interacted with certain neurotransmitters. Their findings revealed there are significant difference between the cathinones and monoamine interaction. (149). The various cathinones have different affinities for the catecholamines and differ in mechanisms of action. Each cathinone can be classified as either a substrate releaser or transport inhibitor. A substrate releaser will enter the axon terminal and disrupt the vesicles that store neurotransmitters until they are released into the synapse. The excess neurotransmitters will be released into the synaptic cleft by reversing the transporter. Substrate releasers ultimately cause a deficit of neurotransmitters in the axon and damage transporters (134, 137). A transport inhibitor prevents the reuptake of cathinones from the synapse (132, 137, 149). Depending on their ability to act as a transport inhibitor, substrate releaser, or a combination of both, synthetic cathinones were initially categorized as either A) cocaine-MDMA-mixed cathinones, B) methamphetamine-like cathinones, or C) pyrovalerone-like cathinones (149) **(Table 1.6)**.

Cocaine-MDMA-mixed cathinones are non-selective monoamine uptake inhibitors; they will inhibit the reuptake of all three neurotransmitters to some degree. This category of cathinone includes mephedrone, methylone, ethylone, butylone, and naphyrone (149). Naphyrone is a stronger dopamine transporter inhibiter than the other four identified, whereas mephedrone and the methylenedioxy-type cathinones have a greater affinity for serotonin transporter inhibition. The higher affinity of mephedrone as a serotonin transporter inhibitor supports the findings of Hadlock *et. al.* (138). All, except naphyrone, were preferential releasers for serotonin. Mephedrone released both dopamine and serotonin.

Methamphetamine-like cathinones, such as cathinone, methcathinone, and flephedrone, are preferential catecholamine transporter inhibitors and dopamine releasers. All have a higher affinity for dopamine transporter inhibition over serotonin transporter inhibition. The third category, pyrovalerone-like cathinones, including MDPV and pyrovalerone, are the most potent and selective dopamine transporter inhibitors with no substrate release activity. MDPV and pyrovalerone were 10 times more potent in their inhibition of the dopamine transporter than mephedrone (149). Additional *in vitro* studies have supported Simmler's study regarding the potency of MDPV as a selective dopamine transporter inhibitor (148, 156). This strong affinity for dopamine reuptake inhibition would increase the abuse and dependence potential of pyrovalerone-like cathinones (152). Regardless of potency and affinity for transporter inhibition or neurotransmitter release, all cathinones increase the concentration of neurotransmitters in the synapse and have a high abuse potential (9, 137, 149).

Table 1.6. Classifications of synthetic cathinones based upon mechanism of action (149).

Non-selective uptake inhibitors (Cocaine-MDMA-mixed cathinones)	**Preferential transporter inhibitors** (Methamphetamine-like cathinones)	**Dopamine transporter inhibitors** (Pyrovalerone-like cathinones)
Mephedrone Methylone Ethylone Butylone Naphyrone	Cathinone Methcathinone Flephedrone (4-FMC)	MDPV Pyrovalerone

Studies have also shown that these drugs may have additive or synergistic effects, especially when two cathinones having two different mechanisms of action are co-administered (156-158). Illicit drug preparations, which may contain more than one cathinone species, could result in increased toxicity and potential for adverse effects.

Pharmacokinetics

In clinical and postmortem biological samples, the identification of the drug may rely on the identification of metabolites. For many drugs, the metabolite has a longer detection window than the parent molecule, making it easier to identify. Detection time of the parent molecule may be shortened due to delayed collection of sample, extensive metabolism, and shorter half-life. An example of this phenomenon is cocaine. Cocaine is extensively metabolized, with less than 10% of the parent molecule detected unchanged. Therefore, the use of cocaine is confirmed through the identification of the primary metabolite, benzoylecgonine. Metabolites have the potential to be pharmacologically active or toxic and can also interact with other substances in the body. From an analytical standpoint, once metabolites are elucidated, they can be incorporated into current detection methods. However, one limitation for incorporating metabolites in analytical methods in accredited forensic laboratories is the lack of commercially available analytical standard

or certified reference material for some substances. This is particularly true for novel psychoactive substances, because their development on the illicit drug market far outpaces pharmacologic research efforts.

Numerous *in vitro* approaches to drug metabolism have been developed, involving the use of human liver microsomes (HLMs), primary hepatocytes, liver preparations, cytosol, liver S9 fractions, supersomes, and microsomes. Microsomal studies can be used to investigate the role of cytochrome P450 (CYP) isozymes. This can be particularly important due to genetic variance and the potential for either adverse drug reactions or drug-drug interactions. Although HLMs are widely used, hepatocytes and liver slices more closely resemble *in vivo* metabolism (98). Through *in vitro* studies, identification and structural elucidation of metabolites and the involvement of specific enzymes can be determined. *In vivo* experiments in humans or animal models may help identify the most abundant metabolites, but significant differences can be observed between species (159-161). Another, less common, method of predicting metabolites is *in silico* experiments using software. *In silico* models produce all potential metabolites and predict which are likely to be the most abundant. The combination of *in vivo* and *in vitro* experiments can support *in silico* predictions and provide an accurate pharmacokinetic profile (98).

The pharmacokinetic profile includes phase I and phase II metabolism. Phase I metabolism involves biotransformations that typically increase polarity to facilitate elimination or conjugation. These biotransformations often include *N*-dealkylation, reduction, oxidation, and hydroxylation. Phase II metabolism involves primarily glucuronidation or sulfation at reactive sites to further increase polarity and excretion. The extent of drug metabolism varies significantly for each drug. *In vivo* and *in vitro*

experiments can predict how much of the drug will be eliminated unchanged, which can help determine which compound should be targeted for identification purposes.

Synthetic cathinone metabolism has been investigated using *in vitro* and *in vivo* experiments (89, 90, 97, 98, 100, 107, 122, 160-168). In order to predict and detect unknown metabolites, knowledge of the parent drug fragmentation is crucial (89, 90, 107, 161, 163, 167). GC-MS has been used to identify metabolites through MS/MS spectra and fragmentation. Again, derivatization is often required to enhance sensitivity of certain function group and elucidate structures (90, 122, 161). High resolution mass spectrometry (HRMS) instruments have become invaluable in structural elucidation of metabolites due to their mass accuracy and high sensitivity and specificity. Through the use of HRMS and GC-MS, the most common metabolic pathways identified for synthetic cathinones involve reduction, hydroxylation, *N*-dealkylation, or a combination thereof. However, biotransformation pathways for the most abundant metabolite vary significantly, depending on the type, or subgroup of cathinone.

Substituted Cathinones

Common metabolic pathways for substituted cathinones include *N*-dealkylation **(A)**, β-keto reduction **(B)**, and hydroxylation to either the aromatic ring substituent or alkyl chain **(C) (Figure 1.8)** (90, 98, 100, 160, 164, 165, 167, 168). The most abundant metabolic pathway varied for the substituted cathinones. Regardless of pathway, metabolism of these types of cathinones is extensive, with unchanged parent drug accounting for 15% or less of the dose (37, 51, 97). Mephedrone has been the most widely studied substituted cathinone (90, 100, 165).

At least seven metabolites of mephedrone have been identified, including hydroxytolyl-mephedrone, nor mephedrone, dihydro mephedrone (mephedrone ephedrine), nor-dihydro mephedrone, nor-hydroxytolyl mephedrone, 4-carboxy mephedrone, and 4-carboxy-dihydro mephedrone (51, 90, 97, 165, 169). The 4-carboxy-dihydro mephedrone was only identified in human urine and not produced by rat liver microsomes (51, 90). The hydroxytolyl metabolites were further metabolized into phase II glucuronides or sulfates (90).

Other substituted cathinones are metabolized in a similar fashion (98, 100, 160, 164, 168). Studies on 3-FMC revealed the most abundant metabolite in rat urine was the reduced "ephedrine form" of the drug, while the most abundant using HLMs was the *N*-dealkylated form (160). A metabolite of 3-FMC was identified where the aromatic ring had been hydroxylated to form hydroxyl-3-FMC (164). This transformation has not been documented for other substituted cathinones. 3,4-DMMC has an interesting metabolic pathway due to the presence of two substituents on the aromatic ring. The methyl groups can be hydroxylated to form either 4-hydroxymethyl-3-methylmethcathinone or 3-hydroxymethyl-4-methylmethcathinone which can be further oxidized to the carboxylic acid. However, these were minor products compared to the reduced form (100).

Cytochrome P450 isoenzymes involved these biotransformations include CYP2D6, CYP2B6, CYP2C19, and CYP2E1 (160, 169). CYP2D6 was the most active enzyme in the hydroxylation of mephedrone into hydroxytolyl mephedrone. *N*-dealkylation of 3-FMC required CYP2B6 and CYP2D6 with some contribution from CYP2C19 and CYP2E1. Based on the structural similarity between the substituted cathinones, other species may involve similar isoenzymes.

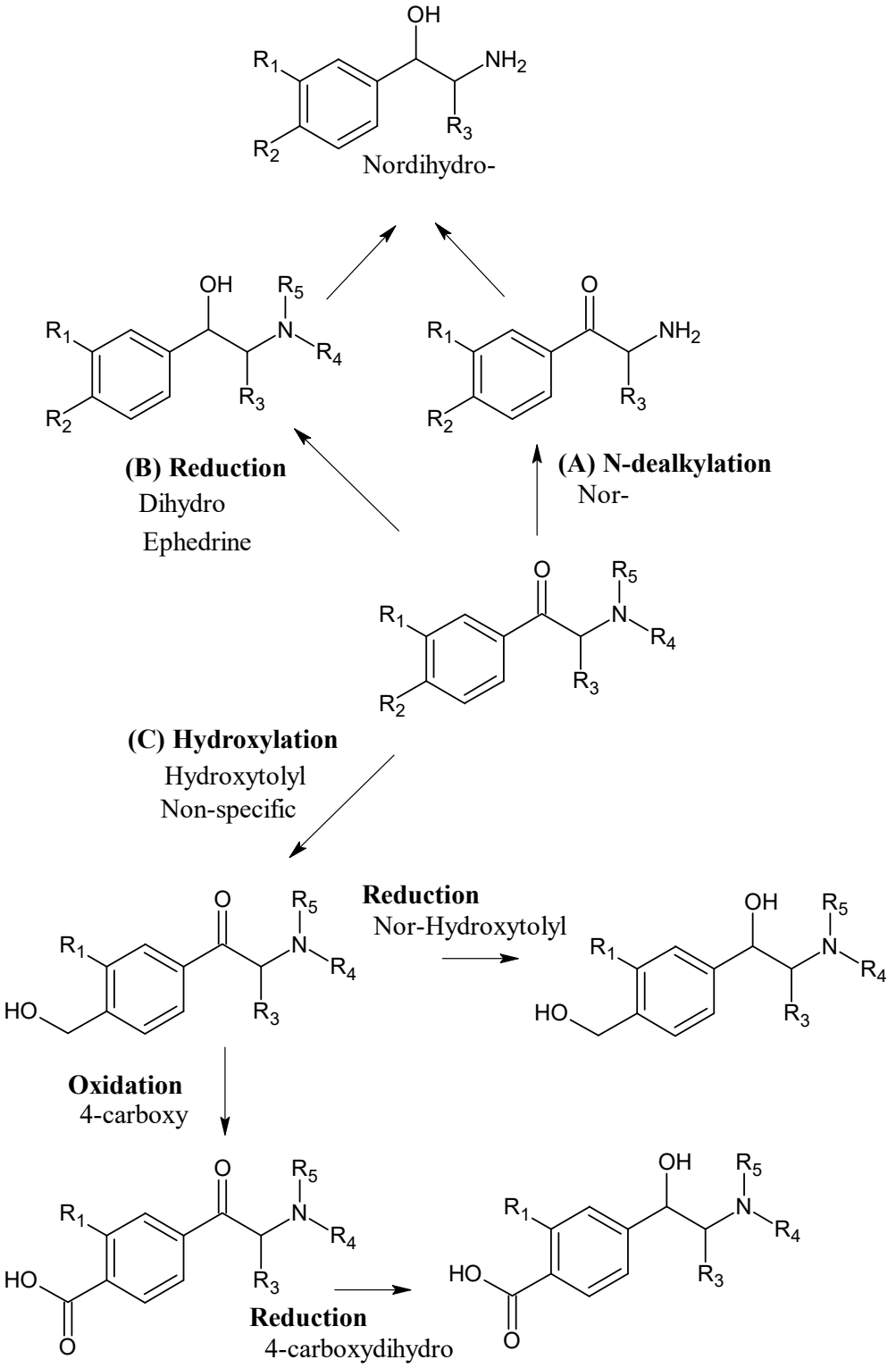


Figure 1.8. Metabolic pathway for substituted and unsubstituted cathinones. Biotransformation (bold) and nomenclature identified for each pathway (51, 90, 97, 165, 169).

Methylenedioxy Cathinones

The methylenedioxy-type cathinones undergo the same biotransformations as their substituted counterparts, including reduction **(A)**, *N*-dealkylation **(B)**, and alkyl chain hydroxylation **(C)**) with the addition of demethylenation **(D)** **(Figure 1.9)**. Demethylenation transforms the methylenedioxy group into two hydroxyl groups forming 3,4-hydroxy methcathinone, also referred to as catechol methcathinone (144, 162, 167, 170). Demethylenation is followed by *O*-methylation using catechol-*O*-methyltransferase (COMT) producing 4-hydroxy-3-methoxymethcathinone (HMMC, 4-OH-3-MeO-MC) or 3-hydroxy-4-methoxymethcathinone (3-OH-4-MeO-MC) **(E)** (**Figure 1.9)** (39, 98, 162, 167, 170). HMMC is the most abundant metabolite in humans and rats (90, 162). This catechol intermediate metabolite and the two methylated metabolites are readily conjugated in phase II metabolism processes (170). While most of the metabolism research involving methylenedioxy-type cathinones has been completed using methylone, comprehensive studies have shown other methylenedioxy analogs follow similar pathways (166, 170).

The nor- form and the ephedrine (or reduced) form of the substituted cathinones are among the most abundant. The methylenedioxy cathinones produce these metabolites, but to a lesser extent (170). While the reduced form has been identified through *in vitro* studies, it is not readily identified through *in vivo* studies (18, 39, 162, 166). Zaitsu *et. al.* did observe the reduction, but only for ethylone and butylone; methylone did not readily metabolize into the ephedrine form (170). Steric hindrance from the methylenedioxy group may inhibit the reduction of the beta-keto group.

The main isoenzyme involved in biotransformation of methylone is CYP2D6; CYP1A2, CYP2B6, and CYP2C19 are involved to a lesser extent (Pedersen, 2013). COMT is involved in the *O*-methylation of the catechol intermediate metabolite.

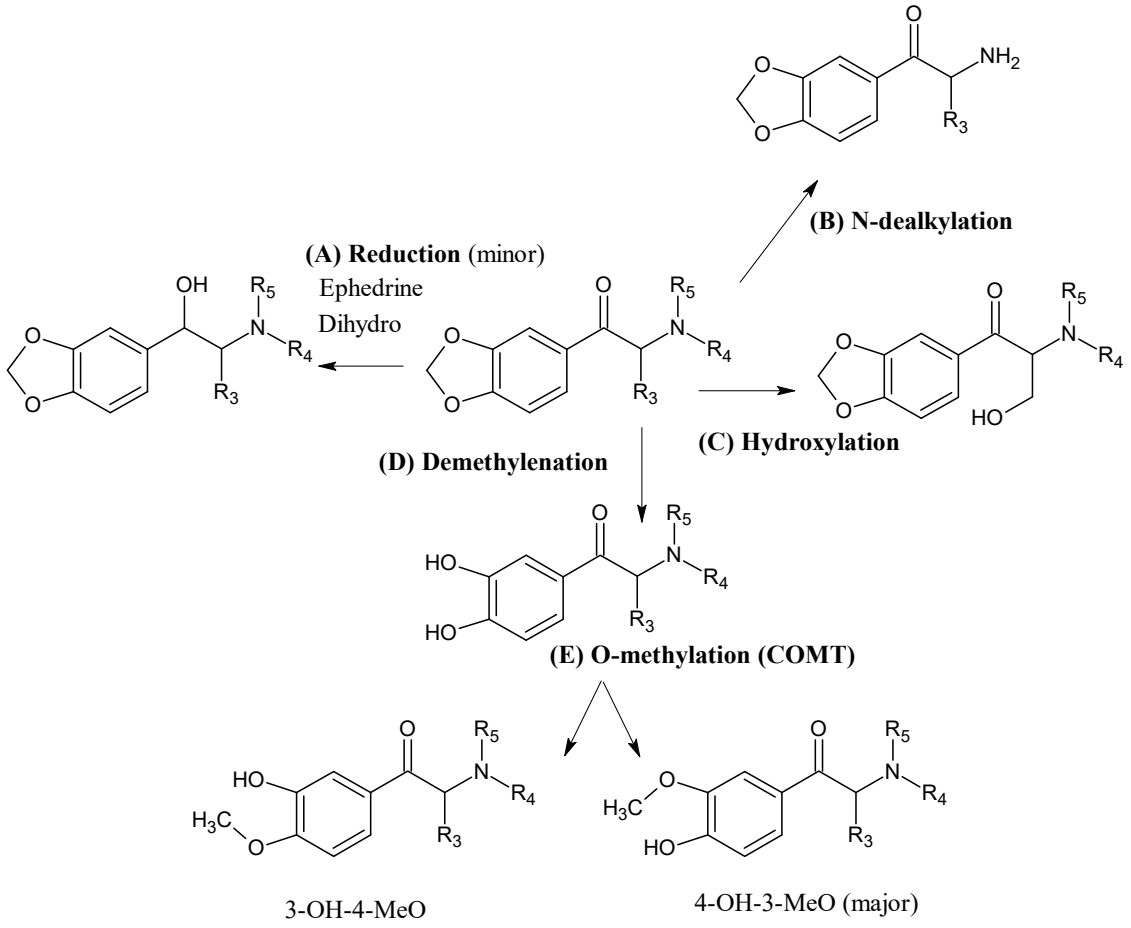


Figure 1.9. Metabolic pathway for methylenedioxy-type cathinones. Biotransformation (bold) and nomenclature identified for each pathway (90, 170).

Pyrrolidinophenone Cathinones

Cathinones containing the pyrrolidinyl group in the absence of the methylenedioxy group can be reduced **(A)** or hydroxylated at either the ring substituent or the alkyl chain **(B) (Figure 1.10)**, with subsequent oxidization to the carboxylic acids. However, the pyrrolidine-type cathinones have distinct biotransformations. The pyrrolidinyl group can be hydroxylated and then further oxidized to form the 2”-oxo (lactam) metabolite **(C & D,**

CD); it can also open to form an aliphatic aldehyde which can be oxidized to a carboxylic acid **(E)**, or it can degrade to form a primary amine **(F) (Figure 1.10)** (97, 122, 159, 171). Alpha-PVP has been the most widely researched pyrrolidinophenone cathinone (98, 122, 159). The metabolism of MPBP (171), pyrovalerone (172, 173), and naphyrone (166) has also been investigated.

The ephedrine (reduced) form has been identified for all studied pyrrolidinophenone cathinones. Hydroxylation has occurred on the alkyl side chain and on the aromatic ring in α-PVP and on the naphthyl group in naphyrone (159, 166). Hydroxylation of the aromatic ring in α-PVP has not been confirmed in humans (159). 4-Hydroxymethyl metabolites have been identified for MPBP and pyrovalerone (171-173). The 2”-oxo metabolite has also been identified consistently, however, in one study, the reduced form was more abundant (159). In both rat and human studies, glucuronides and sulfates have been identified as phase II metabolites (122, 159). Cytochrome P450 isoenzymes involved in the metabolism of these cathinones include CYP2C19 and CYP2D6

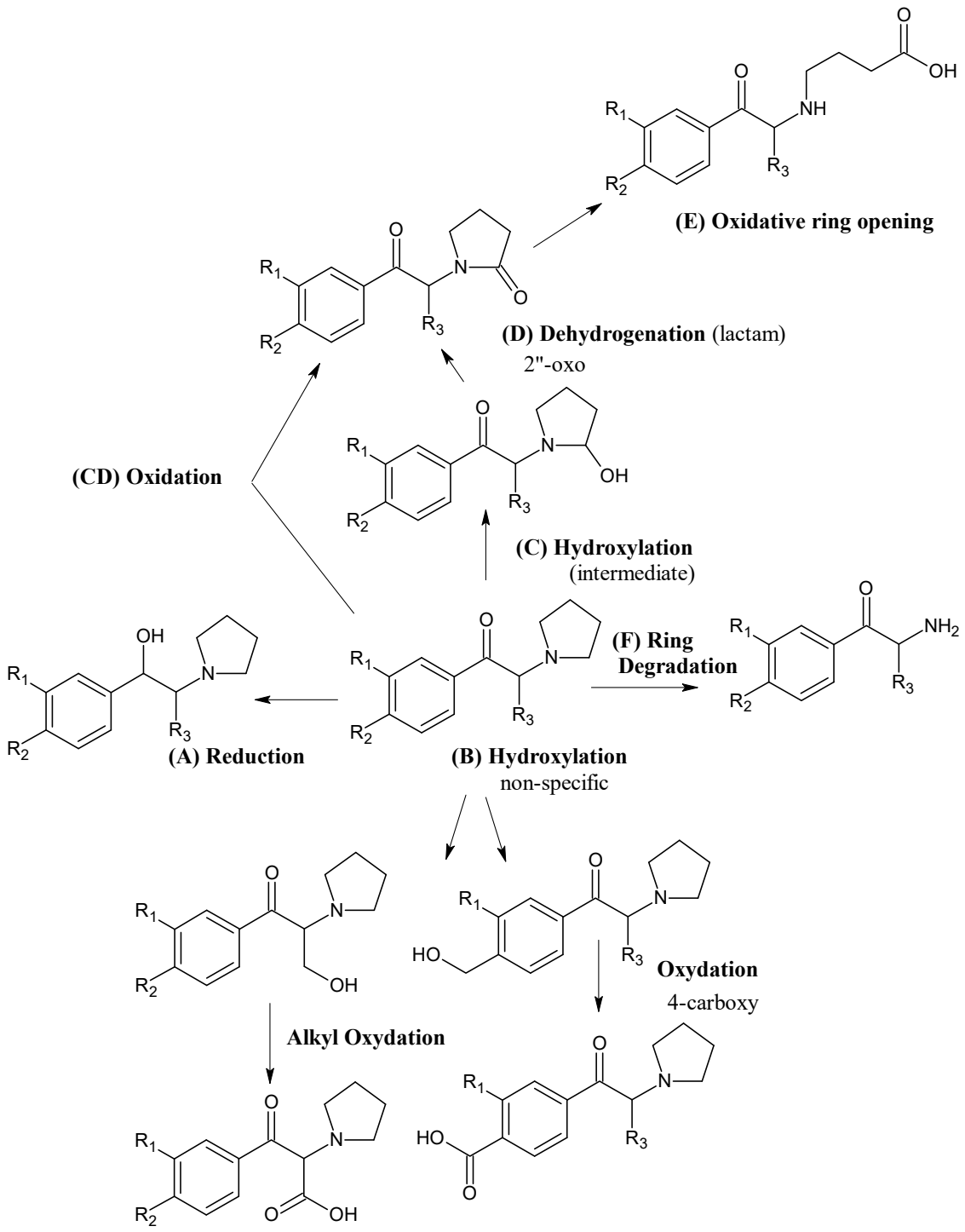


Figure 1.10. Metabolic pathway for pyrrolidine-type cathinones. Biotransformation (bold) and nomenclature identified for each pathway (97, 122, 159, 171).

Methylenedioxy-Pyrrolidinophenone Cathinones

The majority of research on metabolism of cathinones contain both the pyrrolidinyl and methylenedioxy group has been investigated using MDPV. Metabolic pathways for MDPV include reduction to the dihydro (ephedrine) form **(A)**; hydroxylation on the alkyl chain **(B)**; oxidation to form the 2”-oxo metabolite (through intermediate hydroxylation) **(B/C)**; ring degradation to a primary amine **(D)**; ring opening to carboxylic acid **(E)**; demethylenation to form catechol pyrovalerone **(F)**; and *O*-methylation by COMT to form either the 3- or 4-MeO metabolite **(G) (Figure 1.11)** (89, 107, 161, 163, 167). The most abundant metabolite is 4-OH-3-MeO pyrovalerone, similar to the methylenedioxy-type cathinones (161, 163).

Despite the identification of multiple metabolites *in vitro* and *in vivo*, it has been suggested that as much as 80% of MDPV is excreted unchanged (163). Unchanged MDPV has been identified *in vivo* along with its phase I and phase II metabolites (89). Phase II metabolism occurs through glucuronidation or sulfation of hydroxyl and carboxylic acids (161, 163). Demethylenation, the most common metabolic pathway, reportedly involves isoenzymes CYP1A2, CYP2C19, and CYP2D6 (161).

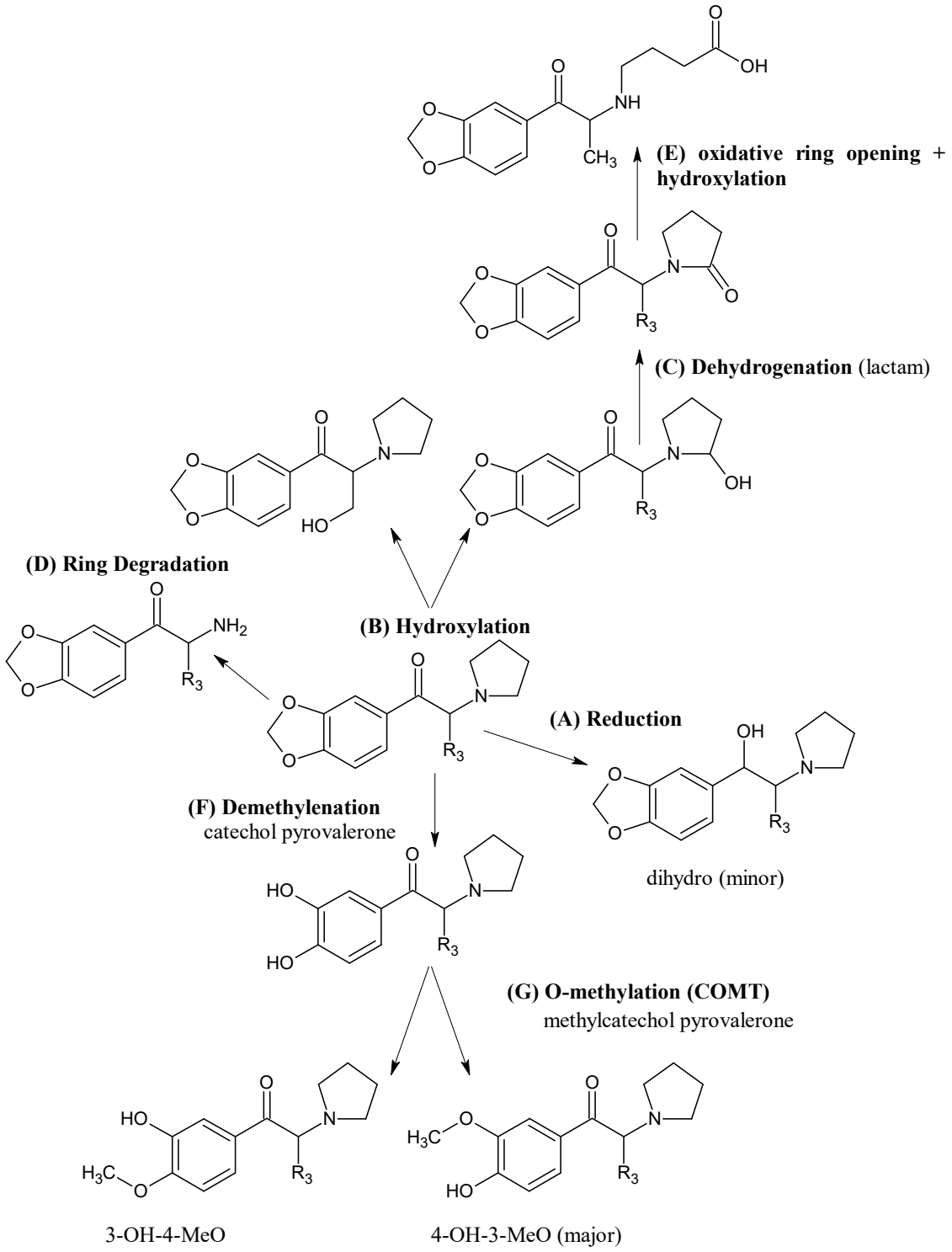


Figure 1.11. Metabolic pathway for pyrrolidine-type cathinones bearing the methylenedioxy group. Biotransformation (bold) and nomenclature identified for each pathway. (107, 161, 163).

Through *in vitro* and *in vivo* studies, metabolites for the different subgroups of cathinones have been proposed and structures elucidated **(Table 1.7)**. When elucidating structures for reduced or hydrolyzed metabolites, both diastereomers must be considered (97, 100, 122, 164). The reduction of the β-ketone or the addition of hydroxyl groups combined with the oxidation to a carboxylic acid makes these metabolites more acidic than the parent compound. Differences in acid-base properties may hamper isolation and detection of parent drug and metabolites. Another complication involves the formation of common metabolites, formed by more than one cathinone species (97, 166). This is especially apparent with *N*-dealkylation among the substituted cathinones and the combination of demethylenation and *N*-dealkylation in the methylenedioxy-type.

Table 1.7. Proposed metabolites for synthetic cathinones.

Cathinone	Metabolites	Metabolic Pathway	Formula	MW	Reference
Mephedrone $C_{11}H_{16}NO$ MW: 178	Hydroxytolyl-mephedrone	4-Me hydroxylation	$C_{11}H_{16}NO_2$	194	(90, 165)
	Normephedrone	*N*-demethylation	$C_{10}H_{14}NO$	164	(90, 165)
	Dihydro-mephedrone	Reduction	$C_{11}H_{18}NO$	180	(90, 165, 169)
	Nor-dihydro-mephedrone	*N*-dealkylation + Reduction	$C_{10}H_{16}NO$	166	(90, 165)
	4-Carboxy-dihydro mephedrone	Oxidation	$C_{11}H_{15}NO_3$	210	(90)
	Nor-hydroxytolyl mephedrone	Hydroxylation + *N*-dealkylation	$C_{10}H_{14}NO_2$	180	(90, 165)
	4-Carboxymethcathinone	Hydoxylation + Oxidation	$C_{11}H_{14}NO_3$	208	(165)
Methcathinone $C_{10}H_{13}NO$ MW: 164	Cathinone	*N*-dealkylation	$C_9H_{11}NO$	150	(167)
4-MEC $C_{12}H_{18}NO$ MW: 192	Hydoxyl-tolyl-MEC	4-Me Hydroxylation	$C_{12}H_{18}NO_2$	208	(168)
	4-Carboxy-MEC	Hydroxylation + Oxidation	$C_{12}H_{16}NO_3$	222	(168)
	Dihydro-4-MEC	Reduction	$C_{12}H_{20}NO$	194	(168)
	4-Carboxy-dihydro-4-MEC	Hydroxylation + Oxidation and Reduction	$C_{12}H_{18}NO_3$	224	(168)
	Nor-4-MEC	*N*-dealkylation	$C_{10}H_{14}NO$	164	(168)
	Nordihydro-4-MEC	*N*-dealkylation + Reduction	$C_{10}H_{16}NO$	166	(168)
3,4-DMMC $C_{12}H_{17}NO$ MW: 192	Dimethylcathinone	*N*-dealkylation	$C_{11}H_{15}NO$	178	(98, 100)
	Dihydro-3,4-DMMC	Reduction	$C_{12}H_{19}NO$	194	(98, 100)

(continued)

Cathinone	Metabolites	Metabolic Pathway	Formula	MW	Reference
	β-OH-DMC	Reduction + *N*-dealkylation	$C_{11}H_{17}NO$	180	(100)
	4-MeO-3-MeMC	Hydroxylation + Oxidation	$C_{12}H_{17}NO_2$	208	(100)
	3-MeO-4-MeMC	Hydroxylation + Oxidation	$C_{12}H_{17}NO_2$	208	(100)
3-FMC $C_{10}H_{12}FNO$ MW: 182	Dihydro-3-FMC	Reduction	$C_{10}H_{14}FNO$	184	(160)
	Nor-3-FMC	*N*-dealkylation	$C_9H_{10}FNO$	168	(160, 164)
	Nordihydro-3-FMC	Reduction + *N*-dealkylation	$C_9H_{12}FNO$	170	(160)
	Hydroxytolyl-3-FMC	Hydroxylation	$C_{10}H_{12}FNO_2$	198	(160, 164)
	Dihydro-hydroxytolyl-3-FMC	Hydroxylation + reduction	$C_{10}H_{14}$ F NO_2	200	(164)
Methylone $C_{11}H_{13}NO_3$ MW: 208	4-OH-3-MeO-MC (HMMC)	Demethylenation + *O*-methylation	$C_{11}H_{15}NO_3$	210	(90, 162, 170)
	3-OH-4-MeO-MC	Demethylenation + *O*-methylation	$C_{11}H_{15}NO_3$	210	(90, 162, 170)
	3,4-Dihydroxymethcathinone	Demethylenation	$C_{10}H_{13}NO_3$	196	(167)
	3,4-MDC	*N*-dealkylation	$C_{10}H_{11}NO_3$	194	(90, 162)
α-PVP $C_{15}H_{21}NO$ MW: 232	Dihydro-α-PVP	Reduction	$C_{15}H_{23}NO$	234	(98, 122, 159)
	2”-oxo-α-PVP	Oxidation	$C_{15}H_{19}NO_2$	246	(98, 122, 159)
	Pyrrodinyl degradation		$C_{11}H_{15}NO$	178	(98, 122, 159)
	Hydroxy-αPVP	Hydroxylation (aliphatic, aromatic)	$C_{15}H_{21}NO_2$	248	(98, 122, 159)
MPBP	Dihydro-MPBP	Reduction	$C_{15}H_{23}NO$	234	(171)

(continued)

Cathinone	Metabolites	Metabolic Pathway	Formula	MW	Reference
$C_{15}H_{21}NO$ MW: 232	2”-oxo-MPBP	Demethylenation + Hydroxylation	$C_{15}H_{19}NO_2$	246	(171)
	4-Me Hydroxylation	Hydroxylation	$C_{15}H_{21}NO_2$	248	(171)
MDPV $C_{16}H_{22}NO_3$ MW: 276	3-OH-3-MeO-MDPV	Demethylenation + *O*-methylation	$C_{16}H_{24}NO_3$	278	(107, 111, 161, 163, 167)
	4-OH-3-MeO-MDPV	Demethylenation + *O*-methylation	$C_{16}H_{24}NO_3$	278	(107, 108, 111, 161, 163, 167)
	2”-oxo-MDPV	Hydroxylation + Dehydrogenation	$C_{16}H_{22}NO_4$	290	(89, 107, 111, 161, 163)
	3,4-Catechol-pyrovalerone	Demethylenation	$C_{15}H_{22}NO_3$	264	(107, 108, 111, 167)
	Dihydro-MDPV	Reduction	$C_{16}H_{24}NO_3$	278	(61)
Naphyrone $C_{19}H_{23}NO$ MW: 282	Naphthyl hydroxylation	Hydroxylation	$C_{19}H_{23}NO_2$	298	(166)
	Alkyl chain hydroxylation	Hydroxylation	$C_{19}H_{23}NO_2$	298	(166)
	Dihydro-naphyrone	Reduction	$C_{19}H_{23}NO$	284	(166)
	Combination	Reduction + Hydroxylation	$C_{19}H_{25}NO_2$	300	(166)

Prevalence

Despite the severe adverse side effects that can occur with synthetic cathinone use, these drugs are still synthesized and distributed worldwide. Synthetic cathinones are being identified across the world in drug seizures and in both antemortem and postmortem toxicology casework. National and international controlled substance monitoring systems have documented a steady increase in use and derivative identification of these harmful drugs.

The National Forensic Laboratory Information System (NFLIS), sponsored by the Drug Enforcement Administration (DEA), compiles data from participating federal, state, and local laboratories regarding identifications in drug seizures. This data assists in monitoring drug trends across the United States (174-176). In 2009 there were only 34 reported cases involving five different synthetic cathinones: mephedrone, MDPV, methylone, methcathinone, and 4-MEC. By the end of 2010, there was an 18-fold increase in case reports, compared with the previous year. By 2015 almost 20,000 cases were reported **(Table 1.8)**. Each year, with the exception of 2015, the number of different cathinones identified in laboratories has increased. The top three synthetic cathinones identified over those six years were a combination of methylone, MDPV, α-PVP, ethylone, and mephedrone. In the 2013-2015 NFLIS report there was a notable decrease in the number of reported cases involving methylone (72% to 2.3% from 2013 to 2015, respectively) and an increase number in the reported ethylone cases (0.1% to 47% in 2013 to 2015, respectively). This shift could be the result of many factors, including increased legislation and monitoring of these substances, as well as precursor availability.

Table 1.8. Number of synthetic cathinone seizures and number of different cathinones identified through NFLIS from 2009 to 2015 (174-176).

Year	Number of Cases	Number of Different Cathinones
2009	34	5
2010	602	11
2011	6,542	21
2012	14,507	33
2013	16,811	33
2014	15,523	42
2015	19,490	35

Internationally, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has identified over 600 different novel psychoactive substances, with synthetic cathinones comprising the second largest group (177). Over 100 different synthetic cathinones have been reported, but only of a select few are monitored by EMCDDA, including methylone and mephedrone. EMCDDA reports the findings from twenty-two European countries and the most commonly identified cathinones in those countries are α-PVP, 3-MMC, ethylone, 4-CMC, and pentedrone. The most frequently identified cathinone varies from country to country, as it does from state to state within the US. Countries and states have performed their own analysis over the years to monitor cathinone trends.

In Italy, a review of 162 drug seizures from 2013-2015 revealed that the majority contained cathinones. The top three cathinones identified were 3-MMC, 4-MEC, and MDPV, two of which are not on EMCDDA most frequently identified list (178). A review of toxicology casework from January 2010 to December 2012 was conducted in a United Kingdom laboratory. Synthetic cathinones were identified in 203 cases, including

antemortem and postmortem specimens. Mephedrone and MDPV were the most common cathinones identified. Of the novel psychoactive groups investigated, synthetic cathinones were not frequently identified in fatalities. However, if a synthetic cathinone was identified in a fatality, it was most often a suicide, which supports suicidal thoughts or tendencies as an adverse effect as mentioned previously (179).

Similar studies were performed in the United States. Forrester identified 474 calls to Texas Poison Control from 2010-2011 involving synthetic cathinones and that the majority of the calls originated from East Texas, close to the Louisiana border (127). In 2013, Warrick performed an analysis of nine Midwestern states for synthetic cathinone cases reported to the National Poison Date System and identified 1,6233 cases. Cases identified per state and county varied, with Ohio and Kentucky having the highest number of reported cases (124). In 2014, Schneir *et. al.* performed a study identifying cathinones from 35 "bath salt" products purchased either online or storefronts. All packages contained warnings such as "do not consume" or "not intended for consumption." Of the 35 items purchased, 32 contained at least one synthetic cathinone, 17 of which contained multiple cathinones. These findings confirm the issues associated with ambiguous or intentionally misleading packaging, and the potential for additive effects. The most prevalent cathinones were MDPV, MDPBP, and methylone (135). An increasing number of seized materials containing synthetic cathinones would imply an increased demand for the product. This demand is reflected in numerous reports of synthetic cathinones to poison control centers and also in antemortem and postmortem toxicology casework.

Synthetic Cathinones in Toxicology Casework

Cathinones have been reported in a variety of antemortem and postmortem toxicology investigations (**Tables 1.9 & 1.10).** The most common antemortem casework involving cathinones involve intoxication, driving under the influence of drugs (DUID), and motor vehicular accidents. Synthetic cathinones have also been identified in drug facilitated sexual assault (DFSA) (93), child endangerment cases (180), and drug possession (181). Antemortem cathinone blood concentrations have ranged from 1.2 – 8,400 ng/mL. Concentrations in urine are much higher, ranging from 34 – 17,420 ng/mL. The majority of postmortem investigations involving synthetic cathinones have been accidental deaths, most often the result of drug intoxication or motor vehicle accidents. Cathinones have been linked to suicides (hanging) and homicides, and have also been identified in samples from natural deaths. Postmortem cathinone concentrations in blood ranged from 1.1 – 27,000 ng/mL.

A comparison of antemortem and postmortem toxicology results clearly shows that like many other drugs, recreational and fatal drug concentrations clearly overlap. The presence of the drug in a postmortem investigation does not necessarily imply that it caused or contributed to death. Among twelve fatalities involving α-PVP presented by Adamowicz, only one death was directly the result of α-PVP intoxication with a blood concentration of 6,200 ng/mL (182). Human performance and postmortem cases were also presented by Marinetti and Antonides. MDPV, pentylone, α-PVP, pyrovalerone, or multiple cathinones in combination with each other were identified. Of the twenty-three postmortem cases presented, ten were ruled accidental due to multiple drug intoxication. Two cases were the direct result of MDPV intoxication. In one case, a meaningful drug

concentration could not be determined due to body decomposition, but the other had a MDPV blood concentration of 91 ng/mL – considerably less than the case presented by Adamowicz (123). These two cases highlight the unpredictable nature of toxicity, due to tolerance. Cathinone concentrations in the blood of living subjects have been reported in excess of 1,000 ng/mL, however most fall below 500 ng/mL **(Table 1.9)**.

Many antemortem and postmortem cases were the result of poly-drug use, including other illicit drugs and/or prescription drugs. In many instances alcohol (54, 56, 94, 102, 115, 182, 183), THC, or synthetic cannabinoids (52, 89, 96, 109, 181, 182) were also identified. Other illicit substances found were methamphetamine (181), cocaine (54, 89, 114-116), MDMA (115), and heroin (55). The most commonly identified prescription drugs were benzodiazepines (66, 89, 102, 109, 111, 112, 115, 116, 184, 185). In several cases, poly-drug use was the result of multiple cathinones (87, 96, 103, 109, 113, 114). The presence of other drugs and the combination of multiple cathinones can further complicate a toxicologists' ability to interpret results and evaluate the pharmacological effects of the cathinone.

Understanding how a drug distributes throughout the body and is absorbed into the tissue can assist in blood concentration interpretation related to death investigations. There have been several postmortem cases that have examined tissue distribution in cathinone-related fatalities (53, 54, 57-59, 63, 67, 113, 118, 121, 123, 186). As expected, concentrations in the urine or stomach contents were higher than concentrations in the tissue or blood. Tissue distribution data for a fatal case involving ethylone was presented by McIntyre *et. al.* This study found that urine and gastric contents were 20,000 and 12,000

ng/mL, respectively, and blood concentrations for peripheral and central blood were 380 and 390 ng/mL, respectively (53).

Liver concentrations also had a tendency to be higher than blood concentrations, but lower than urine or gastric concentrations (53, 58, 63, 121, 123). Due to the liver's primary function in the body – the breakdown and removal of substances – it is not uncommon to observe higher drug concentrations. Distribution studies also provide insight regarding postmortem redistribution, which is discussed later.

Table 1.9. Antemortem cases involving synthetic cathinones.

Drug	Concentration	Investigation	Reference(s)
4-FMC	346 ng/mL (serum) 257 ng/mL (urine)	RDU	(146)
4-MEC	46 ng/mL (blood)	RDU	(181)
α-PVP	6.4-99 ng/mL (blood)	DUID (n=24)	(182)
	1.2-56 ng/mL (blood)	RDU (n=4)	(182)
	63 ng/mL (blood)	DUID	(187)
	230-360 ng/mL (blood)	DUID (n=2)	(147)
	70-100 ng/mL (blood)	RDU (n=2)	(184)
	1-53 ng/mL (serum)	RDU (n=9)	(120)
MDPV	306 ng/mL (blood)	RDU	(109)
	124 ng/mL (blood)	DUID	(109)
	75 ng/mL serum)	RDU	(29)
	<10 – 530 ng/mL (serum)	RDU (n=20)	(89)
	200-8,400 ng/mL (blood)	DUID (n=25)	(112)
	<10-368 ng/mL (blood)	DUID/RDU(n=9)	(123)
	186 ng/mL (serum) 136 ng/mL (urine)	RDU	(146)
	24-241 ng/mL (blood) (n=13) 34-1,386 ng/mL (urine) (n=5)	RDU (n=13)	(5)
	35-55 ng/mL (urine)	RDU (n=1, two time points)	(111)
	21.3-146 ng/mL (serum)	DUID (n=2)	(62)
	140 ng/mL (urine)	RDU	(110)
	40-3,900 ng/mL (urine)	RDU (n=9)	(50)
Mephedrone	80-660 ng/mL (blood)	DUID (n=9)	(188)
	150 ng/mL (serum)	RDU	(114)

(continued)

Drug	Concentration	Investigation	Reference(s)
	1-51 µg/kg (blood) (n=4) 560 µg/kg (urine) (n=1)	DUID (n=4)	(169)
	412 ng/mL (serum)	DUID	(62)
Methcathinone	500 ng/mL (serum), 17,420 ng/mL (urine)	RDU	(189)
Methylone	6 ng/mL (blood)	DUID	(187)
	7 ng/mL (blood)	DUID	(123)
Methedrone	0.2-5 µg/g (blood)	RDU	(94)
Ethylone	<10 ng/mL (blood)	DUID	(187)
Naphyrone	20-30 ng/mL (plasma)	RDU (n=1, two time points)	(190)

RDU: recreational drug use; DUID: driving under the influence of drugs

Table 1.10. Postmortem cases involving synthetic cathinones.

Drug	Concentration	Manner of Death	Reference(s)
3,4-DMMC	3,310 ng/mL (blood)	Accidental	(99)
	27,000 ng/mL (iliac blood) 7,600 ng/mL (urine)	Accidental	(66)
4-MEC	152 ng/mL (blood), 122 ng/mL (urine)	Accidental	(181)
	56 ng/mL (blood), 14,300 ng/mL (urine)	Accidental	(181)
	170-1,730 ng/mL (femoral blood)	Accidental	(102)
	1,200 ng/mL (femoral blood)	Accidental	(103)
α-PVP	1.1-6,200 ng/mL (blood)	Accidental, Suicide	(182)
	174 ng/mL (peripheral blood), 401 ng/mL (urine) 292 ng/g (brain) 122 ng/g (kidney) 190 ng/g (liver) 606 ng/g (gastric contents)	Accidental	(67)
	901 ng/mL (femoral blood) 2610 ng/g (liver) 462 ng/g (kidney) 120 ng/g (brain)	Accidental	(63)

(continued)

Drug	Concentration	Manner of Death	Reference(s)
	4,190 ng/g (stomach contents)		
	458 ng/mL (right heart blood) 442 ng/mL (left heart blood) 654 ng/mL (femoral vein blood) 11,200 ng/mL (urine) 1,030 ng/mL (stomach contents) 518 ng/mL (brain) 681 ng/ml (liver)	Accidental	(121)
	486 ng/mL (blood)	Accidental	(191)
Buphedrone	127 ng/mL (blood)	Accidental	(109)
	3-127 ng/mL (blood)	Accidental	(192)
Ethylone	390 ng/mL (peripheral blood), 380 ng/mL (central blood), 20,000 ng/mL (urine) 1.4 mg/kg (liver) 580 ng/mL (vitreous) 12,000 ng/mL (gastric contents)	Accidental	(53)
	<25-2,572 ng/mL (blood)	Accidental, Suicide, Homicide	(56)
MDPV	17-38 ng/mL (blood)	Accidental, Natural	(109)
	670 ng/mL (urine), 82 ng/mL (serum)	Accidental	(193)
	1,200 ng/mL (cardiac blood)	Accidental	(87)
	39-130 ng/mL (femoral blood), 760-3,800 ng/mL (urine)	Accidental	(88)
	38 ng/mL (blood)	Accidental	(192)
	32-576 ng/mL (serum)	Accidental	(89)
	470 ng/mL (heart blood) 0.53 mg/kg (liver) 0.49 mg/kg (kidney) 580 ng/mL (bile)	Accidental	(58)
	30 ng/mL (heart blood)	Homicide	(58)

(continued)

Drug	Concentration	Manner of Death	Reference(s)
	10-280 ng/mL (heart blood) (n=7) 31 ng/mL (cavity blood) 46-640 ng/mL (femoral blood) (n=7) 18-162 ng/mL (peripheral blood) (n=4) 14-940 ng/mL (vitreous) (n=13) 12-6,080 ng/g (liver) (n=8) 16-896 ng/g (brain) (n=6) 140-1,880 ng/mL (bile) (n=3)	Accidental Suicide Natural Homicide	(123)
	440 ng/mL (femoral blood) 500 ng/mL (heart blood) >5,000 ng/mL (urine) >2,000 ng/mL (gastric contents) 880 ng/mL (bile) 980 ng/g (liver) 120 ng/g (heart)	Accidental	(113)
	700 ng/mL (heart blood) 1,000 ng/mL (peripheral blood)	Accidental	(185)
	170 ng/mL (blood) 1,400 ng/mL (urine)	Accidental	(5)
	46 ng/mL (blood) 1,300 ng/mL (urine)	Accidental	(86)
	11 ng/mL (heart blood)	Not Specified	(64)
Mephedrone	5,500 ng/mL (blood), 7,100 ng/mL (vitreous)	Accidental	(115)
	60-2,100 ng/mL (blood)	Accidental, Suicide	(188)
	500 ng/mL (blood), 198,000 ng/mL (urine)	Accidental	(55)
	1,330 ng/m/L (heart blood), 144,000 ng/mL (urine) 4,520 ng/mL (gastric content) 1,290 ng/mL (bile) 0.79 mg/kg (lung) 0.89 mg/kg (brain)	Accidental	(54)

(continued)

Drug	Concentration	Manner of Death	Reference(s)
	5,100 ng/mL (femoral blood), 186,000 ng/mL (urine), 1.04 g/L (stomach contents)	Accidental	(116)
	1,200-22,000 ng/mL (blood)	Accidental	(92)
	130-2,240 ng/mL (femoral blood)	Accidental	(183)
	14,800 ng/mL (urine) 500 ng/mL (heart blood) 1,900 ng/mL (bile) 38,000 ng/mL (gastric contents)	Accidental	(57)
	2-3,300 ng/mL (blood)	Accidental, Suicide	(130)
	1,300 ng/mL (femoral blood)	Accidental	(103)
	1,097 ng/mL (peripheral blood)	Not Specified	(45)
Methylone	3,400 ng/mL (iliac blood), 3,400 ng/mL (central blood) 4,300 ng/mL (vitreous) 11 mg/kg (liver) 1.7 mg (gastric contents)	Accidental	(52)
	560-3,300 ng/mL (peripheral blood) (n=3) 580-1,000 ng/mL (heart blood) (n=2) 0.88 mg/kg (liver) (n=1) 920-1,400 ng/mL (vitreous) (n=2) 550-230,000 ng/mL (urine) (n=2) 4.5-12 mg/kg (gastric contents) (n=2)	Accidental	(59)
	500 ng/mL (peripheral blood), 39,770 ng/mL (urine) 6,420 ng/mL (bile) 1.47 mg/kg (liver) 0.54 mg/kg (heart) 1.26 mg/kg (kidney)	Accidental	(186)

(continued)

Drug	Concentration	Manner of Death	Reference(s)
	729 ng/mL (heart blood)	Accidental	(123)
	740 ng/mL (central blood) 670 ng/mL (peripheral blood) 38,000 ng/mL (urine) 1.8 mg/kg (liver) 2.3 mg/kg (kidney) 2.1 mg/kg (spleen) 1,800 ng/mL (bile)	Accidental	(58)
	110 ng/mL (heart blood) 200 ng/mL (urine) 0.55 mg/kg (liver) 0.26 mg/kg (kidney) 520 ng/mL (bile)	Suicide	(58)
	60 ng/mL (heart blood) 0.14 mg/kg (liver) 0.16 mg/kg (kidney) 420 ng/mL (bile)	Accidental	(58)
	1,100 ng/mL (heart blood) 220 ng/mL (urine) 1.3 mg/kg (liver) 0.91 mg/kg (kidney)	Homicide	(58)
	3,130 ng/mL (peripheral blood) 6,640 ng/mL (heart blood) 502,000 ng/mL (urine) 35,300 ng/mL (bile) 57,300 ng/mL (gastric contents) 5,040 ng/mL (vitreous)	Accidental	(118)
	700 ng/mL (blood)	Accidental	(95)
	272 ng/mL (blood) 387 ng/g (liver)	Accidental	(194)
	63 ng/mL (heart blood)	Not specified	(64)
Pentedrone	8,794 ng/mL (femoral blood) 100,044 ng/g (liver) 22,102 ng/g (kidney) 13,248 ng/g (brain) 500,534 ng/g (stomach contents)	Accidental	(63)

(continued)

Drug	Concentration	Manner of Death	Reference(s)
	600 ng/mL (blood)	Accidental	(182)
	160 ng/mL (blood) 12,000 ng/mL (urine)	Accidental	(86)
Pyrovalerone	42 ng/mL (femoral blood), 59 ng/mL (heart blood) 24 ng/mL (vitreous) 124 ng/g (liver) 48 ng/g (brain) 70 ng/mL (bile)	Accidental	(123)
MDPBP	9,320 ng/mL (blood)	Accidental	(85)
Methedrone	8.4-9.6 µg/g (femoral blood)	Accidental	(94)
Ethcathinone	5-83 ng/mL (blood)	Accidental, Suicide	(195)
Butylone	20,000 ng/mL (femoral blood) 33 mg/kg (liver)	Suicide	(105)
Methcathinone	210 ng/mL (femoral blood)	Accidental	(105)

Postmortem Redistribution

During postmortem toxicology investigations it is common to determine quantitative values for drugs in multiple specimens. Concentrations reported in case studies significantly increase the knowledge base associated with these emerging drugs. Summarized above are reported postmortem cathinone concentrations in blood, urine, and various tissues **(Table 1.10)**. When interpreting postmortem results, it is important to consider the potential for postmortem redistribution (PMR), or the movement of drugs between tissues and fluids after death (196). Postmortem redistribution has been described as a "toxicological nightmare" because of the interpretive challenges it presents (197). When interpreting antemortem blood and tissue drug concentrations, certain assumptions can be made based upon known physico-chemical properties of the drug. However, these assumptions do not always hold true for postmortem drug concentrations (198). Factors

that effect a drugs' PMR potential have been thoroughly documented and reviewed, but are still not yet thoroughly understood (196, 197, 199-202).

Postmortem redistribution is often characterized by elevated drug concentrations in blood collected from central compartments (e.g. heart blood), relative to those collected peripherally (e.g. femoral vein). The movement of drugs between tissues, organs, and body fluids after death following the disintegration of chemical and anatomical barriers is quite complex (197, 198, 202). Drug movement is driven by the accumulation of drugs in certain organs, changes at the cellular level, and exposure to the environment. Physico-chemical properties of the drug, including volume of distribution (Vd), lipophilicity, pKa and protein binding can influence PMR potential.

Fundamental drug properties determine where a substance will be absorbed and accumulate within the body. These properties include pKa, volume of distribution (Vd), and protein binding affinity. In order to be efficiently absorbed through passive diffusion and produce a pharmacological effect, a drug must be unionized and 'free' or unbound to plasma or tissue proteins. Drug pKa and body cavity pH will determine ionization state and where a drug will be most efficiently absorbed. pH gradients within the body can lead to drug accumulation for charged species, known as "ion trapping". Plasma protein binding affinity (% binding) varies, and drugs which are highly protein bound may distribute less readily. Drugs can also bind to proteins within the tissue, which results in drug accumulation within the tissue itself. The combination of pKa and protein binding will influence a drug's overall Vd. Drugs with higher Vd will predominately distribute into tissue and muscle. Typically, drugs with Vd >3-4 exhibit PMR, however, there are many

exceptions (203, 204). Drugs with higher volume of distribution and potential for ion trapping within cells can be problematic for postmortem interpretation.

After death, the body undergoes changes at a cellular level (196, 198, 205). The changes arise from the depletion of oxygen leading to the cessation of ATP production. As a result, lactic acid builds up, decreasing intracellular pH and sodium concentrations. As the ionic potential increases, the overall integrity of the cell structure becomes compromised. Cellular edema occurs to dilute the sodium concentration (204, 206). Drugs with high pKa values become increasingly ionized in the acidic conditions and remained trapped within the cell. As the integrity of organelle membranes decline, particularly that of lysosomes, autolytic enzymes are released into cytoplasm and cell lysis begins (204, 206). As the cell membrane breaks down, ionized drugs that once relied on active diffusion to cross into a cell now leach out and through passive diffusion move from areas of high concentration to low concentration.

Concentration changes are most significant around certain organs, specifically the gastrointestinal (GI) tract, lungs, and liver. Due to the close proximity of these organs within the torso, drug movement between these organs has been well documented (197, 202, 204, 207). Oral (and intranasal) administration results in high drug concentrations within the stomach and GI tract. After death, drug movement can occur from the GI tract into the cardiac chambers, aorta, left lung, and left lobe of the liver (204, 207). Aspirated stomach contents can also lead to drug movement into the lungs. From the lungs, drugs have been shown to transverse the alveoli into the aorta, left cardiac chamber, and to some extent, the liver (197, 203, 204, 207). Drug concentrations tend to be higher in the liver due to its metabolic and elimination functions. Drugs in the liver redistribute through the

hepatic vein into the inferior vena cava or through passive diffusion into surrounding organs (198, 204, 207). Much of the drug movement that takes place within the thoracic cavity results in artificially elevated concentrations in central blood sources. Subsequent central blood analysis can result in concentrations that are not representative of drug concentrations at time of death, leading to erroneous conclusions.

Drug concentrations can also be altered during putrefactive processes and the introduction of exterior bacteria. As the body decomposes, internal bacteria that was isolated in the GI tract are released and exterior bacteria are introduced. These bacteria break down proteins within the blood, releasing protein bound drugs and increasing free drug concentration (206). As putrefaction occurs, blood will coagulate and thin out, causing changes in blood composition and drug concentration (198, 207). Livor mortis – the gravitational pooling of blood in the body – will also effect blood movement and drug concentrations (198, 204). With several factors influencing postmortem drug movement and producing site-to-site variation, postmortem toxicological analysis is challenging.

To obtain a representative drug concentration at time of death, multiple specimens should be collected, including blood samples from a central source and peripheral source (197, 202). Central blood would be an ideal source due to its large volume; however, it most susceptible to PMR and contamination from internal injuries. Although limited in volume, there are multiple sites from which peripheral blood can be drawn, but the most common and preferred location is the femoral vein (197, 207). A peripheral source is less likely to be effected by PMR or contamination due to its distance from the central cavity. However, there have been some documented cases where peripheral concentrations increased postmortem (199, 208). In order to ensure no contamination from other blood

sources is present, ideally the vein should be visualized and ligated prior to collection (202, 207, 209). However, postmortem sample collection is not standardized; this additional step is often not completed and peripheral blood sources may vary across jurisdictions. The benefit of obtaining specimens from these two independent blood sources is the cardiac to peripheral blood (C/P) ratio, which is useful in predicting PMR potential.

A C/P ratio greater than 1 is often indicative of postmortem redistribution (202, 210). Early studies documented that basic drugs, such as digoxin and tricyclic antidepressants, are more likely to exhibit PMR. In a study done by Dalpe-Scott *et. al.,* C/P ratios for 113 drugs were determined to assess PMR potential. Based upon high C/P ratios, drugs that were most likely to exhibit PMR were basic with a large volume of distribution. However, calculated C/P ratios for a single drug varied (202). Drugs with C/P ratios of 2.4 or higher were considered to exhibit PMR. These included amitriptyline, propoxyphene, doxepin, and chlorpromazine, all with Vd values greater than 6 L/kg. In a more recent study, Han *et. al.* investigated PMR potential for 76 commonly encountered drugs and found that many, including amitriptyline, codeine, diazepam, diphenhydramine, ketamine, lidocaine, propofol, and zolpidem may exhibit PMR, characterized by C/P ratios of 1.2 or higher (210). Zolpidem, with a Vd of 0.6, had a high C/P ratio of 3.74, making it an exception to the notion that drugs with low Vd will not exhibit PMR. These studies show C/P ratios are a good indicator of whether a drug exhibits PMR and should be considered when interpreting drug concentrations. While C/P ratios provide insight about a drug's PMR potential, it is not the only indicator.

Another approach involves the liver to peripheral blood (L/P) ratio (53, 197, 201, 211). L/P ratios for drugs that are known to exhibit PMR are reported to exceed 20, whereas

drugs that do not are often less than 5, allowing for easy differentiation of drugs that exhibit PMR, from those that do not (53, 211). However, caution should be taken when interpreting liver concentrations because the liver can also show concentration changes as a result of PMR. Pounder *et. al.* determined that over time the left side of the liver (closest to the stomach) can have artificially elevated drug concentrations due to drug presence in the stomach. They recommended obtaining liver samples from within the right lobe of the liver to avoid contamination from surrounding tissues (197, 209). Although both C/P and L/P ratios have been proposed, conclusions should not be drawn from single cases. This is evidenced by the wide range of values in multiple studies, and the potential for time-dependent effects, as well as site-dependent variation (199, 202, 210).

Time of sample collection after death is an additional factor that complicates postmortem toxicology interpretation. The postmortem interval between time of death and sampling can impact drug concentration (196, 198, 212). Bacteria present during putrefaction can degrade drugs, therefore lowering concentrations. Drug stability also can significantly impact concentration at time of analysis (199, 201). As postmortem blood becomes more acidic after cell lysis, acid-labile drugs may degrade disproportionately, while others are preserved. Conditions and elements the body may be exposed to immediately after death can also cause drug concentration to decrease through evaporation or degradation (199, 212). Examples include amphetamine and methamphetamine. These drugs have been shown to be unstable and degrade in postmortem samples. They also exhibit PMR (199, 202, 212, 213). With methamphetamine and amphetamine being closely related to synthetic cathinones, information about these drugs can provide insight into the postmortem redistribution potential of new designer drugs.

Methamphetamine and MDMA are structurally similar to synthetic cathinones, with methamphetamine and MDMA being most similar to methcathinone and methylone, respectively. MDMA and methamphetamine are basic drugs (pKa 9.9) with relatively large volumes of distribution (Vd, 3 to 7 L/kg) (17, 214). Plasma protein binding is approximately 65% for MDMA and 10-20% for methamphetamine (17, 214, 215). These physico-chemical properties would indicate that both drugs exhibit some degree of PMR, with MDMA exhibiting more than methamphetamine due to the high percentage of drug released as proteins degrade and bound drugs are released. PMR potential of these drugs has been supported by tissue distribution studies (199, 211, 213, 216-218). Barnhart *et. al.* examined PMR potential in 20 cases involving methamphetamine and discovered that in every case, the peripheral blood concentration was lower than central blood. In five cases, cardiac muscle was available and the concentration of the cardiac muscle was higher than both peripheral and central blood, indicating the elevated central blood could be the result of redistribution of methamphetamine from the cardiac tissue (213). The average C/P ratio for the 20 cases was 2.2. Further investigation of PMR of methamphetamine examined results of 18 methamphetamine positive cases containing peripheral blood, central blood, vitreous humor, and liver samples (211). Almost all cases had an equal or higher concentration in central blood compared to peripheral blood, with the exception of one case, where central blood was 0.25 mg/L and peripheral was 0.26 mg/L. This is concordant with the previous study. McIntyre *et. al.* also investigated the relationship of liver and vitreous concentration to peripheral blood. Both matrices exhibited higher methamphetamine concentrations than peripheral blood. They concluded that methamphetamine does exhibit PMR and that vitreous fluid or liver may be a viable sample

source if blood is unavailable (211). The average C/P and L/P ratios for these 18 cases were 1.6 and 5.7, respectively. Based on these two studies with large sample sizes, methamphetamine exhibits PMR due to drug diffusion from cardiac tissue to central blood sources.

De Letter *et. al.* performed comprehensive analysis on numerous samples obtained from MDMA positive cases (216-218). They noted higher concentrations of MDMA in samples obtained from the central cavity, blood and tissue, rather than peripheral sources. The suspected sources that contributed to the elevated concentrations in the central cavity were the lungs, stomach contents, and liver. They recommended caution and to avoid sampling in areas adjacent to those tissues. C/P and L/P values that could be extrapolated from their data ranged from 1.4 to 4.2 and 7.7 to 8.5, respectively (218). C/P and L/P ratios for MDMA and methamphetamine are listed in **Table 1.11.** These high ratios suggest that MDMA would exhibit PMR. Given the structural similarity of the synthetic cathinones with both MDMA and methamphetamine, it is entirely possible that some drugs within the class are susceptible to PMR.

Table 1.11. C/P and L/P ratios for amphetamine, methamphetamine, and MDMA.

Drug	Cause of Death	Matrix (Concentration)	C/P	L/P (L/kg or mL/g)	Reference
Amphetamine	Drug Intoxication: Fatal Ingestion	PB: 0.4 mg/L CB: 0.7 mg/L L: 0.9 mg/kg	1.6	2.0	(219)
Amphetamine (n=17)	Not Specified	PB: 0.02 – 218 mg/L CB: 0.02 – 0.3 mg/L L: 0.1 – 0.9 mg/kg	0.5 – 2.5, Avg: 1.6	2.8 – 14.9, Avg: 8.1	(211)
Methamphetamine	Drug Intoxication: Fatal Ingestion	PB: 54 mg/L CB: 66 mg/L L: 91 mg/kg	1.2	1.7	(219)
Methamphetamine (n=18)	Not Specified	PB: 0.2 – 1.7 mg/L CB: 0.3 – 2.4 mg/L L: 0.9 – 12 mg/kg	0.9 – 2.4, Avg: 1.6	1.9 – 9.1, Avg: 5.7	(211)
Methamphetamine (n=20)	Not Specified	PB: 0.14 – 4 µg/mL CB: 0.04 – 9 µg/mL	1.2 – 5.8, Avg: 2.2	N/A	(213)
MDMA	Not Specified	TB: 0.5 mg/L Left arm blood: 0.5 mg/L	0.9	N/A	(220)
MDMA	Hyperpyrexia	FB: 2.3 mg/L JB: 3.0 mg/L	1.3	N/A	(220)
MDMA	Not Specified	Left FB: 7.3 mg/L Right FB: 6.2 mg/L HB: 28	L: 3.9 R: 4.6	N/A	(220)

(continued)

Drug	Cause of Death	Matrix (Concentration)	C/P	L/P (L/kg or mL/g)	Reference
MDMA	Drug Intoxication	FB: 1,129 µg/L IVCB: 1,801 µg/L L: 8,904 µg/kg	1.6	7.9	(216)
MDMA	Drug Intoxication	HB: 11 mg/L FB: 2.8 mg/L L: 20 mg/kg	3.9	7.2	(221)
MDMA	Suicide: Hanging	FB: 0.6 mg/L L: 1.8 mg/kg	N/A	3.1	(221)
MDMA	Not Specified	FB: 13 µg/mL Right atrial blood: 57 µg/mL L: 104 µg/g	4.2	7.7	(217)
MDMA	Not Specified	FB: 3.1 µg/mL L: 26 µg/g AB: 4.4 µg/mL	1.4	8.5	(218)

Aorta blood (AB); Femoral blood (FB); Liver (L); Inferior vena cava blood (IVCB); Heart blood (HB); Central blood (CB); Peripheral blood (PB); N/A: not applicable

Physico-chemical properties including Vd, pKa and protein binding are unknown for many synthetic cathinones. **Table 1.12** summarizes the known pKa values for ten cathinones (17). Despite their structural similarity they span almost two pH units (pKa, 7.2 to 8.9). Even less is known concerning the volume of distribution, and protein binding has only been documented for a few synthetic cathinones: methylone (30%), mephedrone (22%) (18, 222). The absence of this information further complicates the prediction of PMR.

Table 1.12. Reported pKa values of synthetic cathinones (17).

Cathinone	pKa
Butylone	7.7
Ethylone	7.8
Mephedrone	8.1
Methcathinone	8.9
Methedrone	7.5
MDPV	8.4
Methylone	7.7
Pentedrone	7.2
Pentylone	8.6
Pyrovalerone	8.2

From fatality case studies reporting tissue distribution, C/P and L/P values can be calculated for select synthetic cathinones **(Table 1.13)**. Marinetti and Antonides reported C/P values in five of their postmortem cases, where four of the cases involved MPDV and the fifth involved pyrovalerone. Cause of the death included drug intoxication, suicide by hanging, and natural causes. C/P values for MDPV ranged from 1.3 to 1.7, with an average of 1.5. The case involving pyrovalerone was accidental, with a C/P value of 1.4 (123). Liver concentrations were also reported for many cases. Seven cases included a peripheral blood source and liver sample, which allowed for L/P values to be estimated. Six of the cases involved MDPV. MDPV L/P ratios ranged from 2.5 to 23, with an average L/P ratio

of 9.9. The seventh case involved pyrovalerone, which had an L/P ratio of 2.9. McIntyre discussed C/P and L/P ratios in their two cases studies involving methylone and ethylone. The C/P and L/P ratios for ethylone were 1.0 and 3.6, respectively (53). The C/P and L/P ratios for methylone were 1.0 and 3.2, respectively (52). Based on the C/P ratios from these cases, MDPV, methylone, ethylone and pyrovalerone should exhibit minimum post mortem redistribution. With the exception of MDPV, the L/P ratios are <5, indicating there is minimum potential for PMR.

Although C/P and L/P ratios were not discussed, additional studies reported central blood, peripheral blood, and liver concentrations for α-PVP, methylone, MDPV, and pentedrone. (58, 59, 63, 67, 113, 121, 186). The estimated C/P and L/P ratios from an additional study involving MDPV and methylone are in concordance with the aforementioned studies (58, 59, 113, 186). The three studies investigating α-PVP related fatalities had similar L/P ratios, ranging from 1.0 to 2.9 (63, 67, 121). Only one case reported central and peripheral blood concentrations for α-PVP (C/P ratios of 1.4 using right heart blood and 1.5 using left heart blood from the same individual) (121). Both C/P and L/P ratios indicate that α-PVP, like the other cathinones, had a minimal potential for PMR. The last case study involved pentedrone, and only an L/P ratio could be calculated, which was 11 (63). This value is between the guidelines for L/P ratio interpretation proposed by McIntyre. More importantly, conclusions drawn from a single case report have limited value, for the reasons described earlier. Existing studies indicate that the potential for this group of novel psychoactive substances to redistribute is minimal based on C/P ratios less than 2 and, on average, low L/P ratios. However, more research is needed.

Table 1.13. C/P and L/P ratios for synthetic cathinones.

Cathinone	Cause of Death	Matrix (Concentration)	C/P	L/P (L/kg)	Reference
MDPV	Drug Intoxication	PB: 162 ng/mL HB: 280 ng/mL L: 3,720 ng/g	1.7	23	(123)
	Drug Intoxication	PB: 18 ng/mL HB: 28 ng/mL L: 52 ng/g	1.6	2.9	(123)
	Drug Intoxication	FB: 129 ng/mL L: 388 ng/g	N/A	3.0	(123)
	Suicide	PB: 102 ng/mL HB: 133 ng/mL L: 256 ng/g	1.3	2.5	(123)
	Natural	PB: 36 ng/mL HB: 56 ng/mL L: 668 ng/g	1.6	19	(123)
	Suicide	FB: 640 ng/mL L: 6,080 ng/g	N/A	9.5	(123)
	Drug Intoxication	FB: 440 ng/mL HB: 500 ng/mL L: 980 ng/g	1.4	2.2	(113)
	Drug Intoxication	HB: 0.7 mg/L PB: 1.0 mg/mL	0.7	N/A	(185)

(continued)

Cathinone	Cause of Death	Matrix (Concentration)	C/P	L/P (L/kg)	Reference
Pyrovalerone	Autoerotic Asphyxia	FB: 42 ng/mL HB: 59 ng/mL L: 124 ng/g	1.4	3.0	(123)
α-PVP	Drug Intoxication	PB: 174 ng/mL L: 190 ng/g	N/A	1.1	(67)
	α-PVP Poisoning	Right HB: 458 ng/mL Left HB: 442 ng/mL FB: 654 ng/mL L: 681 ng/g	R: 1.4 L: 1.5	1.0	(121)
	Drug Intoxication	PB: 901 ng/mL L: 2,610 ng/g	N/A	2.9	(63)
Methylone	Drug Intoxication	PB: 0.50 mg/L L: 1.47 mg/kg	N/A	2.9	(186)
	Drug Intoxication	PB: 0.84 mg/L HB: 1.0 mg/L	1.2	N/A	(59)
	Drug Intoxication	PB: 0.56 mg/L HB: 0.58 mg/L L: 0.88 mg/kg	1.0	1.6	(59)
	Drowning	PB: 3.4 mg/L CB: 3.4 mg/L L: 11 mg/kg	1.0	3.2	(52)
	Drug Intoxication	PB: 0.67 mg/L CB: 0.74 mg/L L: 1.8 mg/kg	1.1	2.7	(58)

(continued)

Cathinone	Cause of Death	Matrix (Concentration)	C/P	L/P (L/kg)	Reference
	Drug Intoxication	PB: 3.13 mg/L CB: 6.64 mg/L	2.1	N/A	(118)
Pentedrone	Drug Intoxication	PB: 8,794 ng/mL L: 100,044 ng/g	N/A	11	(63)
Ethylone	Drug Intoxication	PB: 0.39 mg/L CB: 0.38 mg/L L: 1.4 mg/kg	1.0	3.6	(53)
Butylone	Drug Intoxication	FB: 20 mg/L L: 33 mg/kg	N/A	1.7	(105)

N/A: Not applicable

Cathinone Stability

In order to properly interpret toxicological results, the stability of the suspected drug should be understood. Qualitative and quantitative analysis of a sample reveals the presence and concentration of a drug at the time of analysis, not necessarily at the time of interest. This has important consequences for both antemortem and postmortem investigations, whereby times of interest may be the time of an alleged sexual assault or driving, versus the concentration immediately prior to death. The time between collection and analysis can differ by days, weeks, or months and may have significant implications. During that time, many factors can cause the concentration of a drug to change significantly, or result in a negative or inconclusive finding. These factors include exposure to unfavorable (elevated) temperatures, specimen pH or the biological matrix, and the chemical properties of the drug. Certain functional groups, those with a tendency to become oxidized or reduced, result in a more unstable drug. The stability of common illicit drugs is widely understood (223), however this information is relatively limited for novel psychoactive substances, including synthetic cathinones.

Non-biological Stability

The instability of cathinone, the natural precursor to synthetic cathinones, has been documented since the early 1980s. Szendrei was the first to characterize the stimulant component in the *khat* plant, reporting that cathinone was highly unstable in the presence of oxygen and in alkaline conditions (224). In 1981, Berrang *et. al.* determined that cathinone would dimerize or racemize unless stabilized by an acid, further indicating that cathinone was unstable in basic conditions (225). The instability of cathinone in basic and oxygenated conditions had prevented its isolation and identification until the 1980s, despite

khat being studied years earlier. In order to isolate and identify cathinone, these two studies used fresh plant material that had been freeze dried and frozen until analysis. Thirty years later, in 2010, Chappell and Lee reported that cathinone converted to cathine, the less psychoactive component, during the drying process. In order to prevent that conversion, the harvested plant material should be air-dried or freeze-dried and stored at ambient or cold temperatures. Once dried and stored, cathinone was detectable over 10 years in the plant material, with a 2-3% loss a year when stored at ambient temperature (226).

Like their precursor, synthetic cathinones as a hydrochloric salt powder, are also highly unstable when exposed to oxygen. This was demonstrated by Tsujikawa *et. al.* using ten cathinone derivatives: α-pyrrolidinoheptanophenone (α-PHPP), α-PVP, α-pyrrolidinobutiophenone (α-PBP), α-pyrrolidinopropiophenone (α-PPP), 4-fluoro-α-pyrrolidinovalerophenone (4-F-PVP), 4-methyoxy- α-pyrrolidinopropiophenone (4-MeO-PVP), pyrovalerone, MDPV, *N*-ethylpentedrone, and pentedrone. They concluded that decomposition of the powder occurred mainly at the surface. Furthermore, using α-PHPP, two decomposition products were identified: 2”-oxo- α-PHPP and α-PHPP-*N*-oxide. From the ten synthetic cathinones, conclusions were drawn regarding stability as it related to the various functional groups, including alkyl chain length, benzene ring substituents, and secondary or tertiary amine. After the powders had been exposed to air for 24 hours, the two synthetic cathinones with the lowest residual ratio (<10% remaining) were α-PHPP (a tertiary amine cathinone with a seven-carbon alkyl chain, and no benzene ring substituent) and pyrovalerone (a tertiary amine cathinone with a five carbon alkyl chain and methyl group on the benzene ring). The synthetic cathinone with the least amount of decomposition (61% residual ratio) was pentedrone, a *N*-methylated cathinone with a five-

carbon alkyl chain and no benzene ring substituent. The remaining seven synthetic cathinones had residual ratios between 20 and 32%. It was concluded that structure was related to the stability of these drugs and tertiary amine cathinone appear to be more liable when exposed to oxygen (227).

Tsujikawa *et. al.* also investigated the stability of synthetic cathinones at various pH in aqueous solution. Seven synthetic cathinones were evaluated, including mephedrone, 4-FMC, 3-FMC, 2-FMC, 4-methyoxymethcathinone (4-MeOMC), ethcathinone, and *N,N*-dimethylcathinone (DMC). The synthetic cathinones were stored in aqueous buffers at pH 4, 7, 10, and 12 at ambient temperature. All seven were highly unstable in basic pH solutions (<50% remaining after 12 hours of storage with the exception of 4-MeOMC and DMC). The most stable was DMC, with over 90% remaining after 12 hours of storage at pH 12 and the least stable was 2-FMC, with less than 10% remaining at pH 7. All had over 99% remaining in the pH 4 solution. Adding an antioxidant (*l*-ascorbic acid or sodium sulfite) to the pH 12 solution significantly decreased the amount of degradation that occurred, indicating the presence of oxygen in the solution contributed to degradation of these drugs (228). Maskell *et. al.* investigated the stability of mephedrone in formalin (5, 10, and 20%) at various pH (pH 3.5, 7 and 9.5) over 28 days. Mephedrone was highly unstable at pH 7 and 9.5, with less than 20% remaining by day 3 in both solutions. Due to the unstable nature of mephedrone at basic pH, influence of formalin concentration was insignificant. However, at acidic pH, where mephedrone has shown to be stable, the presence of formalin resulted in degradation (229).

Biological Stability

After the identification of cathinone in the 1980s, analysis of cathinone and its derivatives in biological fluids did not progress until the early 2000s. Many of the stability studies were incorporated into the cathinone method validations using GC or LC. As early as 1989, Morad *et. al.* determined that cathinone was unstable during the extraction process of plasma for an HPLC analysis method. To preserve cathinone during the extraction process, it was necessary to acidify the solvents prior to evaporation (230). In the early 2000s, Paul and Cole investigated the stability of methcathinone and cathinone in urine during the development of their GC-MS detection procedure. Specimens were stored at 2-4°C and -18°C for three months. Losses of almost 80% were observed when stored at 2-4°C for three months, and significant losses were observed by day four. Significant losses were observed after two months of storage at -18°C. They also noted that oxidizing agents should not be used during the extraction process or cathinones will be oxidized to benzoic acid (30).

Concheiro *et. al.* developed a LC-HRMS method for the quantification of 24 synthetic cathinones and four metabolites in urine using a Q-Exactive™ mass spectrometer. Unpreserved urine (pH 7.6) was fortified with the 28 analytes at two concentrations (3 and 300 ng/mL) and stored at room temperature for 24 hours and 4°C for 72 hours. Overall, losses were comparable between the low and high concentration at each storage temperature and time. All were stable when stored at 4°C for 72 hours, except benzedrone (-27% difference). However, instability was observed for over half of the analytes after 24 hours of storage at room temperature, with significant losses observed over all defined analyte groups. While significant losses were observed in all groups, the

majority of pyrrolidinyl-substituted cathinones and metabolites were stable. The authors concluded that the presence of the pyrrolidine group and the conversion of the β-ketone to a hydroxyl in metabolites increased the stability (48). Conchiero *et. al.* also published a method for the determination of 40 novel psychoactive substances in urine and included four new cathinones in addition to the 28 investigated previously. As part of the validation, these new drugs were subjected to the same experimental conditions as the original 28. The four additional cathinones were all pyrrolidinyl-substituted and no instability was observed in any of the conditions (47).

Al-Saffer *et. al.* developed a screening method for LC-MS/MS for the detection of 25 novel psychoactive substances, including 11 synthetic cathinones, in urine. During validation, stability of the compounds was assessed over three months of storage at 22°C, 6°C, and -20°C. Drug-free urine (pH not specified) was fortified at 1,000 ng/mL with all 25 compounds. Over time, the most extreme losses occurred at 22°C, with 3-FMC exhibiting the highest loss and pyrrolidine-type cathinones exhibiting the least (76). At 6 and -20°C, the pyrrolidine-type cathinones were the most stable, with losses remaining within 20% of the original concentration. After extended storage (3 months) at 6°C the remaining cathinones had <15% remaining. While frozen storage did not prevent degradation, many remain within 20% of the starting concentration. Overall, MDPV was consistently the most stable cathinone of the 11 investigated and either buphedrone or 3-FMC was the least stable. As a group, the synthetic cathinones were generally less stable at every condition than the other novel psychoactive substances investigated, except for 4-hydroxy-*N*-methyl-*N*-ethyltrptamine (4-OH-MET), which was less stable than any of the cathinones (76).

Methods have also been developed for the detection of these drugs in blood. Sorenson developed a method for the determination of fifteen cathinones and related ephedrines in blood using LC-MS/MS. Of the fifteen drugs investigated, eight were cathinone derivatives. During the development, stability was assessed for A) pH of the final blood sample extract, B) blood preserved with NaF/potassium oxalate, and C) blood preserved with NaF/citrate buffer (71). The blood preserved with NaF/potassium oxalate resulted in a final pH of 7.4, whereas the blood preserved with NaF/citrate buffer had a final pH of 5.9. The final sample extracts were stored for one week at 20°C. The preserved blood was stored at 20°C and 5°C for one week. The results of their study demonstrated that the stability of these drugs was pH dependent. Final sample extracts had pH values ranging from 2.5 to 8. No significant degradation was observed in extracts with a pH of 4.5 and lower. Significant degradation was observed at pH values greater than 5.5. The same held true for the blood samples; cathinones in the blood preserved with NaF/citrate buffer had less degradation occur than the blood preserved with NaF/potassium oxalate at both temperatures. After two days of storage, all cathinones had at least a 30% loss in the potassium oxalate blood, compared to 10% loss in the citrate buffer blood (71).

Ammann *et. al.* investigated processed sample stability, freeze-thaw stability and short-term stability over 6 weeks. Using LC-MS/MS, 25 synthetic cathinone and ephedrines were evaluated in blood. Blood specimens and extracts were stored at 4°C. The extracts for the processed sample stability were stable for over 24 hours. In stored blood, all drugs met the acceptance criteria of within 80-120% of controls in the short-term and freeze-thaw experiments (77).

While the stability of some synthetic cathinones have been reported, findings are often incidental, short-term or limited in scope due to the fact that they have been incorporated into method development and validation studies. However, Johnson and Botch-Jones investigated the stability of MDPV and mephedrone, a tertiary and secondary amine cathinone, respectively, using HPLC-QTrap. Urine, blood, and serum were fortified with all four drugs at 1,000 ng/mL and stored at 22, 4, and -20°C for two weeks. MDPV was within 20% of initial concentration in all matrices at all temperatures over the two week period, while mephedrone was only stable at -20°C. At 4°C, mephedrone remained within 20% of the initial concentration in plasma and urine, but there was a 30% loss on day 7 and over 50% loss after 14 days in blood. At 22°C, mephedrone was unstable in all three matrices: in whole blood by day two with approximately 30% loss, in plasma by day 4 with a 90% loss, and urine at day 7 with an approximate 35% loss. After 14 days mephedrone was undetectable in blood and plasma, and had over a 50% loss in urine. The pH of the urine was unspecified, however, based on the previous studies these results are more comparable to an acidic urine (<pH 6) (117). These results also highlight the stabilizing effect of the pyrrolidinyl group on MDPV compared to the secondary amine in mephedrone.

Soh and Elliot investigated the stability of thirteen novel psychoactive substances, including one secondary amine cathinone, 4-MEC. The stability of the drugs was investigated in blood and plasma stored at 20°C for at least 28 days using HPLC-DAD analysis. Included in their investigation was the identification of degradation products using LC-MS/MS and UHPLC-Q/TOF-MS. 4-MEC was more stable in plasma compared to blood. After seven days of storage, there was a 92% loss in blood compared to 50% in

plasma. After 14 days, 4-MEC was undetectable in blood, but remained detectable in plasma over the 28 day period. The HPLC-DAD analysis revealed the presence of a peak at a difference wavelength (212 nm) compared to 4-MEC (262 nm). LC-MS/MS and Q/TOF-MS analysis determined it was most likely dihydro-4-MEC, as a result of the beta-ketone being reduced to a hydroxyl (104).

Busardo *et. al.* investigated the stability of mephedrone in antemortem (AM) and postmortem (PM) blood. AM and PM blood were pooled, fortified at 1 mg/mL, and stored at 20°C, 4°C, and -20°C. The effect a preservative on mephedrone stability was also investigated. PM and AM blood was either unpreserved, preserved with 3% ethylenediaminetetraacetic acid (EDTA), or preserved with 1.67%/0.2% sodium fluoride/potassium oxalate (NaF/KOx). The final pH of the blood was not stated after the addition of the preservative. Samples were stored for six months. Mephedrone was found to be most stable when stored at -20°C and least stable at 20°C. The blood with no preservative exhibited more degradation than either of the preservatives. Mephedrone in the blood preserved with NaF/KOx was the most stable over the six months. Mephedrone was detectable for over 87 days in PM blood preserved with NaF/KOx compared to 80 days when preserved with EDTA. Comparing antemortem to postmortem blood, mephedrone was more stable in antemortem blood (91). From their findings, in order to prevent mephedrone from degrading in blood, antemortem or postmortem samples should be preserved with NaF/KOx and stored at -20°C. The two common additives are contained in grey-top evaluated blood tubes.

While most published studies focus on urine and blood, toxicologists analyze a variety of matrices. Miller *et. al.* investigated the stability of ten synthetic cathinones in

oral fluid (OF). OF specimens examined consisted of neat (unpreserved) OF (pH 8), OF-Quantisal® (pH 6), and OF-Oral-Eze (pH 7). Specimens were stored at room temperature, 4°C, and -20°C for one month. OF was fortified at 2.5 and 150 ng/mL. Their findings revealed that all ten cathinones were most stable in Quantisal and least stable in neat OF stored at room temperature and 4°C. All synthetic cathinones were stable in the three OF mixtures when stored at -20°C. As with the previous studies, MDPV (a tertiary amine cathinone containing the pyrrolidinyl group) was the most stable out of the 10, followed by α-PVP. Cathinone, methcathinone, ethcathinone were among the least stable. Despite being a tertiary amine cathinone, naphyrone also was considerably unstable, indicating that the naphthyl group may influence the stability of this particular cathinone more so than the stabilizing pyrrolidinyl group (65). A comprehensive list of stability studies related to non-biological and biological material can be found in **Table 1.14.**

Table 1.14. Summary of cathinone and synthetic cathinones stability studies in non-biological and biological matrices.

Cathinone	Matrix	Storage Temperature	Storage Time	Conclusion	Reference
Non-biological Studies					
Cathinone	Dried plant	Not Specified	Not Specified	Unstable in basic conditions Unstable in oxygenated conditions	(224)
Cathinone	Synthesized powder	Not Specified	Not Specified	Unstable in basic conditions Racemizes and dimerizes	(225)
Cathinone	Dried plant	Room Temperature	10 years	Stable when air-dried Cathinone conversion to cathine occurs in drying process	(226)
10 cathinones	Hydrochloric salts	Room Temperature	24 hours	Unstable in air Structural dependence Identification of 2”-oxo and N-oxide for α-PHPP	(227)
7 cathinones	Aqueous buffers (pH 4, 7, 10, 12)	22°C	48 hours	Degradation dependent on pH Degradation dependent on chemical structure Identification of 3 degradation products for 4-MMC	(228)
Mephedrone	Formalin solution (5, 10, 20%) pH (3.5, 7, 9.5)	Not Specified	28 days	Degradation was pH dependent Presence of formalin resulted in degradation More degradation occurred in higher concentration of formalin	(229)

(continued)

Cathinone	Matrix	Storage Temperature	Storage Time	Conclusion	Reference
Biological Studies					
Cathinone	Plasma	N/A	N/A	Stable in salt form only Dimerizes after solvent is removed Acidify solvent prior to removal	(230)
Cathinone Methcathinone	Urine	2 - 4°C -18°C	3 months	Both stable for 3 days at 2-4°C Both stable for 2 months at -18°C	(30)
32 Cathinones	Urine (pH 7.6)	Room temperature 4°C	72 hours	Instability at Room Temp. after 24 hours Stable at 4C for 72 hrs except benzedrone, 4-FMC, naphyrone Hydroxyl metabolites and pyrrolidinyl derivatives most stable	(47, 48)
11 cathinones	Urine	22°C 6°C -20°C	3 months	Instability occurred at all temperatures MDPV consistently most stable Buphedrone and 3-FMC least stable	(76)
8 cathinones	Preserved blood: NaF/KOx (pH 7.4) NaF/Cit (pH 5.9)	20°C 5°C	7 days	More stable at 5°C More stable in NaF/KOx	(71)
25 cathinones	Blood	4°C	6 weeks	All stable	(77)

(continued)

Cathinone	Matrix	Storage Temperature	Storage Time	Conclusion	Reference
MDPV Mephedrone	Blood Urine Serum	22°C 4°C -20°C	14 days	Matrix and storage temperature significant More stable in urine, followed by plasma More stable at -20°C MDPV more stable than mephedrone	(117)
4-MEC	Blood Plasma	20 - 23°C	37 days	More stable in plasma Identification of dihydro-4-MEC	(104)
Mephedrone	AM & PM blood: no preservative EDTA NaF/KOx	20°C 4°C -20°C	6 months	More stable in NaF/KOx More stable at -20°C AM more stable than PM	(91)
10 cathinones	Oral fluid neat Quantisal Oral-Eze	Room temperature 4°C -20°C	One month	More stable in Quantisal at room temperature and 4°C All stable at -20°C in 3 OF mixtures	(65)

N/A: Not applicable

Synthetic Cathinone Degradation

In addition to providing information regarding the stability of these novel drugs, some studies further investigated products that remain after the parent drug degraded. While information is limited, degradation products and pathways have been proposed (104, 227-229). It was shown several decades ago that cathinones can be easily oxidized and lost during extraction. The result of this oxidation is dimerization to dimethyldiphenylpyrazine or benzoic acid (23, 225). While these may not be directly related to degradation in biological matrices, the majority of biological samples are subjected to sample preparation and extraction, possibly leading to the formation of these decomposition products.

Some of the degradation products that form as the result of drug breakdown during long term storage overlap with identified metabolites. As a result, degradation product determination can be challenging. Most degradation studies have been completed using non-biological matrices, including aqueous or formalin solutions, while just a few having been involved a biological matrix (104, 228, 229). Dihydro-4-MEC was proposed as a degradation product by Soh and Elliott, however, their analysis was completed in plasma and blood. Therefore the formation of this product could be the result of metabolism (104). However, 4-MEC is known to have more than one metabolite and no other metabolites were identified in their sample. It was noted that the decrease of 4-MEC and increase in the dihydro- breakdown product was not proportional (104). This lack of relationship could indicate that the dihydro breakdown product is minor compared to other, undetected, products.

Another common degradation product and metabolite is the formation of 2”-oxo derivatives for cathinones bearing a tertiary amine. The formation of a 2”-oxo species was

identified for α-PVP, MDPV, and pyrovalerone (227). This was identified from powders exposed to air, therefore the formation of this product was not attributed to metabolism. This analysis also revealed the formation of *N*-oxide degradation product for the tertiary amines and *N*-dealkylation for the secondary amine cathinone, pentedrone (227). The proposed degradation pathway for cathinones bearing a tertiary amine is depicted in **Figure 1.12.**

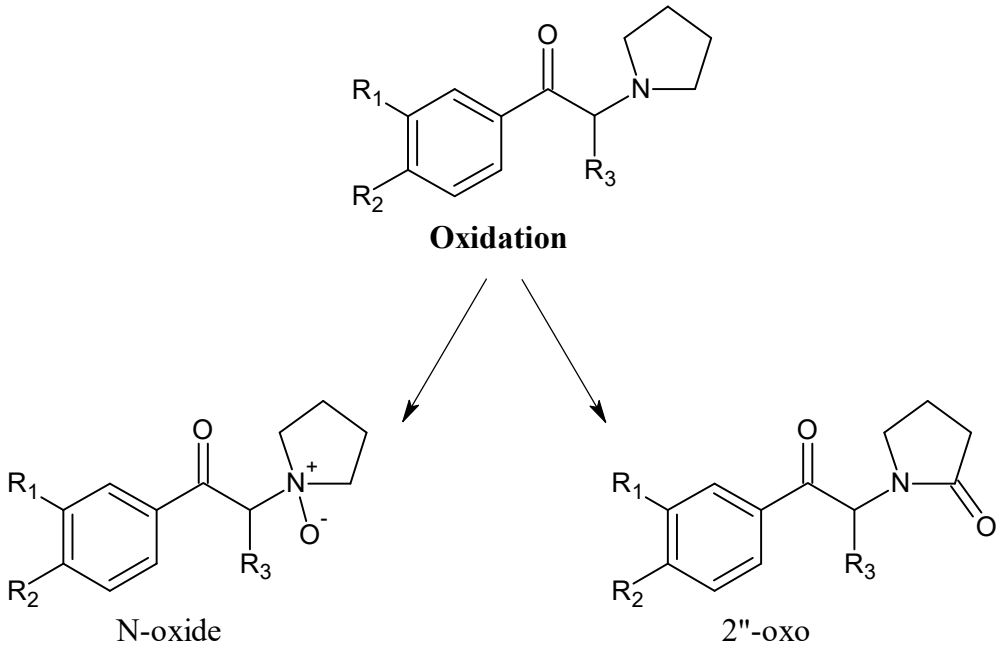


Figure 1.12. Degradation pathway for tertiary amine cathinones (227).

Aqueous solutions and formalin solutions were used to investigate the breakdown of mephedrone (228, 229). Tsujikawa *et. al.* proposed a three-step degradation path for mephedrone, beginning with oxidative deamination to form propanedione **(Figure 1.13A)**. The propanedione is further oxidized and cleaved to form benzoic acid (**Figure 1.13B)**, which then forms benzamide through amidation (**Figure 1.13C)** (228). Benzamide was the most abundant degradant while the others increased then decreased in abundance over the

48 hour period. Maskell *et. al.* reported the formation of a *N*-alkylated degradation product when mephedrone was in formalin solution (229). The degradation products and pathways for secondary amine synthetic cathinones are depicted in **Figure 1.13** and all identified degradation products for secondary and tertiary amine cathinones are in **Table 1.15**.

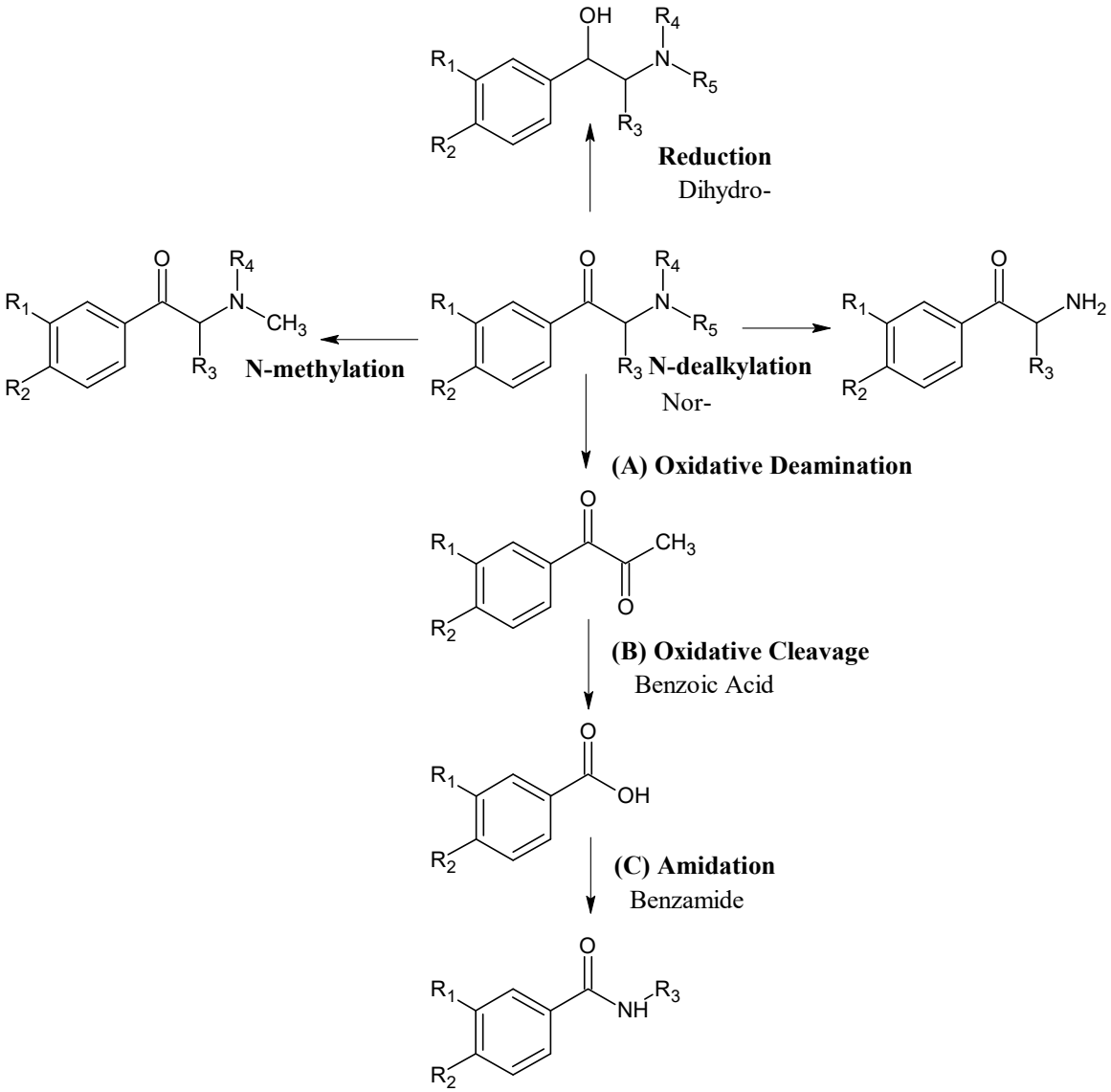


Figure 1.13. Proposed degradation pathway for secondary amine cathinones (227-229).

Table 1.15. Identified degradation products for select synthetic cathinones.

Cathinone	Degradation Product	Reference
4-MEC	Dihydro-4-MEC	(104)
Pyrrolidine Cathinones α-PVP MDPV Pyrovalerone	2”-oxo *N*-oxide	(227)
Pentedrone	*N*-dealkylation	(227)
Mephedrone	Propanedione	(228)
	Benzamide	(228)
	Benzoic Acid	(228)
	N-alkylation	(229)

Statement of the Problem

The instability of cathinone was initially documented in the 1980s and the investigation of the derivatives’ stability in multiple matrices has received increased attention over the last decade due to a rise in cathinone related fatalities. From these studies, it can be concluded that synthetic cathinone stability is significantly influenced by pH, storage temperature, and chemical structure. However, as Miller *et. al*. noted, their study was limited due to a lack of authentic samples (65). While many of the studies, particularly those included in method development, had authentic samples, stability was not investigated. Another limitation was storage time. Most studies had a maximum storage time of only one month. Only Busardo *et. al.* stored their mephedrone fortified blood for six months. Due to forensic backlogs, specimens may be stored for several weeks and sometimes months. During this time, not only can the concentration of the drug decrease, but the pH of the specimen could potentially change. Urinary pH has been shown to increase over 2 pH units after room temperature storage for one day. This could be problematic for alkaline labile drugs. This rise in pH is thought to be the result of carbon

dioxide loss from the breakdown of urea and uric acid (231, 232). Changes in urinary pH are minimized at cooler storage temperatures.

A final factor that can be concluded from these studies is the influence of chemical characteristics. It has been suggested that synthetic cathinones containing the pyrrolidinyl group are relatively stable under some conditions. However, the majority of studies investigated only a limited number of cathinones. To fully understand how structural features of the cathinone species influence stability in biological evidence, a comprehensive and systematic study is needed.

References

1. Chung, H., Lee, J. and Kim, E. (2016) Trends of novel psychoactive substances (NPSs) and their fatal cases. *Forensic Toxicology,* **34**, 1-11.

2. Elliott, S., Sedefov, R. and Evans-Brown, M. (2017) Assessing the toxicological significance of new psychoactive substances in fatalities. *Drug Testing and Analysis,* 1-7.

3. Katz, D. P., Bhattacharya, D., Bhattacharya, S., Deruiter, J., Clark, C. R., Suppiramaniam, V.*, et al.* (2014) Synthetic cathinones: "a khat and mouse game". *Toxicology Letters,* **229**, 349-356.

4. Capriola, M. (2013) Synthetic cathinone abuse. *Clinical Pharmacology,* **5**, 109-115.

5. Spiller, H. A., Ryan, M. L., Weston, R. G. and Jansen, J. (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. *Clinical Toxicology,* **49**, 499-505.

6. Zawilska, J. B. and Wojcieszak, J. (2013) Designer cathinones—an emerging class of novel recreational drugs. *Forensic Science International,* **231**, 42-53.

7. Rosenbaum, C. D., Carreiro, S. P. and Babu, K. M. (2012) Here today, gone tomorrow…and back again? A review of herbal marijuana alternatives (K2, spice), synthetic cathinones (bath salts), kratom, salvia divinorum, methoxetamine, and piperazines. *Journal of Medical Toxicology,* **8**, 15-32.

8. Kelly, J. P. (2011) Cathinone derivatives: a review of their chemistry, pharmacology and toxicology. *Drug Testing and Analysis,* **3**, 439-453.

9. Coppola, M. and Mondola, R. (2012) Synthetic cathinones: chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as “bath salts” or “plant food”. *Toxicology Letters,* **211**, 144-149.

10. Drug Enforcement Administration. (2011) Schedules of controlled substances: Temporary placement of three synthetic cathinones in Schedule I. Final order. *Federal Registry,* **76**, 65371-65375.

11. National Conference of State Legislatures (NCSL), 2015. Synthetic drug threats. Updated 13 Jan 2015: http://www.ncsl.org/research/civil-and-criminal-justice/synthetic-drug-threats.aspx. Last accessed April 2017.

12. Drug Enforcement Administration. (2014) Schedules of controlled substances: Temporary placement of 10 synthetic cathinones in Schedule I. Final order. *Federal Registry,* **79**, 12938-12943

13. Banks, M. L., Worst, T. J., Rusyniak, D. E. and Sprague, J. E. (2014) Synthetic cathinones (“bath salts”). *The Journal of Emergency Medicine,* **46**, 632-642.

14. Paillet-Loilier, M., Cesbron, A., Le Boisselier, R., Bourgine, J. and Debruyne, D. (2014) Emerging drugs of abuse: current perspectives on substituted cathinones. *Substance Abuse and Rehabilitation,* **5**, 37-52.

15. Zhou, M. J., Bouazzaoui, S., Jones, L. E., Goodrich, P., Bell, S. E. J., Sheldrake, G. N.*, et al.* (2015) Isolation and structural determination of non-racemic tertiary cathinone derivatives. *Organic & Biomolecular Chemistry,* **13**, 9629-9636.

16. Gibbons, S. and Zloh, M. (2010) An analysis of the ‘legal high’ mephedrone. *Bioorganic & Medicinal Chemistry Letters,* **20**, 4135-4139.

17. Baselt, R.C. (2017) *Disposition of Toxic Drugs and Chemicals in Man 11th Edition.* Seal Beach, CA: Biomedical Publications, 2017, Print.

18. López-Arnau, R., Martínez-Clemente, J., Carbó, M. l., Pubill, D., Escubedo, E. and Camarasa, J. (2013) An integrated pharmacokinetic and pharmacodynamic study of a new drug of abuse, methylone, a synthetic cathinone sold as "bath salts". *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* **45**, 64-72.

19. Braun, U., Shulgin, A. T. and Braun, G. (1980) Centrally active N-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylenedioxyamphetamine). *Journal of Pharmaceutical Sciences,* **69**, 192-195.

20. Dal Cason, T. A. (1997) The characterization of some 3,4-methylenedioxycathinone (MDCATH) homologs. *Forensic Science International,* **87**, 9-53.

21. Kalix, P. (1981) Cathinone, an alkaloid from khat leaves with an amphetamine-like releasing effect. *Psychopharmacology,* **74**, 269-270.

22. Gregg, R. A. and Rawls, S. M. (2014) Behavioral pharmacology of designer cathinones: a review of the preclinical literature. *Life Sciences,* **97**, 27-30.

23. DeRuiter, J., Hayes, L., Valaer, A., Clark, C. R. and Noggle, F. T. (1994) Methcathinone and designer analogues: synthesis, stereochemical analysis, and analytical properties. *Journal of Chromatographic Science,* **32**, 552-564.

24. Babu, N. (2013) DFT studies of molecular structure, equilibium constant for keto-enol tautomerism and geometrical isomerism (E-Z) of 2-amino-1-phenylpropan-

1-one (cathinone). *Advances in Applied Science Research: Pelagia Research Library,* **4**, 147-153.

25. O'Byrne, P. M., Kavanagh, P. V., McNamara, S. M. and Stokes, S. M. (2013) Screening of stimulants including designer drugs in urine using a liquid chromatography tandem mass spectrometry system. *Journal of Analytical Toxicology,* bks091.

26. Leffler, A. M., Smith, P. B., de Armas, A. and Dorman, F. L. (2014) The analytical investigation of synthetic street drugs containing cathinone analogs. *Forensic Science International,* **234**, 50-56.

27. Zuba, D. (2012) Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods. *Trac-Trends in Analytical Chemistry,* **32**, 15-30.

28. Allegretti, P., de las Mercedes, S., Castro, E. and Furlong, J. (2007) Tautomeric equilibria studies by mass spectrometry. *World Journal of Chemistry,* **2**, 25-62.

29. Truscott, S. M., Crittenden, N. E., Shaw, M. A., Middleberg, R. A. and Jortani, S. A. (2013) Violent behavior and hallucination in a 32-year-old patient. *Clinical Chemistry,* **59**, 612-615.

30. Paul, B. D. and Cole, K. A. (2001) Cathinone (khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. *Journal of Analytical Toxicology,* **25**, 525-530.

31. Nieddu, M., Burrai, L., Trignano, C. and Boatto, G. (2014) Cross-reactivities of 39 new amphetamine designer drugs on three abuse drugs urinary screening tests. *Forensic Toxicology,* **32**, 132-138.

32. Nieddu, M., Burrai, L., Baralla, E., Pasciu, V., Varoni, M. V., Briguglio, I., *et al.* (2016) ELISA detection of 30 new amphetamine designer drugs in whole blood, urine and oral fluid using neogen® "amphetamine" and "methamphetamine/mdma" kits. *Journal of Analytical Toxicology,* **40**, 492-497.

33. de Castro, A., Lendoiro, E., Fernández-Vega, H., Steinmeyer, S., López-Rivadulla, M. and Cruz, A. (2014) Liquid chromatography tandem mass spectrometry determination of selected synthetic cathinones and two piperazines in oral fluid. Cross reactivity study with an on-site immunoassay device. *Journal of Chromatography A,* **1374**, 93-101.

34. Verstraete, A. G. (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. *Therapeutic Drug Monitoring,* **26**, 200-205.

35. Grueninger, D., Englert, R. (2011) Determination of the amphetamine-like designer drugs methcathinone and 4-methylmethcathinone in urine by LC-MS/MS. *Annales de Toxicologie Analytique,* **23**, 7-14.

36. Swortwood, M. J., Hearn, W. L. and DeCaprio, A. P. (2014) Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays. *Drug Testing and Analysis,* **6**, 716-727.

37. Toennes, S. W. and Kauert, G. F. (2002) Excretion and detection of cathinone, cathine, and phenylpropanolamine in urine after kath chewing. *Clin Chem,* **48**, 1715-1719.

38. Petrie, M., Lynch, K. L., Ekins, S., Chang, J. S., Goetz, R. J., Wu, A. H. B., *et al.* (2013) Cross-reactivity studies and predictive modeling of "bath salts" and other

amphetamine-type stimulants with amphetamine screening immunoassays. *Clinical Toxicology,* **51**, 83-91.

39. Pedersen, A. J., Dalsgaard, P. W., Rode, A. J., Rasmussen, B. S., Müller, I. B., Johansen, S. S., *et al.* (2013) Screening for illicit and medicinal drugs in whole blood using fully automated SPE and ultra-high-performance liquid chromatography with TOF-MS with data-independent acquisition. *Journal of Separation Science,* **36**, 2081-2089.

40. Guale, F., Shahreza, S., Walterscheid, J. P., Chen, H.-H., Arndt, C., Kelly, A. T., *et al.* (2013) Validation of LC–TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens. *Journal of Analytical Toxicology,* **37**, 17-24.

41. Birkler, R. I. D., Telving, R., Ingemann-Hansen, O., Charles, A. V., Johannsen, M. m. r. a. d. and Andreasen, M. F. m. r. a. d. (2012) Screening analysis for medicinal drugs and drugs of abuse in whole blood using ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC–TOF-MS)—toxicological findings in cases of alleged sexual assault. *Forensic Science International,* **222**, 154-161.

42. Nielsen, M. K. K., Johansen, S. S., Dalsgaard, P. W. and Linnet, K. (2010) Simultaneous screening and quantification of 52 common pharmaceuticals and drugs of abuse in hair using UPLC–TOF-MS. *Forensic Science International,* **196**, 85-92.

43. Sundström, M., Pelander, A., Angerer, V., Hutter, M., Kneisel, S. and Ojanperä, I. (2013) A high-sensitivity ultra-high performance liquid chromatography/high-

resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine. *Analytical and Bioanalytical Chemistry,* **405**, 8463-8474.

44. Adamowicz, P. and Tokarczyk, B. (2016) Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. *Drug Testing and Analysis,* **8**, 652-667.

45. Vaiano, F., Busardò, F. P., Palumbo, D., Kyriakou, C., Fioravanti, A., Catalani, V., *et al.* (2016) A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. *Journal of Pharmaceutical and Biomedical Analysis,* **129**, 441-449.

46. Neifeld, J. R., Regester, L. E., Holler, J. M., Vorce, S. P., Magluilo, J. J., Ramos, G., *et al.* (2016) Ultrafast screening of synthetic cannabinoids and synthetic cathinones in urine by rapidfire-tandem mass spectrometry. *Journal of Analytical Toxicology,* **40**, 379-387.

47. Concheiro, M., Castaneto, M., Kronstrand, R. and Huestis, M. A. (2015) Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography–high resolution mass spectrometry and library matching. *Journal of Chromatography A,* **1397**, 32-42.

48. Concheiro, M., Anizan, S., Ellefsen, K. and Huestis, M. A. (2013) Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. *Analytical and Bioanalytical Chemistry,* **405**, 9437-9448.

49. Minakata, K., Yamagishi, I., Nozawa, H., Hasegawa, K., Wurita, A., Gonmori, K., *et al.* (2014) MALDI-TOF mass spectrometric determination of four pyrrolidino cathinones in human blood. *Forensic Toxicology,* **32**, 169-175.

50. Ojanperä, I. A., Heikman, P. K. and Rasanen, I. J. (2011) Urine analysis of 3, 4-methylenedioxypyrovalerone in opioid-dependent patients by gas chromatography–mass spectrometry. *Therapeutic Drug Monitoring,* **33**, 257-263.

51. Olesti, E., Pujadas, M., Papaseit, E., Pérez-Mañá, C., Pozo, Ó. J., Farré, M., *et al.* (2017) GC-MS quantification method for mephedrone in plasma and urine: Application to human pharmacokinetics. *Journal of Analytical Toxicology,* **41**, 100-106.

52. McIntyre, I. M., Hamm, C. E., Aldridge, L. and Nelson, C. L. (2013) Acute methylone intoxication in an accidental drowning - a case report. *Forensic Science International,* **231**, e1-3.

53. McIntyre, I. M., Hamm, C. E., Sherrard, J. L., Gary, R. D., Burton, C. G. and Mena, O. (2014) Acute 3,4-methylenedioxy-N-ethylcathinone (ethylone) intoxication and related fatality: a case report with postmortem concentrations. *Journal of Analytical Toxicology,*

54. Gerace, E., Petrarulo, M., Bison, F., Salomone, A. and Vincenti, M. (2014) Toxicological findings in a fatal multidrug intoxication involving mephedrone. *Forensic Science International,* **243**, 68-73.

55. Dickson, A. J., Vorce, S. P., Levine, B. and Past, M. R. (2010) Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin. *Journal of Analytical Toxicology,* **34**, 162-168.

56. Lee, D., Chronister, C. W., Hoyer, J. and Goldberger, B. A. (2015) Ethylone-related deaths: toxicological findings. *Journal of Analytical Toxicology,* **39**, 567-571.

57. Aromatario, M., Bottoni, E., Santoni, M. and Ciallella, C. (2012) New "lethal highs": a case of a deadly cocktail of GHB and mephedrone. *Forensic Science International,* **223**, e38-e41.

58. Cawrse, B. M., Levine, B., Jufer, R. A., Fowler, D. R., Vorce, S. P., Dickson, A. J., *et al.* (2012) Distribution of methylone in four postmortem cases. *Journal of Analytical Toxicology,* **36**, 434-439.

59. Pearson, J. M., Hargraves, T. L., Hair, L. S., Massucci, C. J., Frazee, C. C., Garg, U., *et al.* (2012) Three fatal intoxications due to methylone. *Journal of Analytical Toxicology,* **36**, 444-451.

60. Peters, J. R., Keasling, R., Brown, S. D. and Pond, B. B. (2016) Quantification of synthetic cathinones in rat brain using HILIC–ESI-MS/MS. *Journal of Analytical Toxicology,* **40**, 718-725.

61. Paul, M., Ippisch, J., Herrmann, C., Guber, S. and Schultis, W. (2014) Analysis of new designer drugs and common drugs of abuse in urine by a combined targeted and untargeted LC-HR-QTOFMS approach. *Analytical and Bioanalytical Chemistry,* **406**, 4425-4441.

62. Maas, A., Wippich, C., Madea, B. and Hess, C. (2015) Driving under the influence of synthetic phenethylamines: a case series. *International Journal of Legal Medicine,* **129**, 997-1003.

63. Sykutera, M., Cychowska, M. and Bloch-Boguslawska, E. (2015) A fatal case of pentedrone and α-pyrrolidinovalerophenone poisoning. *Journal of Analytical Toxicology,* **39**, 324-329.

64. Swortwood, M. J., Boland, D. M. and DeCaprio, A. P. (2013) Determination of 32 cathinone derivatives and other designer drugs in serum by comprehensive LC-QQQ-MS/MS analysis. *Anal Bioanal Chem,* **405**, 1383-1397.

65. Miller, B., Kim, J. and Concheiro, M. (2017) Stability of synthetic cathinones in oral fluid samples. *Forensic Science International,* **274**, 13-21.

66. Usui, K., Aramaki, T., Hashiyada, M., Hayashizaki, Y. and Funayama, M. (2014) Quantitative analysis of 3,4-dimethylmethcathinone in blood and urine by liquid chromatography–tandem mass spectrometry in a fatal case. *Legal Medicine,* **16**, 222-226.

67. Potocka-Banaś, B., Janus, T., Majdanik, S., Banaś, T., Dembińska, T. and Borowiak, K. (2017) Fatal intoxication with α-PVP, a synthetic cathinone derivative. *Journal of Forensic Sciences,* **62**, 553-556.

68. Pasin, D., Bidny, S. and Fu, S. (2015) Analysis of new designer drugs in post-mortem blood using high-resolution mass spectrometry. *Journal of Analytical Toxicology,* **39**, 163-171.

69. Bijlsma, L., Sancho, J. V., Hernández, F. and Niessen, W. M. A. (2011) Fragmentation pathways of drugs of abuse and their metabolites based on QTOF MS/MS and MS^E accurate-mass spectra. *Journal of Mass Spectrometry,* **46**, 865-875.

70. Smith, J. P., Sutcliffe, O. B. and Banks, C. E. (2015) An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). *Analyst,* **140**, 4932-4948.

71. Sorensen, 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**, 727-736.

72. Kerrigan, S., Savage, M., Cavazos, C. and Bella, P. (2015) Thermal degradation of synthetic cathinones: implications for forensic toxicology. *Journal of Analytical Toxicology,* bkv099.

73. Westphal, F., Junge, T., Girreser, U., Greibl, W. and Doering, C. (2012) Mass, NMR and IR spectroscopic characterization of pentedrone and pentylone and identification of their isocathinone by-products. *Forensic Science International,* **217**, 157-167.

74. McDermott, S. D., Power, J. D., Kavanagh, P. and O'Brien, J. (2011) The analysis of substituted cathinones. part 2: An investigation into the phenylacetone based isomers of 4-methylmethcathinone and n-ethylcathinone. *Forensic Science International,* **212**, 13-21.

75. Archer, R. P. (2009) Fluoromethcathinone, a new substance of abuse. *Forensic Science International,* **185**, 10-20.

76. Al-Saffar, Y., Stephanson, N. N. and Beck, O. (2013) Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine—experience from the Swedish population. *Journal of Chromatography B,* **930**, 112-120.

77. Ammann, D., McLaren, J. M., Gerostamoulos, D. and Beyer, J. (2012) Detection and quantification of new designer drugs in human blood: part 2 – designer cathinones. *Journal of Analytical Toxicology,* **36**, 381-389.

78. Li, X., Uboh, C. E., Soma, L. R., Liu, Y., Guan, F., Aurand, C. R., *et al.* (2014) Sensitive hydrophilic interaction liquid chromatography/tandem mass spectrometry method for rapid detection, quantification and confirmation of cathinone-derived designer drugs for doping control in equine plasma. *Rapid Communications in Mass Spectrometry,* **28**, 217-229.

79. Gwak, S., Arroyo-Mora, L. E. and Almirall, J. R. (2015) Qualitative analysis of seized synthetic cannabinoids and synthetic cathinones by gas chromatography triple quadrupole tandem mass spectrometry. *Drug Testing and Analysis,* **7**, 121-130.

80. Joshi, M., Cetroni, B., Camacho, A., Krueger, C. and Midey, A. J. (2014) Analysis of synthetic cathinones and associated psychoactive substances by ion mobility spectrometry. *Forensic Science International,* **244**, 196-206.

81. Fornal, E. (2014) Study of collision-induced dissociation of electrospray-generated protonated cathinones. *Drug Testing and Analysis,* **6**, 705-715.

82. Fornal, E. (2013) Identification of substituted cathinones: 3,4-methylenedioxy derivatives by high performance liquid chromatography–quadrupole time of flight mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis,* **81–82**, 13-19.

83. Fornal, E. (2013) Formation of odd-electron product ions in collision-induced fragmentation of electrospray-generated protonated cathinone derivatives: aryl -

primary amino ketones. *Rapid Communications in Mass Spectrometry,* **27**, 1858-1866.

84. Tsujikawa, K., Kuwayama, K., Kanamori, T., Iwata, Y. T. and Inoue, H. (2013) Thermal degradation of alpha-pyrrolidinopentiophenone during injection in gas chromatography/mass spectrometry. *Forensic Science International,* **231**, 296-299.

85. Wiergowski, M., Woźniak, M. K., Kata, M. and Biziuk, M. (2016) Determination of MDPBP in postmortem blood samples by gas chromatography coupled with mass spectrometry. *Monatshefte für Chemie - Chemical Monthly,* **147**, 1415-1421.

86. Liveri, K., Constantinou, M. A., Afxentiou, M. and Kanari, P. (2016) A fatal intoxication related to MDPV and pentedrone combined with antipsychotic and antidepressant substances in Cyprus. *Forensic Science International,* **265**, 160-165.

87. Namera, A., Urabe, S., Saito, T., Torikoshi-Hatano, A., Shiraishi, H., Arima, Y., *et al.* (2013) A fatal case of 3,4-methylenedioxypyrovalerone poisoning: Coexistence of alpha-pyrrolidinobutiophenone and alpha-pyrrolidinovalerophenone in blood and/or hair. *Forensic Toxicology,* **31**, 338-343.

88. Wright, T. H., Cline-Parhamovich, K., Lajoie, D., Parsons, L., Dunn, M. and Ferslew, K. E. (2013) Deaths involving methylenedioxypyrovalerone (MDPV) in upper east Tennessee. *Journal of Forensic Sciences,* **58**, 1558-1562.

89. Grapp, M., Kaufmann, C. and Ebbecke, M. (2017) Toxicological investigation of forensic cases related to the designer drug 3,4-methylenedioxypyrovalerone

(MDPV): Detection, quantification and studies on human metabolism by GC-MS. *Forensic Science International,* **273**, 1-9.

90. Meyer, M. R., Wilhelm, J., Peters, F. T. and Maurer, H. H. (2010) Beta-keto amphetamines: Studies on the metabolism of the designer drug mephedrone and toxicological detection of mephedrone, butylone, and methylone in urine using gas chromatography–mass spectrometry. *Analytical & Bioanalytical Chemistry,* **397**, 1225-1233.

91. Busardò, F. P., Kyriakou, C., Tittarelli, R., Mannocchi, G., Pantano, F., Santurro, A.*, et al.* Assessment of the stability of mephedrone in ante-mortem and post-mortem blood specimens. *Forensic Science International,* **256**, 28-37.

92. Torrance, H. and Cooper, G. (2010) The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland. *Forensic Science International,* **202**, e62-e63.

93. Hagan, K. S. and Reidy, L. (2015) Detection of synthetic cathinones in victims of sexual assault. *Forensic Science International,* **257**, 71-75.

94. Wikström, M., Thelander, G., Nyström, I. and Kronstrand, R. (2010) Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone). *Journal of Analytical Toxicology,* **34**, 594-598.

95. Carbone, P., Carbone, D. L., Carstairs, S. and Luzi, S. A. (2012) Sudden cardiac death associated with methylone use. *American Journal of Clinical Pathology,* **138**, A320-A320.

96. Klavž, J., Gorenjak, M. and Marinšek, M. (2016) Suicide attempt with a mix of synthetic cannabinoids and synthetic cathinones: Case report of non-fatal

intoxication with AB-CHMINACA, AB-FUBINACA, alpha-PHP, alpha-PVP and 4-CMC. *Forensic Science International,* **265**, 121-124.

97. Uralets, V., Rana, S., Morgan, S. and Ross, W. (2014) Testing for designer stimulants: metabolic profiles of 16 synthetic cathinones excreted free in human urine. *Journal of Analytical Toxicology,* **38**, 233-241.

98. Tyrkkö, E., Pelander, A., Ketola, R. A. and Ojanperä, I. (2013) In silico and in vitro metabolism studies support identification of designer drugs in human urine by liquid chromatography/quadrupole-time-of-flight mass spectrometry. *Analytical and Bioanalytical Chemistry,* **405**, 6697-6709.

99. Sykutera, M. and Bloch-Bogusławska, E. (2015) A fatal case of 3, 4-dimethylmethcathinone poisoning. *Problems of forensic sciences,* **102**, 138-148.

100. Shima, N., Katagi, M., Kamata, H., Matsuta, S., Nakanishi, K., Zaitsu, K.*, et al.* (2013) Urinary excretion and metabolism of the newly encountered designer drug 3, 4-dimethylmethcathinone in humans. *Forensic Toxicology,* **31**, 101-112.

101. Alvarez, J. C., Etting, I., Abe, E., Villa, A. and Fabresse, N. (2017) Identification and quantification of 4-methylethcathinone (4-MEC) and 3,4-methylenedioxypyrovalerone (MDPV) in hair by LC-MS/MS after chronic administration. *Forensic Science International,* **270**, 39-45.

102. Smith, P., Cole, R., Hamilton, S., West, K., Morley, S. and Maskell, P. (2016) Reporting two fatalities associated with the use of 4-methylethcathinone (4-MEC) and a review of the literature. *Journal of Analytical Toxicology,* **40**, 553-560.

103. Rojek, S., Kłys, M., Maciów-Głąb, M., Kula, K. and Strona, M. (2014) Cathinones derivatives-related deaths as exemplified by two fatal cases involving

methcathinone with 4-methylmethcathinone and 4-methylethcathinone. *Drug Testing and Analysis,* **6**, 770-777.

104. Soh, Y. N. A. and Elliott, S. (2014) An investigation of the stability of emerging new psychoactive substances. *Drug Testing and Analysis,* **6**, 696-704.

105. Rojek, S., Kłys, M., Strona, M., Maciów, M. and Kula, K. (2012) "Legal highs"—toxicity in the clinical and medico-legal aspect as exemplified by suicide with bk-MBDB administration. *Forensic Science International,* **222**, e1-e6.

106. Balikova, M., Zidkova, M., Oktabec, Z., Maresova, V., Linhart, I., Himl, M.*, et al.* (2013) The abuse of 3,4-methylenedioxypyrrolidinobutyrophenone (MDPBP): a case report. *Journal of Forensic Toxicology and Pharmacology,* 2.

107. Ibáñez, M., Pozo, Ó. J., Sancho, J. V., Orengo, T., Haro, G. and Hernández, F. (2016) Analytical strategy to investigate 3,4-methylenedioxypyrovalerone (MDPV) metabolites in consumers' urine by high-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry,* **408**, 151-164.

108. Anizan, S., Ellefsen, K., Concheiro, M., Suzuki, M., Rice, K. C., Baumann, M. H.*, et al.* (2014) 3, 4-methylenedioxypyrovalerone (MDPV) and metabolites quantification in human and rat plasma by liquid chromatography–high resolution mass spectrometry. *Analytica Chimica Acta,* **827**, 54-63.

109. Adamowicz, P., Gil, D., Skulska, A. and Tokarczyk, B. (2013) Analysis of MDPV in blood—determination and interpretation. *Journal of Analytical Toxicology,* **37**, 308-312.

110. Borek, H. A. and Holstege, C. P. (2012) Hyperthermia and multiorgan failure after abuse of "bath salts" containing 3,4-methylenedioxypyrovalerone. *Annals of Emergency Medicine,* **60**, 103-105.

111. Bertol, E., Mari, F., Boscolo Berto, R., Mannaioni, G., Vaiano, F. and Favretto, D. (2014) A mixed MDPV and benzodiazepine intoxication in a chronic drug abuser: Determination of MDPV metabolites by LC-HRMS and discussion of the case. *Forensic Science International,* **243**, 149-155.

112. Kriikku, P., Wilhelm, L., Schwarz, O. and Rintatalo, J. (2011) New designer drug of abuse: 3, 4-methylenedioxypyrovalerone (MDPV). Findings from apprehended drivers in Finland. *Forensic Science International,* **210**, 195-200.

113. Wyman, J. F., Lavins, E. S., Engelhart, D., Armstrong, E. J., Snell, K. D., Boggs, P. D., *et al.* (2013) Postmortem tissue distribution of mdpv following lethal intoxication by "bath salts". *Journal of Analytical Toxicology,* **37**, 182-185.

114. Wood, D., Davies, S., Puchnarewicz, M., Button, J., Archer, R., Ovaska, H., *et al.* (2010) Recreational use of mephedrone (4-methylmethcathinone, 4-MMC) with associated sympathomimetic toxicity. *Journal of Medical Toxicology,* **6**, 327-330.

115. Adamowicz, P., Tokarczyk, B., Stanaszek, R. and Slopianka, M. (2013) Fatal mephedrone intoxication—a case report. *Journal of Analytical Toxicology,* **37**, 37-42.

116. Lusthof, K. J., Oosting, R., Maes, A., Verschraagen, M., Dijkhuizen, A. and Sprong, A. G. A. (2011) A case of extreme agitation and death after the use of mephedrone in the Netherlands. *Forensic Science International,* **206**, E93-E95.

117. Johnson, R. D. and Botch-Jones, S. R. (2013) The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *Journal of Analytical Toxicology,* **37**, 51-55.

118. Barrios, L., Grison-Hernando, H., Boels, D., Bouquie, R., Monteil-Ganiere, C. and Clement, R. (2016) Death following ingestion of methylone. *International Journal of Legal Medicine,* **130**, 381-385.

119. Ellefsen, K. N., Concheiro, M., Suzuki, M., Rice, K. C., Elmore, J. S., Baumann, M. H., *et al.* (2015) Quantification of methylone and metabolites in rat and human plasma by liquid chromatography-tandem mass spectrometry. *Forensic Toxicology,* **33**, 202-212.

120. Umebachi, R., Aoki, H., Sugita, M., Taira, T., Wakai, S., Saito, T., *et al.* (2016) Clinical characteristics of α-pyrrolidinovalerophenone (α-PVP) poisoning. *Clinical Toxicology,* **54**, 563-567.

121. Hasegawa, K., Suzuki, O., Wurita, A., Minakata, K., Yamagishi, I., Nozawa, H., *et al.* (2014) Postmortem distribution of α-pyrrolidinovalerophenone and its metabolite in body fluids and solid tissues in a fatal poisoning case measured by LC-MS-MS with the standard addition method. *Forensic Toxicology,* **32**, 225-234.

122. Shima, N., Katagi, M., Kamata, H., Matsuta, S., Sasaki, K., Kamata, T., *et al.* (2014) Metabolism of the newly encountered designer drug α-pyrrolidinovalerophenone in humans: Identification and quantitation of urinary metabolites. *Forensic Toxicology,* **32**, 59-67.

123. Marinetti, L. J. and Antonides, H. M. (2013) Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results. *Journal of Analytical Toxicology,* **37**, 135-146.

124. Warrick, B. J., Hill, M., Hekman, K., Christensen, R., Goetz, R., Casavant, M. J., *et al.* (2013) A 9-state analysis of designer stimulant,"bath salt," hospital visits reported to poison control centers. *Annals of Emergency Medicine,* **62**, 244-251.

125. Batisse, A., Fortias, M., Bourgogne, E., Gregoire, M., Sec, I. and Djezzar, S. (2014) Case series of 21 synthetic cathinones abuse. *Journal of Clinical Psychopharmacology,* **34**, 411-413.

126. Ezaki, J., Ro, A., Hasegawa, M. and Kibayashi, K. (2016) Fatal overdose from synthetic cannabinoids and cathinones in Japan: demographics and autopsy findings. *The American Journal of Drug and Alcohol Abuse,* **42**, 520-529.

127. Forrester, M. B. (2012) Synthetic cathinone exposures reported to Texas poison centers. *The American Journal of Drug and Alcohol Abuse,* **38**, 609-615.

128. Palamar, J. J., Acosta, P., Sherman, S., Ompad, D. C. and Cleland, C. M. (2016) Self-reported use of novel psychoactive substances among attendees of electronic dance music venues. *The American Journal of Drug and Alcohol Abuse,* **42**, 624-632.

129. Carhart-Harris, R. L., King, L. A. and Nutt, D. J. (2011) A web-based survey on mephedrone. *Drug and Alcohol Dependence,* **118**, 19-22.

130. Loi, B., Corkery, J. M., Claridge, H., Goodair, C., Chiappini, S., Gimeno Clemente, C., *et al.* (2015) Deaths of individuals aged 16–24 years in the UK after

using mephedrone. *Human Psychopharmacology: Clinical and Experimental,* **30**, 225-232.

131. Wood, D. M. and Dargan, P. I. (2012) Mephedrone (4-methylmethcathinone): what is new in our understanding of its use and toxicity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* **39**, 227-233.

132. Zawilska, J. B. and Andrzejczak, D. (2015) Next generation of novel psychoactive substances on the horizon – a complex problem to face. *Drug and Alcohol Dependence,* **157**, 1-17.

133. Prosser, J. and Nelson, L. (2012) The toxicology of bath salts: a review of synthetic cathinones. *Journal of Medical Toxicology,* **8**, 33-42.

134. McGraw, M. and McGraw, L. Bath salts: not as harmless as they sound. *Journal of Emergency Nursing,* **38**, 582-588.

135. Schneir, A., Ly, B. T., Casagrande, K., Darracq, M., Offerman, S. R., Thornton, S., *et al.* (2014) Comprehensive analysis of "bath salts" purchased from California stores and the internet. *Clinical Toxicology,* **52**, 651-658.

136. Karch, S. B. (2015) Cathinone neurotoxicity ("the 3Ms"). *Current Neuropharmacology,* **13**, 21-25.

137. Baumann, M. H., Partilla, J. S. and Lehner, K. R. (2013) Psychoactive "bath salts": not so soothing. *European Journal of Pharmacology,* **698**, 1-5.

138. Hadlock, G. C., Webb, K. M., McFadden, L. M., Chu, P. W., Ellis, J. D., Allen, S. C., *et al.* (2011) 4-methylmethcathinone (mephedrone): Neuropharmacological effects of a designer stimulant of abuse. *Journal of Pharmacology and Experimental Therapuetics S,* **339**, 530-536.

139. Katselou, M., Papoutsis, I., Nikolaou, P., Spiliopoulou, C. and Athanaselis, S. (2016) A-PVP ("flakka"): A new synthetic cathinone invades the drug arena. *Forensic Toxicology,* **34**, 41-50.

140. Winstock, A. R., Mitcheson, L. R., Deluca, P., Davey, Z., Corazza, O. and Schifano, F. (2011) Mephedrone, new kid for the chop? *Addiction,* **106**, 154-161.

141. Miotto, K., Striebel, J., Cho, A. K. and Wang, C. (2013) Clinical and pharmacological aspects of bath salt use: a review of the literature and case reports. *Drug and Alcohol Dependence,* **132**, 1-12.

142. Rech, M. A., Donahey, E., Cappiello Dziedzic, J. M., Oh, L. and Greenhalgh, E. (2015) New drugs of abuse. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy,* **35**, 189-197.

143. German, C. L., Fleckenstein, A. E. and Hanson, G. R. (2014) Bath salts and synthetic cathinones: an emerging designer drug phenomenon. *Life Sciences,* **97**, 2-8.

144. Winstock, A., Mitcheson, L., Ramsey, J., Davies, S., Puchnarewicz, M. and Marsden, J. (2011) Mephedrone: use, subjective effects and health risks. *Addiction,* **106**, 1991-1996.

145. Penders, T. M., Gestring, R. E. and Vilensky, D. A. (2012) Intoxication delirium following use of synthetic cathinone derivatives. *The American Journal of Drug and Alcohol Abuse,* **38**, 616-617.

146. Thornton, S. L., Gerona, R. R. and Tomaszewski, C. A. (2012) Psychosis from a bath salt product containing flephedrone and MDPV with serum, urine, and product quantification. *Journal of Medical Toxicology,* **8**, 310-313.

147. Rojek, S., Kula, K., Maciów-Głąb, M. and Kłys, M. (2016) New psychoactive substance α-PVP in a traffic accident case. *Forensic Toxicology,* **34**, 403-410.

148. Eshleman, A. J., Wolfrum, K. M., Hatfield, M. G., Johnson, R. A., Murphy, K. V. and Janowsky, A. (2013) Substituted methcathinones differ in transporter and receptor interactions. *Biochemical Pharmacology,* **85**, 1803-1815.

149. Simmler, L. D., Buser, T. A., Donzelli, M., Schramm, Y., Dieu, L. H., Huwyler, J.*, et al.* (2013) Pharmacological characterization of designer cathinones in vitro. *British Journal of Pharmacology,* **168**, 458-470.

150. Gygi, M. P., Gibb, J. W. and Hanson, G. R. (1996) Methcathinone: an initial study of its effects on monoaminergic systems. *Journal of Pharmacology and Experimental Therapuetics,* **276**, 1066.

151. Gygi, M. P., Fleckenstein, A. E., Gibb, J. W. and Hanson, G. R. (1997) Role of endogenous dopamine in the neurochemical deficits induced by methcathinone. *Journal of Pharmacology and Experimental Therapuetics,* **283**, 1350-1355.

152. Watterson, L. R., Kufahl, P. R., Nemirovsky, N. E., Sewalia, K., Grabenauer, M., Thomas, B. F.*, et al.* (2014) Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone (mdpv). *Addiction Biology,* **19**, 165-174.

153. Gatch, M. B., Dolan, S. B. and Forster, M. J. (2015) Comparative behavioral pharmacology of three pyrrolidine-containing synthetic cathinone derivatives. *Journal of Pharmacology and Experimental Therapuetics,* **354**, 103.

154. den Hollander, B., Rozov, S., Linden, A.-M., Uusi-Oukari, M., Ojanperä, I. and Korpi, E. R. (2013) Long-term cognitive and neurochemical effects of “bath salt”

designer drugs methylone and mephedrone. *Pharmacology Biochemistry and Behavior,* **103**, 501-509.

155. Cozzi, N. V., Sievert, M. K., Shulgin, A. T., Jacob, P. and Ruoho, A. E. (1999) Inhibition of plasma membrane monoamine transporters by β-ketoamphetamines. *European Journal of Pharmacology,* **381**, 63-69.

156. Araújo, A. M., Valente, M. J., Carvalho, M., Dias da Silva, D., Gaspar, H., Carvalho, F.*, et al.* (2015) Raising awareness of new psychoactive substances: Chemical analysis and in vitro toxicity screening of 'legal high' packages containing synthetic cathinones. *Archives of Toxicology,* **89**, 757-771.

157. De Felice, L. J., Glennon, R. A. and Negus, S. S. (2014) Synthetic cathinones: Chemical phylogeny, physiology, and neuropharmacology. *Life Sciences,* **97**, 20-26.

158. Cameron, K. N., Kolanos, R., Solis, E., Glennon, R. A. and De Felice, L. J. (2013) Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter. *British Journal of Pharmacology,* **168**, 1750-1757.

159. Sauer, C., Peters, F. T., Haas, C., Meyer, M. R., Fritschi, G. and Maurer, H. H. (2009) New designer drug α-pyrrolidinovalerophenone (PVP): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. *Journal of Mass Spectrometry,* **44**, 952-964.

160. Meyer, M. R., Vollmar, C., Schwaninger, A. E., Wolf, E. and Maurer, H. H. (2012) New cathinone-derived designer drugs 3-bromomethcathinone and 3-

fluoromethcathinone: Studies on their metabolism in rat urine and human liver microsomes using GC-MS and LC–high-resolution MS and their detectability in urine. *Journal of Mass Spectrometry,* **47**, 253-262.

161. Meyer, M. R., Du, P., Schuster, F. and 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.

162. Kamata, H. T., Shima, N., Zaitsu, K., Kamata, T., Miki, A., Nishikawa, M.*, et al.* (2006) Metabolism of the recently encountered designer drug, methylone, in humans and rats. *Xenobiotica,* **36**, 709-723.

163. Strano-Rossi, S., Cadwallader, A. B., de la Torre, X. and Botre, F. (2010) Toxicological determination and in vitro metabolism of the designer drug methylenedioxypyrovalerone (MDPV) by gas chromatography/mass spectrometry and liquid chromatography/quadrupole time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry,* **24**, 2706-2714.

164. Pawlik, E., Plässer, G., Mahler, H. and Daldrup, T. (2012) Studies on the phase I metabolism of the new designer drug 3-fluoromethcathinone using rabbit liver slices. *International Journal of Legal Medicine,* **126**, 231-240.

165. Khreit, O. I. G., Grant, M. H., Zhang, T., Henderson, C., Watson, D. G. and Sutcliffe, O. B. (2013) Elucidation of the phase I and phase II metabolic pathways of (±)-4′-methylmethcathinone (4-MMC) and (±)-4′-(trifluoromethyl)methcathinone (4-TFMMC) in rat liver hepatocytes using LC-

MS and LC-MS^2. *Journal of Pharmaceutical and Biomedical Analysis,* **72**, 177-185.

166. Mueller, D. M. and Rentsch, K. M. (2012) Generation of metabolites by an automated online metabolism method using human liver microsomes with subsequent identification by LC-MS^n, and metabolism of 11 cathinones. *Analytical and Bioanalytical Chemistry,* **402**, 2141-2151.

167. Chen, X. (2015) Simultaneous determination of four designer drugs and their major metabolites by liquid chromatography–mass spectrometry. *Journal of Chromatography B,* **992**, 1-7.

168. Helfer, A. G., Turcant, A., Boels, D., Ferec, S., Lelièvre, B., Welter, J., *et al.* (2015) Elucidation of the metabolites of the novel psychoactive substance 4-methyl-n-ethyl-cathinone (4-MEC) in human urine and pooled liver microsomes by GC-MS and LC-HR-MS/MS techniques and of its detectability by GC-MS or LC-MS^n standard screening approaches. *Drug Testing and Analysis,* **7**, 368-375.

169. Pedersen, A. J., Reitzel, L. A., Johansen, S. S. and Linnet, K. (2013) In vitro metabolism studies on mephedrone and analysis of forensic cases. *Drug Testing and Analysis,* **5**, 430-438.

170. Zaitsu, K., Katagi, M., Kamata, H. T., Kamata, T., Shima, N., Miki, A., *et al.* (2009) Determination of the metabolites of the new designer drugs bk-MBDB and bk-MDEA in human urine. *Forensic Science International,* **188**, 131-139.

171. Peters, F. T., Meyer, M. R., Fritschi, G. and Maurer, H. H. (2005) Studies on the metabolism and toxicological detection of the new designer drug 4′-methyl-α-

pyrrolidinobutyrophenone (MPBP) in rat urine using gas chromatography–mass spectrometry. *Journal of Chromatography B,* **824**, 81-91.

172. Michaelis, W., Russel, J. H. and Schindler, O. (1970) Metabolism of pyrovalerone hydrochloride. *Journal of Medicinal Chemistry,* **13**, 497-503.

173. Shin, H.-S., Shin, Y.-S. O., Lee, S. and Park, B.-B. (1996) Detection and identification of pyrovalerone and its hydroxylated metabolite in the rat. *Journal of Analytical Toxicology,* **20**, 568-572.

174. U.S. Drug Enforcement Administration: Diversion Control Division (2011) National forensic laboratory information system special report: Synthetic cannabinoids and synthetic cathinones reported in NFLIS: 2009-2010. Springfield, VA: U.S. Drug Enforcement Administration.

175. U.S. Drug Enforcement Administration: Diversion Control Division (2011) National forensic laboratory information system special report: Synthetic cannabinoids and synthetic cathinones reported in NFLIS: 2010-2013. Springfield, VA: U.S. Drug Enforcement Administration.

176. U.S. Drug Enforcement Administration: Diversion Control Division (2016) Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2013-2015. Springfield, VA: U..S. Drug Enforcement Administration.

177. European Monitoring Centre for Drugs and Drug Addiction. (2017) European Drug Report 2017: Trends and Developments. Available at http://www.emcdda.europa.eu/.

178. Odoardi, S., Romolo, F. S. and Strano-Rossi, S. (2016) A snapshot on NPS in Italy: distribution of drugs in seized materials analysed in an Italian forensic

laboratory in the period 2013–2015. *Forensic Science International,* **265**, 116-120.

179. Elliott, S. and Evans, J. (2014) A 3-year review of new psychoactive substances in casework. *Forensic Science International,* **243**, 55-60.

180. Pichini, S. s. p. i. i., Rotolo, M. C., García, J., Girona, N., Leal, L., García-Algar, O., *et al.* (2014) Neonatal withdrawal syndrome after chronic maternal consumption of 4-methylethcathinone. *Forensic Science International,* **245**, e33-e35.

181. Gil, D., Adamowicz, P., Skulska, A., Tokarczyk, B. and Stanaszek, R. (2013) Analysis of 4-MEC in biological and non-biological material—three case reports. *Forensic Science International,* **228**, e11-e15.

182. Adamowicz, P., Gieroń, J., Gil, D., Lechowicz, W., Skulska, A., Tokarczyk, B., *et al.* (2016) Blood concentrations of α-pyrrolidinovalerophenone (α-PVP) determined in 66 forensic samples. *Forensic Toxicology,* **34**, 227-234.

183. Maskell, P. D., De Paoli, G., Seneviratne, C. and Pounder, D. J. (2011) Mephedrone (4-methylmethcathinone)-related deaths. *Journal of Analytical Toxicology,* **35**, 188-191.

184. Dumestre-Toulet, V., Brault, S., Labadie, M. and Penouil-Pucheu, F. (2017) Madness with five dollars: two new cases of non-lethal poisoning flakka (α-PVP). *Toxicologie Analytique et Clinique,* **29**, 111-116.

185. Kesha, K., Boggs, C. L., Ripple, M. G., Allan, C. H., Levine, B., Jufer-Phipps, R., *et al.* (2013) Methylenedioxypyrovalerone ("bath salts"), related death: case report and review of the literature. *Journal of Forensic Sciences,* **58**, 1654-1659.

186. Shimomura, E. T., Briones, A. J., Warren, W. S., Addison, J. W., Knittel, J. L., Shoemaker, S. A., *et al.* (2016) Case report of methylone, oxymorphone and ethanol in a fatality case with tissue distribution. *Journal of Analytical Toxicology,*

187. Knoy, J. L., Peterson, B. L. and Couper, F. J. (2014) Suspected impaired driving case involving α-pyrrolidinovalerophenone, methylone and ethylone. *Journal of Analytical Toxicology,* **38**, 615-617.

188. Cosbey, S. H., Peters, K. L., Quinn, A. and Bentley, A. (2013) Mephedrone (methylmethcathinone) in toxicology casework: a Northern Ireland perspective. *Journal of Analytical Toxicology,* **37**, 74-82.

189. Belhadj-Tahar, H. and Sadeg, N. (2005) Methcathinone: a new postindustrial drug. *Forensic Science International,* **153**, 99-101.

190. Derungs, A., Schietzel, S., Meyer, M. R., Maurer, H. H., Krähenbühl, S. and Liechti, M. E. (2011) Sympathomimetic toxicity in a case of analytically confirmed recreational use of naphyrone (naphthylpyrovalerone). *Clinical Toxicology,* **49**, 691-693.

191. Saito, T., Namera, A., Osawa, M., Aoki, H. and Inokuchi, S. (2013) SPME–GC–MS analysis of α-pyrrolidinovaleorophenone in blood in a fatal poisoning case. *Forensic Toxicology,* **31**, 328-332.

192. Zuba, D., Adamowicz, P. and Byrska, B. (2013) Detection of buphedrone in biological and non-biological material – two case reports. *Forensic Science International,* **227**, 15-20.

193. Murray, B. L., Murphy, C. M. and Beuhler, M. C. (2012) Death following recreational use of designer drug “bath salts” containing 3, 4-methylenedioxypyrovalerone (MDPV). *Journal of Medical Toxicology,* **8**, 69-75.

194. Kovács, K., Tóth, A. R. and Kereszty, É. M. (2012) A new designer drug: methylone related death. *Orvosi Hetilap,* **153**, 271-276.

195. Adamowicz, P., Gieron, J., Gil, D., Lechowicz, W., Skulska, A. and Tokarczyk, B. (2016) The prevalence of new psychoactive substances in biological material - a three-year review of casework in Poland. *Drug Testing and Analysis, **8**, 63-70.*

196. Cook, D. S., Braithwaite, R. A. and Hale, K. A. (2000) Estimating antemortem drug concentrations from postmortem blood samples: the influence of postmortem redistribution. *Journal of Clinical Pathology,* **53**, 282.

197. Pounder, D. J. and Jones, G. R. (1990) Post-mortem drug redistribution — a toxicological nightmare. *Forensic Science International,* **45**, 253-263.

198. Skopp, G. (2010) Postmortem toxicology. *Forensic Science, Medicine, and Pathology,* **6**, 314-325.

199. Drummer, O. H. (2004) Postmortem toxicology of drugs of abuse. *Forensic Science International,* **142**, 101-113.

200. Leikin, J. B. and Watson, W. A. (2003) Post-mortem toxicology: what the dead can and cannot tell us. *Journal of Toxicology: Clinical Toxicology,* **41**, 47-56.

201. Yarema, M. C. and Becker, C. E. (2005) Key concepts in postmortem drug redistribution. *Clinical Toxicology,* **43**, 235-241.

202. Dalpe-Scott, M., Degouffe, M., Garbutt, D. and Drost, M. (1995) A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. *Canadian Society of Forensic Science Journal,* **28**, 113-121.

203. Hilberg, T., *et al.* (1999) The extent of postmortem drug redistribution in a rat model. *Journal of Forensic Sciences,* **44**, 956-962.

204. Pélissier-Alicot, A.-L., Gaulier, J.-M., Champsaur, P. and Marquet, P. (2003) Mechanisms underlying postmortem redistribution of drugs: a review. *Journal of Analytical Toxicology,* **27**, 533-544.

205. Ferner, R. E. (2008) Post-mortem clinical pharmacology. *British Journal of Clinical Pharmacology,* **66**, 430-443.

206. Butzbach, D. M. (2010) The influence of putrefaction and sample storage on post-mortem toxicology results. *Forensic Science, Medicine, and Pathology,* **6**, 35-45.

207. Jones, G. R. and Pounder, D. J. (1987) Site dependence of drug concentrations in postmortem blood—a case study. *Journal of Analytical Toxicology,* **11**, 186-190.

208. Apple, F. S. (2011) A better understanding of the interpretation of postmortem blood drug concentrations. *Journal of Analytical Toxicology,* **35**, 381-383.

209. Dinis-Oliveira, R. J., Carvalho, F., Duarte, J. A., Remião, F., Marques, A., Santos, A., *et al.* (2010) Collection of biological samples in forensic toxicology. *Toxicology Mechanisms and Methods,* **20**, 363-414.

210. Han, E., Kim, E., Hong, H., Jeong, S., Kim, J., In, S., *et al.* (2012) Evaluation of postmortem redistribution phenomena for commonly encountered drugs. *Forensic Science International,* **219**, 265-271.

211. McIntyre, I., Hamm, C. and Bader, E. (2011) Postmortem methamphetamine distribution. *Journal of Forensic Research,* **2**, 1-3.

212. Gerostamoulos, D., Beyer, J., Staikos, V., Tayler, P., Woodford, N. and Drummer, O. H. (2012) The effect of the postmortem interval on the redistribution of drugs: A comparison of mortuary admission and autopsy blood specimens. *Forensic Science, Medicine, and Pathology,* **8**, 373-379.

213. Barnhart, F. E., Fogacci, J. R. and Reed, D. W. (1999) Methamphetamine—a study of postmortem redistribution. *Journal of Analytical Toxicology,* **23**, 69-70.

214. Garrett, E. R., Seyda, K. and Marroum, P. (1991) High performance liquid chromatographic assays of the illicit designer drug "ecstasy", a modified amphetamine, with applications to stability, partitioning and plasma protein binding. *Acta Pharm Nord,* **3**, 9-14.

215. Huestis, M. A. and Cone, E. J. (2007) Methamphetamine disposition in oral fluid, plasma, and urine. *Annals of the New York Academy of Sciences,* **1098**, 104-121.

216. Dams, R., De Letter, E. A., Mortier, K. A., Cordonnier, J. A., Lambert, W. E., Piette, M. H. A., *et al.* (2003) Fatality due to combined use of the designer drugs MDMA and PMA: a distribution study. *Journal of Analytical Toxicology,* **27**, 318-323.

217. De Letter, E. A., Bouche, M.-P. L. A., Van Bocxlaer, J. F., Lambert, W. E. and Piette, M. H. A. (2004) Interpretation of a 3,4-methylenedioxymethamphetamine (MDMA) blood level: discussion by means of a distribution study in two fatalities. *Forensic Science International,* **141**, 85-90.

218. De Letter, E. A., Clauwaert, K. M., Lambert, W. E., Van Bocxlaer, J. F., De Leenheer, A. P. and Piette, M. H. A. (2002) Distribution study of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine in a fatal overdose. *Journal of Analytical Toxicology,* **26**, 113-118.

219. Logan, B., Weiss, E. and Harruff, R. (1996) Case report: distribution of methamphetamine in a massive fatal ingestion. *Journal of Forensic Sciences*, **41**, 322-323.

220. Elliott, S. P. (2005) MDMA and MDA concentrations in antemortem and postmortem specimens in fatalities following hospital admission. *Journal of Analytical Toxicology,* **29**, 296-300.

221. Rohrig, T. P. and Prouty, R. W. (1992) Tissue distribution of methylenedioxymethamphetamine. *Journal of Analytical Toxicology,* **16**, 52-53.

222. Martínez-Clemente, J., López-Arnau, R., Carbó, M., Pubill, D., Camarasa, J. and Escubedo, E. (2013) Mephedrone pharmacokinetics after intravenous and oral administration in rats: relation to pharmacodynamics. *Psychopharmacology,* **229**, 295-306.

223. Kerrigan, S. (2013) Sampling, Storage and Stability, in: A. Negrusz, G. Cooper (Eds.), Clarke's Analytical Forensic Toxicology 2 Ed, Pharmaceutical Press, London, England, pp 335-347

224. Szendrei, K. (1980) The Chemistry of Khat. *Bulletin on Narcotics,* **32,** 5-36.

225. Berrang, B. D., Lewin, A. H. and Carroll, F. I. (1982) Enantiomeric α-aminopropiophenones (cathinone): preparation and investigation. *The Journal of Organic Chemistry,* **47**, 2643-2647.

226. Chappell, J. S. and Lee, M. M. (2010) Cathinone preservation in khat evidence via drying. *Forensic Science International,* **195**, 108-120.

227. Tsujikawa, K., Yamamuro, T., Kuwayama, K., Kanamori, T., Iwata, Y. T. and Inoue, H. (2015) Instability of the hydrochloride salts of cathinone derivatives in air. *Forensic Science International,* **248**, 48-54.

228. Tsujikawa, K., Mikuma, T., Kuwayama, K., Miyaguchi, H., Kanamori, T., Iwata, Y. T.*, et al.* (2012) Degradation pathways of 4-methylmethcathinone in alkaline solution and stability of methcathinone analogs in various pH solutions. *Forensic Science International,* **220**, 103-110.

229. Maskell, P. D., Seetohul, L. N., Livingstone, A. C., Cockburn, A. K., Preece, J. and Pounder, D. J. (2013) Stability of 3,4-methylenedioxymethampetamine (MDMA), 4-methylmethcathinone (mephedrone) and 3-trifluromethylphenylpiperazine (3-TFMPP) in formalin solution. *Journal of Analytical Toxicology,* **37**, 440-446.

230. Morad, A., Al-Meshal, I., Nasir, M. and El-Feraly, F. (1989) High-performance liquid chromatographic determination of (−)-cathinone in plasma. *Chromatographia,* **27**, 201-204.

231. Cook, J. D., Strauss, K. A., Caplan, Y. H., LoDico, C. P. and Bush, D. M. (2007) Urine pH: the effects of time and temperature after collection. *Journal of Analytical Toxicology,* **31**, 486-496.

232. Fura, A., Harper, T. W., Zhang, H., Fung, L. and Shyu, W. C. (2003) Shift in pH of biological fluids during storage and processing: effect on bioanalysis. *Journal of Pharmaceutical and Biomedical Analysis,* **32**, 513-522.

CHAPTER II

IDENTIFICATION AND QUANTIFICATION OF SYNTHETIC CATHINONES IN BLOOD AND URINE USING LIQUID CHROMATOGRAPHY-QUADRUPOLE/TIME OF FLIGHT (LC-Q/TOF) MASS SPECTROMETRY[1]

This dissertation follows the style and format of *The Journal of Analytical Toxicology*.

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Abstract

Synthetic cathinones continue to present a formidable challenge to forensic toxicology laboratories despite the fact that they are often encountered in impaired driving and death investigations. Due to limitations in immunoassay-based screening technologies, many forensic toxicology laboratories must rely on more labor intensive chromatographic-based screening approaches in order to detect these drugs in biological evidence. Solid phase extraction (SPE) and liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry were used to identify twenty-two synthetic cathinones in urine and blood. Target drugs included methcathinone, ethcathinone, pentedrone, buphedrone, 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (4-FMC), 4-methylethcathinone (4-MEC), 4-ethylmethcathinone (4-EMC), mephedrone, methedrone, 3,4-dimethylmethcathinone (3,4-DMMC), ethylone, butylone, pentylone, eutylone, methylone, methylenedioxypyrovalerone (MDPV), 4-methylpyrrolidinobutiophenone (MPBP), 3,4-methylenedioxypyrrolidinobutiophenone (MDPBP), α-pyrrolidinopentiphenone (α-PVP), pyrovalerone, and naphyrone. A total of nine deuterated internal standards were employed. Using traditional reversed phase chromatography all positional isomers, including 3-FMC and 4-FMC, were separated in 12 mins. The procedure was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation. Extraction efficiencies were 84-104% and 81-93% in urine and blood, respectively. Limits of quantitation in both matrices were 0.25 – 5 ng/mL. Precision, bias and matrix effect were all within acceptable thresholds and the assay was free from more than fifty interferences. The validated method was used to identify cathinones in authentic urine case samples (n=20) and these results

highlight important considerations for cathinone stability and the subsequent interpretation of results.

Keywords: Cathinone, Designer drug, Liquid chromatography-mass spectrometry, High resolution mass spectrometry

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Introduction

The proliferation of new psychoactive substances (NPSs) continues to present a variety of challenges from both a public health and public safety standpoint. Abuse of NPSs has increased significantly in recent years and many drugs within this class are now well-established as recreational drugs. This is certainly true of the synthetic cannabinoids and cathinones, both of which have had far-reaching consequences on the field of forensic toxicology over the past decade.

The sought after effects of synthetic cathinones are similar to those of other stimulants and may include increased energy, mood enhancement, exhilaration and psychoactive effects. Adverse effects include panic attacks, tremors, depression and psychosis. Synthetic cathinones are also associated with neurological, cardiovascular and psychopathological symptoms such as tachycardia, hallucinations, delusions, violence, aggressive tendencies and death (1-3). Due to the severity of these adverse consequences and an increase in synthetic cathinone-related emergency room visits and fatal intoxications, the Drug Enforcement Administration (DEA) has scheduled more than a dozen synthetic cathinones since 2011 (4, 5). Enforcement actions are often confounded by the swift appearance of new analogs. For this reason, many states have legislatively enforced “general class bans” as part of an ongoing effort to discourage cathinone abuse (6).

Despite efforts to curb their appeal, synthetic cathinone use is now widespread and there are numerous reports of their involvement in criminal and death investigations. The proliferation of these new drugs presents a significant challenge to forensic toxicology laboratories. The rate at which new drugs are being introduced can significantly outpace the ability of many forensic toxicology laboratories to develop and validate methods of analysis. Moreover, many of the newer drugs are not readily detected using traditional approaches for screening and confirmation and may require more specialized and costly instrumental techniques.

Immunoassay screening has not proven effective for the large number of drugs within the synthetic cathinone class. Antibody cross-reactivity using commercial amphetamine-based immunoassays are highly variable (7, 8). While biochip array and antibody-based assays have been reported for some of the cathinones, the rapid emergence of new structural analogs presents a formidable challenge for immunoassay-based screening technologies.

Cathinones are known to undergo a variety of phase I transformations including *N*-dealkylation, reduction, hydroxylation, oxidation and demethylenation. Despite a growing body of research on cathinone metabolism, commercial metabolite standards are only available for a relatively small number of cathinones. For this reason, most toxicology methods tend to target the parent drug, and this approach has proven successful due to a sufficiently high concentration of parent drug present in biological samples (9, 10) .

Gas chromatography-mass spectrometry (GC-MS) remains the most widely used technique in forensic toxicology in large part due to its robustness, specificity, and low capital outlay relative to other hyphenated chromatographic techniques. However,

synthetic cathinones may undergo thermal degradation during GC analysis. Noggle and DeRuiter (11) were the first to document this phenomenon in 1994 using methcathinone. Archer and Tsujikawa (12, 13) observed similar in-situ degradation using 4-FMC and α-PVP. More recently this phenomenon was demonstrated using eighteen synthetic cathinones (14). Unfortunately, these oxidative breakdown products can be difficult to separate from their parent drug and share many of the same characteristic ions. Furthermore, cathinones undergo extensive fragmentation during electron impact ionization (EI) and the strong tendency of these beta-keto amphetamines to undergo alpha cleavage can result in mass spectra with a limited number of diagnostic ions (15-18). EI spectra are heavily dominated by the immonium ion of relatively low mass, while lower intensity acylium and arylium ions tend to lack specificity due to common benzylic substituents among the cathinones.

Liquid chromatography-mass spectrometry (LC-MS) approaches can facilitate a larger complement of diagnostic ions because electrospray ionization (ESI) conditions can be carefully controlled and optimized to meet analyte-dependent needs. Hyphenated LC techniques have been described for many of the synthetic cathinones. However, Ammann (19) was the first to report the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) to simultaneously identify a large number of cathinones (twenty-five) in blood. More recently, high resolution mass spectrometry (HRMS) has been used to detect cathinones in blood (20) and urine (21-23). Although traditional triple quadrupole mass spectrometry is considered to offer the greatest sensitivity, HRMS approaches such as quadrupole-time of flight and Orbitrap™ offer improved mass accuracy, enhanced

selectivity, and the opportunity for retrospective data interrogation, the latter of which is a distinct advantage for such a rapidly evolving drug class.

One of the greatest challenges within the cathinone class are the number of isobaric compounds. This is of particular importance when certain positional isomers are scheduled, while others are not. Since many of these isomers share the same precursor, product ions and fragmentation pathways, mass separation is not always feasible. Hence, significant emphasis must be placed on chromatographic separation in order to make a definitive identification. While most reported LC methods have separated several isomers, the fluorinated derivatives present the greatest challenge (10, 19) and many published reports do not include multiple fluorinated isomers in their assays for this reason. Li (24) was the first to report the separation of 3- and 4-fluoromethcathinone using hydrophilic interaction liquid chromatography (HILIC) tandem mass spectrometry, following unsuccessful use of traditional reversed phase approaches.

In this report we describe the separation of all isomer pairs, including 3-FMC and 4-FMC using a traditional C18 column and HRMS. We present a validated method for the quantitative determination of twenty-two synthetic cathinones in urine and blood using LC-Q/TOF MS. The method was used to reanalyze authentic urine samples after ten months of refrigerated storage. The quantitative assay was developed as part of an on-going study to systematically evaluate cathinone stability in biological evidence. For this reason, a total of sixteen secondary amines (four unsubstituted and twelve ring-substituted) and six tertiary amines (pyrrolidines) were selected (**Figure 2.1**).

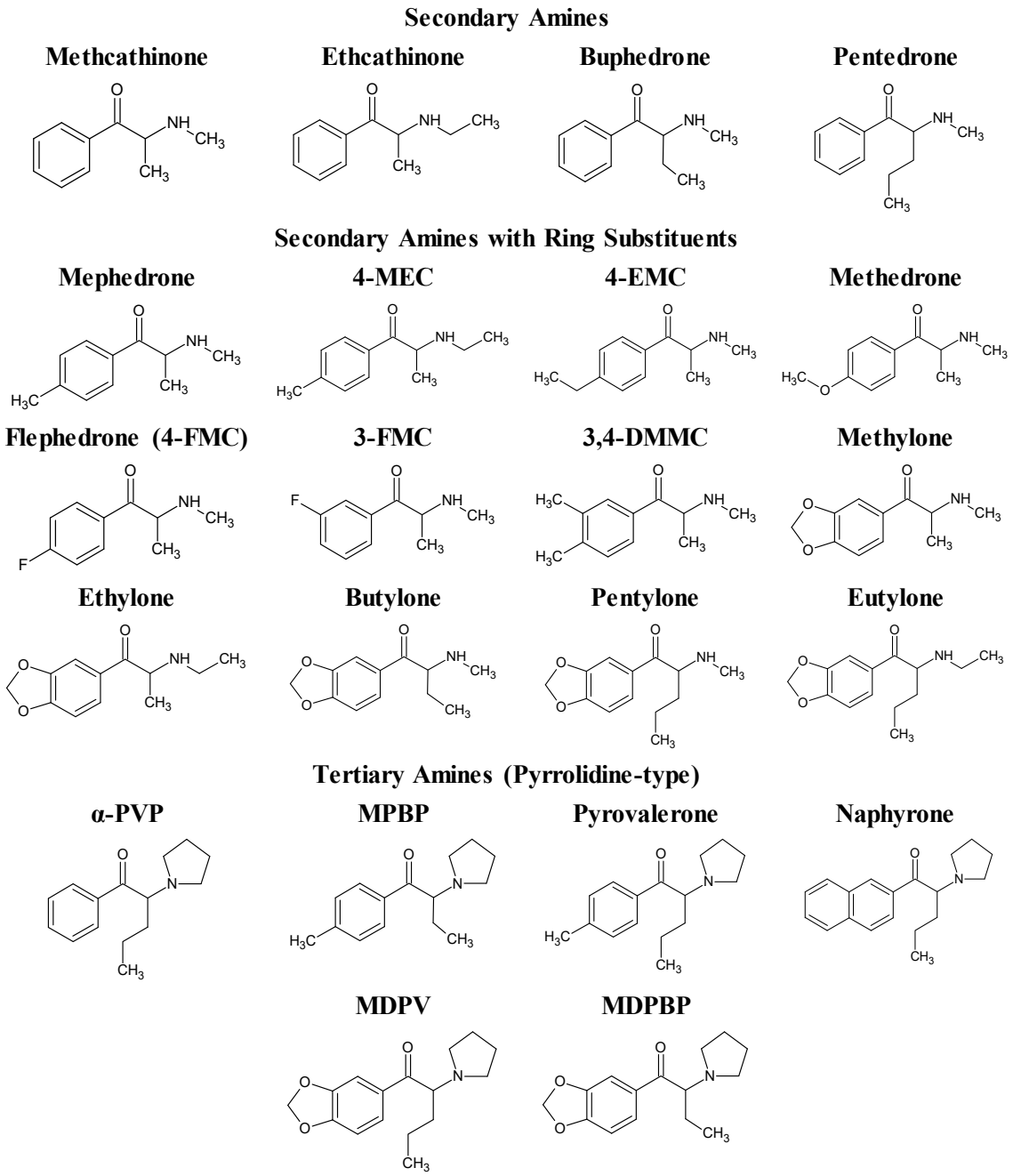

Figure 2.1. Target analytes included in the study.

Materials and Methods

Chemicals and reagents

Reference standards including 3,4-DMMC, 3-FMC, 4-EMC, 4-FMC, 4-MEC, α-PVP, buphedrone, butylone, ethcathinone, ethylone, eutylone, MDPBP, MDPV, methcathinone, methedrone, methylone, mephedrone, MPBP, naphyrone, pentedrone, pentylone, pyrovalerone and internal standards (butylone-D3, ethylone-D3, naphyrone-D3, α-PVP-D8, pentylone-D3, eutylone-D5, methylone-D3, mephedrone-D3 and MDPV-D8) were purchased from Cerilliant Corp. (Round Rock, TX, USA). Reference materials were purchased as methanolic 1.0 mg/mL standards with the exception of deuterated analogs (0.1 mg/mL). Pooled drug-free urine purchased from Utak Laboratories (Valencia, CA, USA) was preserved with 1% sodium fluoride prior to use. Bovine blood preserved with 1% sodium fluoride and 0.2% potassium oxalate was purchased from Quad Five (Ryegate, Montana, USA).

Dichloromethane, isopropyl alcohol and glacial acetic acid were obtained from Mallinckrodt Chemicals (St. Louis, MO, USA) and methanol (LCMS grade), concentrated hydrochloric acid, acetonitrile (LCMS grade) and dibasic sodium phosphate (Na_2HPO_4, ACS grade) were obtained from J.T. Baker (Center Valley, MA, USA). Hexane (Optima®) and ethyl acetate (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and formic acid (>95%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Concentrated ammonium hydroxide was obtained from Macron Fine Chemicals (Center Valley, MA, USA) and monobasic sodium phosphate (NaH_2PO_4, ACS grade) was obtained from VWR (Radnor, PA, USA). Deionized water was purified in-house using a Millipore Direct-Q® UV Water Purification system (Billerica, MA, USA). PolyChrom Clin II 3 cc

(35 mg) solid phase extraction (SPE) columns were obtained from SPEware (Baldwin Park, CA, USA).

Instrumental analysis

Nitrogen was generated using a Genius 3040 nitrogen generator (Peak Scientific, Billerica, MA, USA). SPE was performed using a JT Baker vacuum manifold and extracts were evaporated to dryness under nitrogen using a TurboVap LV® concentration workstation (Caliper Life Sciences, Hopkinton, MA, USA). An Agilent Technologies 6530 LC-Q/TOF MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 1290 Infinity autosampler was used to analyze samples. Separation was achieved using an Agilent Technologies Series 1200 LC system equipped with an Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm particle size) and an Agilent Poroshell 120 EC-C18 guard column (2.1 x 5 mm, 2.7 µm particle size) in a thermostatically controlled column compartment (35ºC).

The mobile phase consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile (B). A flow rate of 0.4 mL/min was maintained using the gradient elution profile as follows: 96% A and 4% B (0 – 0.5 mins); 10% B (0.5 – 5 mins); 40% B (5 – 11 mins); 100%B (12 mins). The column was rinsed with 100% B for 1 minute before re-equilibration. The total acquisition time was 12 mins and the target compounds eluted between 3 and 11 mins.

The LC-Q/TOF MS was equipped with an ESI source in positive mode with Jet Stream technology under the following conditions: drying gas (N_2), 13 L/min; drying gas temperature, 200ºC; nebulizer, 20 psi; sheath gas temperature, 250ºC; nitrogen sheath gas flow, 12 L/min; capillary voltage, 4000 V; nozzle voltage, 0 V; fragmentor, 150 V;

skimmer, 65V. Agilent MassHunter software was used for acquisition, qualitative and quantitative analysis. Following optimization of collision induced dissociation (CID) voltages, a minimum of two transition ions were selected with a mass tolerance of 5 ppm using targeted MS/MS acquisition. Precursor ion resolution of 7,000 (m/z 164-287) and MS/MS resolution of 6,000 was observed at full width half maximum (FWHM). Precursor and product ions, collision energies, retention times and the internal standard for each drug are summarized in **Table 2.1**. Precursor ions were selected in the quadrupole using a 1 Dalton (Da) window. Data was acquired using a mass range of 40-1000 Da, with a MS scan rate of 8 spectra/sec and a MS/MS Scan rate of 3 spectra/sec. During the selection of product ions, greater emphasis was placed on specificity rather than sensitivity. Non-specific losses (including water loses) that have been utilized in publications elsewhere were not considered acceptable.

Table 2.1. Transition ions, collision energies (CE), retention time (RT), and internal standard selection. Analytes are listed in retention time order and ion ratios are shown in parentheses.

Cathinone	Precursor Ion (m/z)	Product Ions (m/z)	CE (V)	RT (min)	Internal Standard
Methcathinone	164.1075	131.0731 (100%) 105.0703 (25%)	20	3.394	Mephedrone-D3
3-FMC	182.0976	149.0634 (100%) 123.0605 (15%)	20	3.938	Mephedrone-D3
4-FMC	182.0976	149.0636 (100%) 123.0605 (22%)	20	4.094	Mephedrone-D3
Methylone	208.0968	160.0757 (100%) 132.0807 (37%)	20	4.133	Methylone-D3

(continued)

Cathinone	Precursor Ion (m/z)	Product Ions (m/z)	CE (V)	RT (min)	Internal Standard
Ethcathinone	178.1226	131.0721 (100%) 117.0586 (34%) 105.0700 (50%)	20	4.302	Butylone-D3
Ethylone	222.1125	174.1222 (100%) 146.0958 (79%)	30	5.153	Ethylone-D5
Methedrone	194.1176	161.0833 (100%) 146.0598 (41%) 135.0803 (22%)	20	5.291	Mephedrone-D3
Buphedrone	178.1226	131.0731 (100%) 91.0549 (58%) 145.0880 (14%)	20	5.442	Mephedrone-D3
Butylone	222.1125	174.0914 (100%) 146.0964 (84%)	30	6.259	Butylone-D3
Mephedrone	178.1226	145.0889 (100%) 119.0853 (14%)	20	6.444	Mephedrone-D3
Eutylone	236.1281	188.1069 (100%) 174.0547 (104%) 161.0598 (26%)*	30	6.901	Eutylone-D5
4-MEC	192.1383	145.0886 (100%) 159.1041 (33%)* 131.0738 (30%)	20	7.185	Mephedrone-D3
MDPBP	262.1438	161.0597 (100%) 191.0704 (80%) 112.1125 (96%)	20	7.225	Eutylone-D5
Pentedrone	192.1383	132.0810 (100%) 91.0546 (68%)	20	7.505	Mephedrone-D3
Pentylone	236.1281	188.1070 (100%) 175.0682 (40%)	30	7.813	Pentylone-D3
3,4-DMMC	192.1383	159.1043 (100%) 144.0802 (24%)	20	8.055	Methylone-D3
α-PVP	232.1696	161.0954 (100%) 91.0549 (367%)	20	8.159	α-PVP-D8

(continued)

Cathinone	Precursor Ion (m/z)	Product Ions (m/z)	CE (V)	RT (min)	Internal Standard
4-EMC	192.1383	145.0889 (100%) 105.0701 (10%)	20	8.232	Mephedrone-D3
MPBP	232.1696	161.0960 (100%) 133.1010 (48%) 112.1120 (61%)	20	8.410	Naphyrone-D5
MDPV	276.1594	205.0857 (100%) 126.1277 (137%) 175.0756 (116%)	20	8.444	MDPV-D8
Pyrovalerone	246.1852	175.1110 (100%) 126.1280 (63%) 105.0701 (212%)	20	9.450	Naphyrone-D5
Naphyrone	282.1852	211.1122 (100%) 126.1280 (37%) 141.0701 (143%)	20	10.774	Naphyrone-D5

Preparation of standards and reagents

Working standards containing all twenty-two target compounds were prepared in methanol at 0.01, 0.1 and 1.0 µg/mL for the fortification of urine, and 0.02, 0.2, and 2.0 µg/mL for the fortification of blood. The combined internal standard solution consisted of nine isotopically labelled standards in methanol at 0.25 µg/mL and 0.5 µg/mL for urine and blood, respectively. Phosphate buffer (pH 6, 0.1M) was prepared from 0.1M solutions of mono and dibasic sodium phosphate, and acidic methanol consisted of concentrated hydrochloric acid diluted in methanol (2%, v/v). The elution solvent which was prepared daily, consisted of 2% concentrated ammonium hydroxide in 95:5 dichloromethane/isopropyl alcohol (v/v).

Urine extraction

Internal standard solution (0.25 µg/mL) was added to 1.0 mL urine to achieve a final concentration of 25 ng/mL. Urine was diluted with 2.0 mL of pH 6.0 phosphate buffer

(0.1M) and briefly vortexed. Samples were transferred to PolyChrom Clin II SPE columns (3 cc columns, 35 mg) and allowed to flow through under gravity or sufficient vacuum to maintain constant flow (approximately 1 mL/min). Columns were rinsed with 1.0 mL deionized water followed by 1.0 mL of 1 M acetic acid. After drying columns for five mins on full vacuum, samples were washed successively using hexane (1.0 mL), ethyl acetate (1.0 mL) and methanol (1.0 mL). Cathinones were eluted using two 0.5 mL aliquots of elution solvent. Acidic methanol (30 μL) was added to each extract prior to evaporation under nitrogen at 50°C. Extracts were reconstituted in 25 μL of a 50:50 mixture of Mobile Phase A/B and 1 μL was injected onto the LC-Q/TOF MS for analysis.

Blood extraction

Internal standard solution (0.5 μg/mL) was added to 2.0 mL blood to achieve a final concentration of 25 ng/mL. A protein precipitation was performed with the addition of 4.0 mL of cold acetonitrile while vortex mixing, followed by centrifugation at 4000 rpm for 5 mins. The supernatant was decanted and diluted with 6.0 mL of pH 6.0 phosphate buffer (0.1M) and briefly vortexed. Samples were transferred to SPE columns (3 cc columns, 35 mg) and extracted in a manner analogous to urine. Extracts were reconstituted in 25 μL of a 50:50 mixture of Mobile Phase A/B and 1 μL was injected onto the LC-Q/TOF MS for analysis.

Assay validation

Assay performance was evaluated in terms of extraction efficiency, calibration model, precision, bias, limit of detection (LOD), limit of quantification (LOQ), matrix effects, interference, ion suppression, carryover, processed sample stability, and dilution integrity in accordance with published recommendations (25).

The extraction efficiency in blood (100 ng/mL) and urine (25 ng/mL) was determined by direct comparison of extracted and non-extracted samples. Urine and blood containing internal standard (25 ng/mL) was extracted in the presence and absence of the target compounds. Samples extracted without target compounds were fortified with equivalent drug post-extraction (prior to evaporation and reconstitution). Analytical recovery was calculated by comparing the relative peak area (drug/IS) for extracted samples (n=4) with the mean relative peak are for the non-extracted samples (n=4).

Limits of detection and quantitation were established using drug-free blood and urine fortified with reference materials. Three sources of drug-free matrix were analyzed in duplicate over three independent runs. The LOD was the lowest concentration of drug that produced a reportable result (signal to noise ratio of 3:1 or more; retention time ±2% of the standard; ion ratios ± 20%). Limit of quantitation was determined contemporaneously and was defined as the lowest concentration of drug to produce a quantitative value within 20% of the expected value, a S/N ratio of 10:1 or more, retention time ±2% of the standard, ion ratios within 20% and acceptable precision and bias.

Precision and bias were evaluated in urine (10, 100 and 1,000 ng/mL) and blood (20, 100, 1,000 ng/mL) using three samples of pooled fortified matrix at three concentrations (low, medium, high) over five runs. Within-run precision was calculated for each concentration (n=3) over each of the five runs. Between-run precision was calculated for each concentration over all five runs (n=15). Bias was evaluated contemporaneously with precision using the same concentrations over five days. Tolerance for bias and precision was 20%.

The calibration model was established in accordance with SWGTOX recommendations using a minimum of six non-zero calibrators over five independent runs. Calibration models were evaluated visually and analytically using the correlation coefficient (R^2), standardized residual plots and the F-test to determine the significance of the quadratic term (α=0.05). A total of seven non-zero calibrators were used in urine (5, 10, 50, 100, 250, 500, and 1,000 ng/mL) and eight in blood (5, 10, 50, 100, 250, 500, 750, and 1,000 ng/mL).

Interferences associated with the biological matrix, isotopically labeled internal standards, common drugs and structurally related compounds were systematically evaluated. Matrix interferences were evaluated using ten drug-free blood and urine samples from independent sources in the absence of internal standard. Ion contributions arising from the use of stable isotope internal standards were evaluated by fortifying drug-free urine and blood with internal standard (25 ng/mL) and monitoring the signal of the target analytes. In a similar fashion, ion contributions associated with high concentrations of drug (1,000 ng/mL) were evaluated in the absence of internal standard. Drug interferences were evaluated using four categories of compounds: common amphetamines, structurally related designer drugs, common drugs and other therapeutic drugs of significance**.** Twenty-two common drugs, ten common amphetamine-type drugs and fifteen structurally related designer drugs were selected. Diethylpropion (amfepramone, Tenuate®) and bupropion (Wellbutrin®, Zyban®) are therapeutically used cathinones. These were included in the interference study, together with hydroxybupropion (metabolite) and ropivacaine (Naropin®) (due to its mass spectral similarity to MDPV). Putrefactive amines were also included for the interference study in blood. Interferences were assessed using negative

and positive controls. A 10 to 100-fold excess of interferent (relative to the target drug) was employed for interference testing. The negative control consisted of drug-free urine or blood fortified with internal standard (25 ng/mL) and 1000 ng/mL of interferent; positive controls contained internal standard (25 ng/mL), interferent (1,000 ng/mL) and target cathinones at a ten-fold and hundred-fold lower concentration (100 and 10 ng/mL, respectively). Interferences associated with more than fifty drugs were evaluated in total and these are summarized in **Table 2.2**.

Table 2.2. Summary of compounds included in the interference study. Compounds were separated into five groups: common drugs (n=22), amphetamine-like drugs (n=10), designer drugs (n=15), other drugs (n=4), and putrefactive amines (n=4).

Common Drugs	**Amphetamine-Like Drugs**	**Designer Drugs**	**Other Drugs**	**Putrefactive Amines (Blood Only)**
Alprazolam	Amphetamine	2C-B	Bupropion	Putrescine
Amitriptyline	Methamphetamine	2C-C	Diethylpropion	Phenethylamine
Caffeine	MDA	2C-D	Hydroxy-bupropion	Tryptamine
Cocaine	MDEA	2C-E	Ropivacaine	Tyramine
Codeine	MDMA	2C-H		
Cotinine	MBDB	2C-I		
Cyclobenzaprine	Ephedrine	2C-T-2		
Dextromethorphan	Pseudoephedrine	2C-T-4		
Diazepam	Phentermine	2C-T-7		
Diphenhydramine	Phenylpropanolamine	4-MTA		
Hydrocodone		DOB		
Ketamine		DOC		
Methadone		DOI		
Morphine		DOET		
Nicotine		DOM		
Nordiazepam				
Oxazepam				
Oxycodone				
Phencyclidine				
Propoxyphene				
Tramadol				
Zolpidem				

4-bromo-2,5-dimethoxyphenethylamine (2C-B), 2,5-dimethoxy-4-chlorophenethylamine (2C-C), 2,5-dimethoxy-4methylphenethylamine (2C-D), 2,5-dimethoxy-4-ethylphenethylamine (2C-E), 2,5-dimethoxyphenethylamine (2C-H), 2,5-dimethoxy-4-iodophenethylamine (2C-I), 2,5-dimethoxy4-ethylthiophenethylamine (2C-T-2), 2,5-dimethoxy-4-isopropylthiophenethylamine (2C-T-4), 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7), 4-methylthioamphetamine (4-MTA), 2,5-dimethoxy-4-bromoamphetamine (DOB), 2,5-dimethoxy-4-chloroamphetamine (DOC), 2,5-dimethoxy-4-ethylamphetamine (DOET), 2,5-dimethoxy-4-iodoamphetamine (DOI), 2,5-dimethoxy-4-methylamphetamine (DOM).

Matrix effects were quantitatively assessed using post-extraction addition at two concentrations (20 ng/mL and 200 ng/mL for urine; 50 ng/mL and 500 ng/mL for blood). Ten drug-free matrices from independent sources (n=2) were extracted in the absence of drug and fortified with drug post-extraction. Ion suppression or enhancement was calculated by comparing the mean peak areas of drug in matrix with the drug in mobile phase (no matrix).

Carryover was assessed by analyzing a negative control immediately following the injection of a high control (1,000, 2,500, and 5,000 ng/mL). Carryover was present when the negative control produced a reportable result (signal to noise ratio of 3:1 or more, retention time ±2% and ion ratios within 20% of expected). The influence of sample dilution was evaluated using urine or blood fortified at 1,000 ng/mL. Dilution integrity for urine was determined using two- and four-fold dilutions in 0.1M pH 6.0 phosphate buffer (to achieve final volume of 1.0 mL) prior to extraction. Dilution integrity for blood using two- and four-fold dilutions was determined by direct precipitation of 0.5 mL or 1.0 mL blood with 4.0 mL of cold acetonitrile. Quantitative results were evaluated and calculated concentrations within 20% of the expected concentration were deemed acceptable. The stability of processed samples was assessed by extracting samples (25 ng/mL and 350 ng/mL) in triplicate and analyzing the same extracts over a period of up to 48 hours. The samples were stored in the refrigerated autosampler tray and were considered stable until the quantitative result produced a bias exceeding ± 20%.

Authentic unpreserved urine samples (n=20) from cathinone users were reanalyzed using the LC-Q/TOF method in accordance with an Institutional Review Board (IRB)

approved study. Specimens provided by Redwood Toxicology Laboratories (RTL) were reanalyzed and compared with the original results.

Results and Discussion

Synthetic cathinones can thermally degrade during GC-MS analysis. This oxidative degradation is characterized by the loss of two hydrogens to produce a 2,3-enamine or an imine (11, 14). As part of the routine ionization optimization process, the possibility of thermal degradation was considered. Data was acquired using a non-targeted (full scan) method. Precursor ions for known (-2 Da) degradation products were not present for any of the drugs, demonstrating that heated conditions inside the ESI source did not result in thermal degradation of cathinones.

An overlaid chromatogram depicts the chromatographic separation of all twenty-two cathinones, including 3- and 4-FMC (**Figure 2.2**). Individual extracted ion chromatograms and MS/MS spectra, with tentative structures for quantifier and qualifier ions, for each cathinone are located in **Appendix A**. **Table 2.3** summarizes structural losses that produce product ions. Proposed fragments for common losses in secondary, unsubstituted cathinones, secondary, substituted cathinones, secondary, methylenedioxy-type cathinone, and tertiary (pyrrolidine-type) cathinones bearing the methylenedioxy group presented in **Figure 2.3**. Mass accuracy for a 100 ng/mL control in both matrices was within 5 ppm **(Table 2.4).** Extraction efficiencies were 84-104% in urine at 25 ng/mL (n=4) and 81-93% in blood at 100 ng/mL (n=4) (**Table 2.5**).

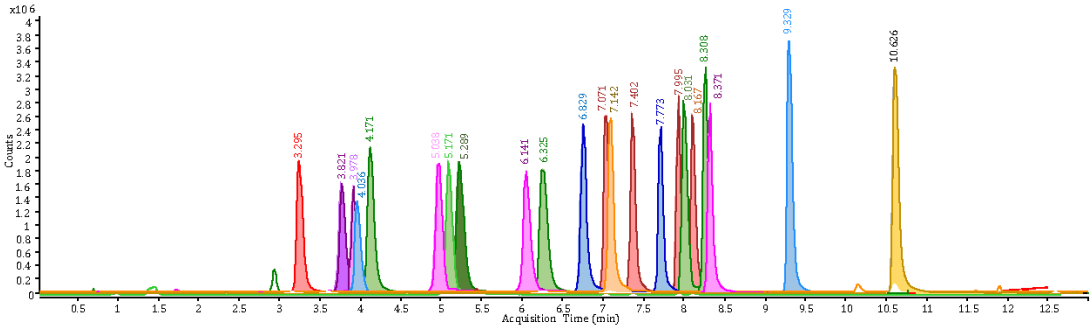

Figure 2.2. Chromatographic separation of cathinones, including positional isomers in a representative extract (100 ng/mL). Internal standards are excluded for clarity. methcathinone (3.295); 3-FMC (3.821); 4-FMC (3.978); methylone (4.036); ethcathinone (4.171); ethylone (5.038); methedrone (5.171); buphedrone (5.298); butylone (6.141); mephedrone (6.325); eutylone (6.829); 4-MEC (7.071); MDPBP (7.142); pentedrone (7.402); pentylone (7.773); 3,4-DMMC (7.995); α-PVP (8.031); 4-EMC (8.167); MPBP (8.308); MDPV (8.371); pyrovalerone (9.329); naphyrone (10.626).

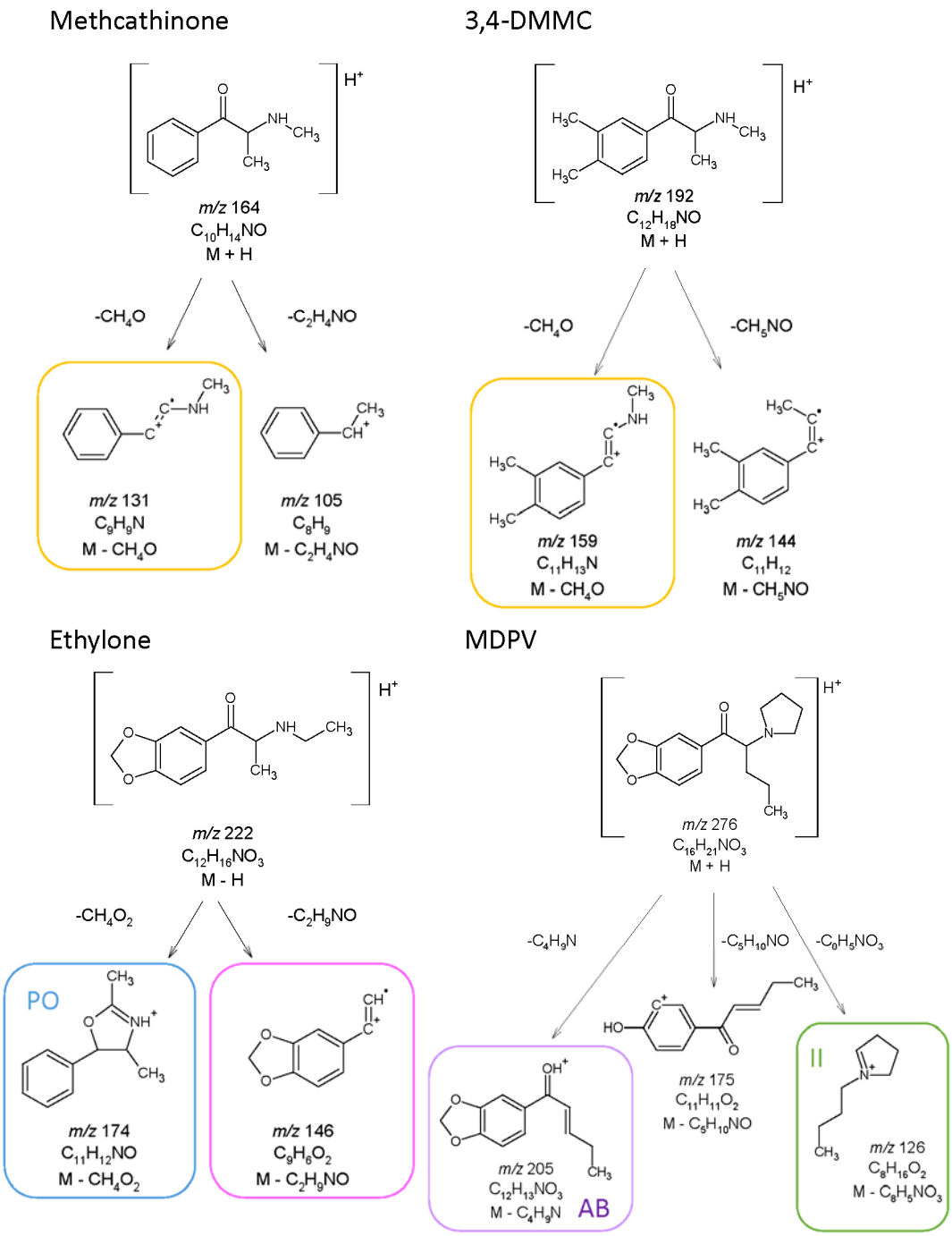

Figure 2.3. Proposed fragmentation pathways for common losses. Common losses include CH_4O in unsubstituted and substituted cathinones (orange, methcathinone, 3,4-DMMC), methylenedioxy loss to form phenyloxazole (blue) and loss of $R_3R_4R_5NO$ (red) in methylenedioxy-type cathinones, and dissocation of pyrrolidinyl to form alkyldioxybenzoyloxonium ion (purple) and immonium ion (green) in tertiary amine cathinones.

Table 2.3. Proposed fragmentation for quantifier (bold) and qualifier transitions.

Cathinone	Precursor Ion (m/z)	Product Ions (m/z)	Loss
Methcathinone	164.1075	**131.0731**	$-CH_4O$
		105.0703	$-C_2H_4NO$
3-FMC	182.0976	**149.0634**	$-CH_4O$
		123.0605	$-C_2H_4NO$
4-FMC	182.0976	**149.0636**	$-CH_4O$
		123.0605	$-C_2H_4NO$
Methylone	208.0968	**160.0757**	$-CH_4O_2$ (-MD)
		132.0807	$-C_3H_9NO$
Ethcathinone	178.1226	**131.0721**	$-C_2H_6O$
		117.0586	$-C_3H_6NO$
		105.0700	$-C_2H_6NO$
Ethylone	222.1125	**174.1222**	$-CH_4O_2$ (-MD)
		146.0958	$-C_3H_9NO$
Methedrone	194.1176	**161.0833**	$-CH_4O$
		146.0598	$-C_2H_7O$
		135.0803	$-C_2H_4NO$
Buphedrone	178.1226	**131.0731**	$-C_2H_6O$
		91.0549	Tropylium
		145.0880	$-CH_4O$
Butylone	222.1125	**174.0914**	$-CH_4O_2$ (-MD)
		146.0964	$-C_3H_9NO$
Mephedrone	178.1226	**145.0889**	$-CH_4O$
		119.0853	$-C_2H_4NO$
Eutylone	236.1281	**188.1069**	$-CH_4O_2$ (-MD)
		174.0547	$-C_3H_{11}N$
		161.0598*	$-C_3H_8NO$
4-MEC	192.1383	**145.0886**	$-C_2H_6O$
		159.1041*	$-CH_4O$
		131.0738	$-C_2H_6NO$
MDPBP	262.1438	**161.0597**	$-C_5H_{10}NO$
		191.0704	$-C_4H_9N$ (-PYR)
		112.1125	$-C_8H_5O_3$

(continued)

Cathinone	Precursor Ion (m/z)	Product Ions (m/z)	Loss
Pentedrone	192.1383	**132.0810**	$-C_3H_7O$
		91.0546	Tropylium
Pentylone	236.1281	**188.1070**	$-CH_4O_2$ (-MD)
		175.0682	$-C_3H_8O$
3,4-DMMC	192.1383	**159.1043**	$-CH_4O$
		144.0802	$-CH_5NO$
α-PVP	232.1696	**161.0954**	$-C_4H_9N$ (-PYR)
		91.0549	Tropylium
4-EMC	192.1383	**145.0889**	$-CH_4NO$
		105.0701	$-C_4H_8NO$
MPBP	232.1696	**161.0960**	$-C_4H_9N$ (-PYR)
		133.1010	$-C_5H_8NO$
		112.1120	$-C_8H_7O$
MDPV	276.1594	**205.0857**	$-C_4H_9N$ (-PYR)
		126.1277	$-C_8H_5O_3$
		175.0756	$-C_8H_{16}NO$
Pyrovalerone	246.1852	**175.1110**	$-C_4H_9N$ (-PYR)
		126.1280	$-C_8H_7O$
		105.0701	$-C_8H_{16}NO$
Naphyrone	282.1852	**211.1122**	$-C_4H_9N$ (-PYR)
		126.1280	$-C_{11}H_7O$
		141.0701	$-C_{11}H_8$

*ion transition not included in blood acquisition. MD: methylenedioxy, PYR: pyrrolidine group

Table 2.4. Accurate mass for 100 ng/mL control in urine and blood.

			Blood		Urine	
Cathinone	**Formula**	**Exact Mass [M+H]**	**Accurate Mass**	**Difference (ppm)**	**Accurate Mass**	**Difference (ppm)**
3,4-DMMC	$C_{12}H_{17}NO$	192.1383	192.1385	1.04	192.1388	2.60
3-FMC	$C_{10}H_{12}FNO$	182.0976	182.0976	0.00	182.0975	-0.55
4-EMC	$C_{12}H_{17}NO$	192.1383	192.1383	0.00	192.1385	1.04
4-FMC	$C_{10}H_{12}FNO$	182.0976	182.0978	1.10	182.0977	0.55
4-MEC	$C_{12}H_{17}NO$	192.1383	192.1389	3.12	192.1386	1.56
α-PVP	$C_{15}H_{21}NO$	232.1696	232.1699	1.29	232.1702	2.58
Buphedrone	$C_{11}H_{15}NO$	178.1226	178.1231	2.81	178.1228	1.12
Butylone	$C_{12}H_{15}NO_3$	222.1125	222.1135	4.50	222.1128	1.35
Ethcathinone	$C_{11}H_{15}NO$	178.1226	178.1229	1.68	178.1228	1.12
Ethylone	$C_{12}H_{15}NO_3$	222.1125	222.1132	3.15	222.1128	1.35
Eutylone	$C_{13}H_{17}NO_3$	236.1281	236.1298	2.70	236.1287	2.54
MDPBP	$C_{15}H_{19}NO_3$	262.1438	262.1436	-0.76	262.1443	1.91
MDPV	$C_{16}H_{21}NO_3$	276.1594	276.1592	-0.72	276.1603	3.26
Mephedrone	$C_{11}H_{15}NO$	178.1226	178.1227	0.56	178.1229	1.68
Methcathinone	$C_{10}H_{13}NO$	164.1070	164.1069	-0.61	164.1072	1.22
Methedrone	$C_{11}H_{15}NO_2$	194.1176	194.1178	1.03	194.1178	1.03
Methylone	$C_{11}H_{13}NO_3$	208.0968	208.0969	0.48	208.0971	1.44
MPBP	$C_{15}H_{21}NO$	232.1696	232.1702	2.58	232.1703	3.02
Naphyrone	$C_{19}H_{23}NO$	282.1852	282.1856	1.42	282.1861	3.19
Pentedrone	$C_{12}H_{17}NO$	192.1383	192.1384	0.52	192.1388	2.60
Pentylone	$C_{13}H_{17}NO_3$	236.1281	236.1282	0.42	236.1288	2.96
Pyrovalerone	$C_{16}H_{23}NO$	246.1852	246.1863	4.47	246.1862	4.06

Table 2.5. Extraction efficiencies in urine (25 ng/mL) and blood (100 ng/mL) using replicate analyses (n=4).

Cathinone	Mean Extraction Efficiency (%)	
	Urine	Blood
3,4-DMMC	96 ± 7	83 ± 35
3-FMC	84 ± 12	81 ± 19
4-EMC	97 ± 4	87 ± 17
4-FMC	90 ± 9	86 ± 12
4-MEC	101 ± 4	85 ± 10
α-PVP	94 ± 4	84 ± 21
Buphedrone	95 ± 5	85 ± 11
Butylone	98 ± 3	87 ± 9
Ethcathinone	89 ± 4	87 ± 15
Ethylone	98 ± 3	87 ± 11
Eutylone	98 ± 3	93 ± 10
MDPBP	94 ± 3	87 ± 9
MDPV	95 ± 4	88 ± 20
Methcathinone	93 ± 10	83 ± 20
Methedrone	104 ± 6	84 ± 13
Methylone	99 ± 4	83 ± 20
Mephedrone	97 ± 7	82 ± 24
MPBP	93 ± 4	91 ± 7
Naphyrone	95 ± 4	88 ± 12
Pentedrone	95 ± 5	88 ± 11
Pentylone	100 ± 5	88 ± 17
Pyrovalerone	92 ± 4	90 ± 12

Following visual, analytical, and statistical evaluation of calibration models, a weighted (1/x) quadratic model was selected for all analytes in both matrices. The calibration curves for the chosen model and the residual plots for a representative drug (MDPV) are depicted in **Figure 2.4**. Residual plots and calibration curves for all twenty-two cathinones in blood and urine are located in **Appendix B and C**, respectively. The coefficients of determination (R^2) were all above 0.99 or 0.98 for all models. Upon visual assessment using residual plots, the data did not appear to be randomly dispersed, indicating a non-linear (quadratic)

model should be used. Statistical evaluation further indicated a weighted (1/x) curve was optimal.

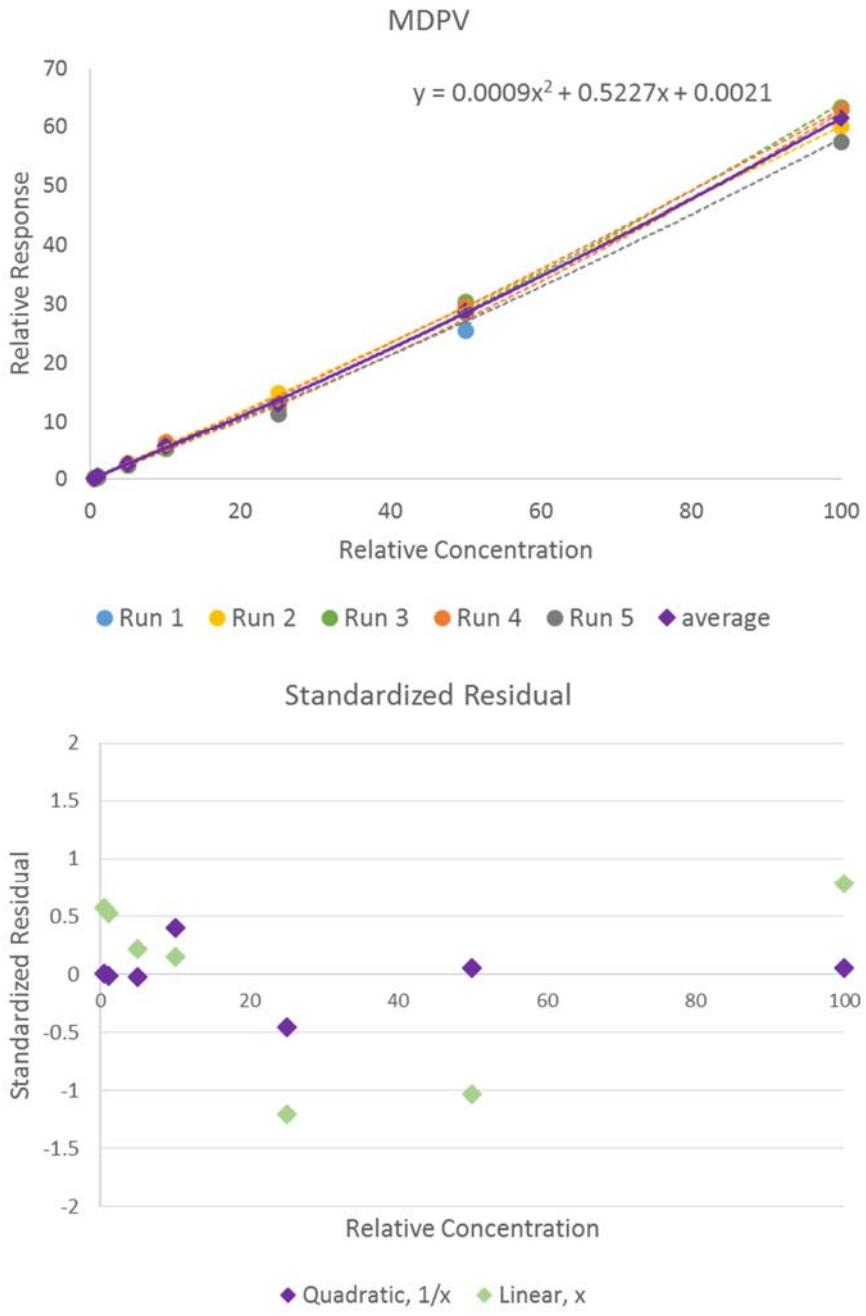

Figure 2.4. Combined calibration curve of weighted (1/x) quadratic model of MDPV over five independent extractions. Calibration curve expressed as relative response and relative concentration. Overlaid residual plots for linear unweighted and quadratic weighted (1/x) shown below.

Limits of detection and quantitation for the twenty-two synthetic cathinones in blood ranged from 1-5 ng/mL (n=18), significantly lower than previously published literature using HRMS (50-100 ng/mL) (20). Limits of detection in urine ranged from 0.25-5 ng/mL (n=18). Bias, precision and signal to noise (S/N) ratios at the limits of detection and quantitation are summarized in **Tables 2.6 and 2.7.** Extracted ion chromatograms (EICs) for all drugs at the limit of quantitation in urine and blood are shown in **Figures 2.5 and 2.6**, respectively.

Table 2.6. Limits of detection and quantitation in urine. The mean, standard deviation (SD), signal to noise ratio (S/N), bias, and CV (%) at the LOQ are summarized for each drug (n=18).

Cathinone	LOD (ng/mL)	LOQ (ng/mL)	Mean ± SD (ng/mL)	S/N	Bias (%)	CV (%)
3,4-DMMC	5	5	5.0 ± 0.3	23:1	-0.7	6.4
3-FMC	1	2	9.9 ± 0.6	349:1	-0.9	6.4
4-EMC	2	5	5.1 ± 0.3	43:1	2.3	5.3
4-FMC	1	1	0.9 ± 0.1	358:1	-8.5	5.9
4-MEC	1	1	0.9 ± 0.05	1438:1	-8.1	5.3
α-PVP	2	2	2.0 ± 0.2	103:1	-1.7	9.4
Buphedrone	2	2	2.0 ± 0.1	159:1	1.4	6.3
Butylone	1	2	2.0 ± 0.2	44:1	1.6	8.3
Ethcathinone	1	2	2.0 ± 0.1	578:1	0.0	6.6
Ethylone	1	5	4.9 ± 0.4	623:1	-0.7	7.1
Eutylone	5	5	4.9 ± 0.3	338:1	-1.1	5.5
MDPBP	0.5	5	5.2 ± 0.2	556:1	4.3	4.4
MDPV	1	2	2.1 ± 0.1	967:1	4.0	6.9
Mephedrone	2	2	2.1 ± 0.1	95:1	3.5	5.3
Methcathinone	0.25	0.25	0.24 ± 0.02	241:1	-2.3	9.9

(continued)

Cathinone	LOD (ng/mL)	LOQ (ng/mL)	Mean ± SD (ng/mL)	S/N	Bias (%)	CV (%)
Methedrone	1	1	0.9 ± 0.1	102:1	-2.0	14.2
Methylone	0.25	1	0.9 ± 0.1	1323:1	-0.8	9.4
MPBP	1	5	4.9 ± 0.4	239:1	-1.0	7.7
Naphyrone	0.5	0.5	0.27 ± 0.02	666:1	15.3	6.6
Pentedrone	5	5	5.1 ± 0.3	489:1	1.5	6.4
Pentylone	1	5	4.7 ± 0.3	125:1	-5.4	5.3
Pyrovalerone	0.25	0.25	0.27 ± 0.02	235:1	8.7	8.0

Table 2.7. Limits of detection and quantitation in blood. The mean, standard deviation (SD), signal to noise (S/N), bias, and CV (%) at the LOQ are summarized for each drug (n=18).

Cathinone	LOD (ng/mL)	LOQ (ng/mL)	Mean ± SD (ng/mL)	S/N	Bias (%)	CV (%)
3,4-DMMC	2	2	1.87 ± 0.13	196:1	-6.4	6.9
3-FMC	2	2	2.01 ± 0.19	69:1	0.0	9.2
4-EMC	1	1	1.02 ± 0.06	70:1	1.0	5.9
4-FMC	5	5	5.07 ± 0.43	128:1	1.5	8.4
4-MEC	5	5	4.98 ± 0.37	72:1	-0.5	7.4
α-PVP	2	2	1.85 ± 0.16	16:1	-7.6	8.6
Buphedrone	5	5	4.94 ± 0.39	117:1	-1.2	7.8
Butylone	2	2	1.92 ± 0.17	63:1	-4.0	8.8
Ethcathinone	5	5	4.82 ± 0.39	155:1	-3.6	8.2
Ethylone	2	2	1.96 ± 0.19	67:1	-3.0	10.0
Eutylone	5	5	4.80 ± 0.39	46:1	-4.0	8.2
MDPBP	5	5	4.80 ± 0.25	84:1	-4.0	5.2
MDPV	2	2	1.86 ± 0.14	55:1	-7.3	7.4
Mephedrone	2	2	1.99 ± 0.13	181:1	-0.4	6.6
Methcathinone	2	2	1.91 ± 0.16	155:1	-4.8	8.6

(continued)

Cathinone	LOD (ng/mL)	LOQ (ng/mL)	Mean ± SD (ng/mL)	S/N	Bias (%)	CV (%)
Methedrone	2	2	1.90 ± 0.17	104:1	-6.6	8.9
Methylone	2	2	1.92 ± 0.09	305:1	-4.9	4.5
MPBP	2	2	1.87 ± 0.16	75:1	-6.5	8.3
Naphyrone	1	1	1.00 ± 0.07	33:1	0.7	6.9
Pentedrone	5	5	5.05 ± 0.35	195:1	0.9	6.8
Pentylone	5	5	4.77 ± 0.42	61:1	-4.6	8.9
Pyrovalerone	1	2	1.86 ± 0.13	152:1	-6.9	6.8

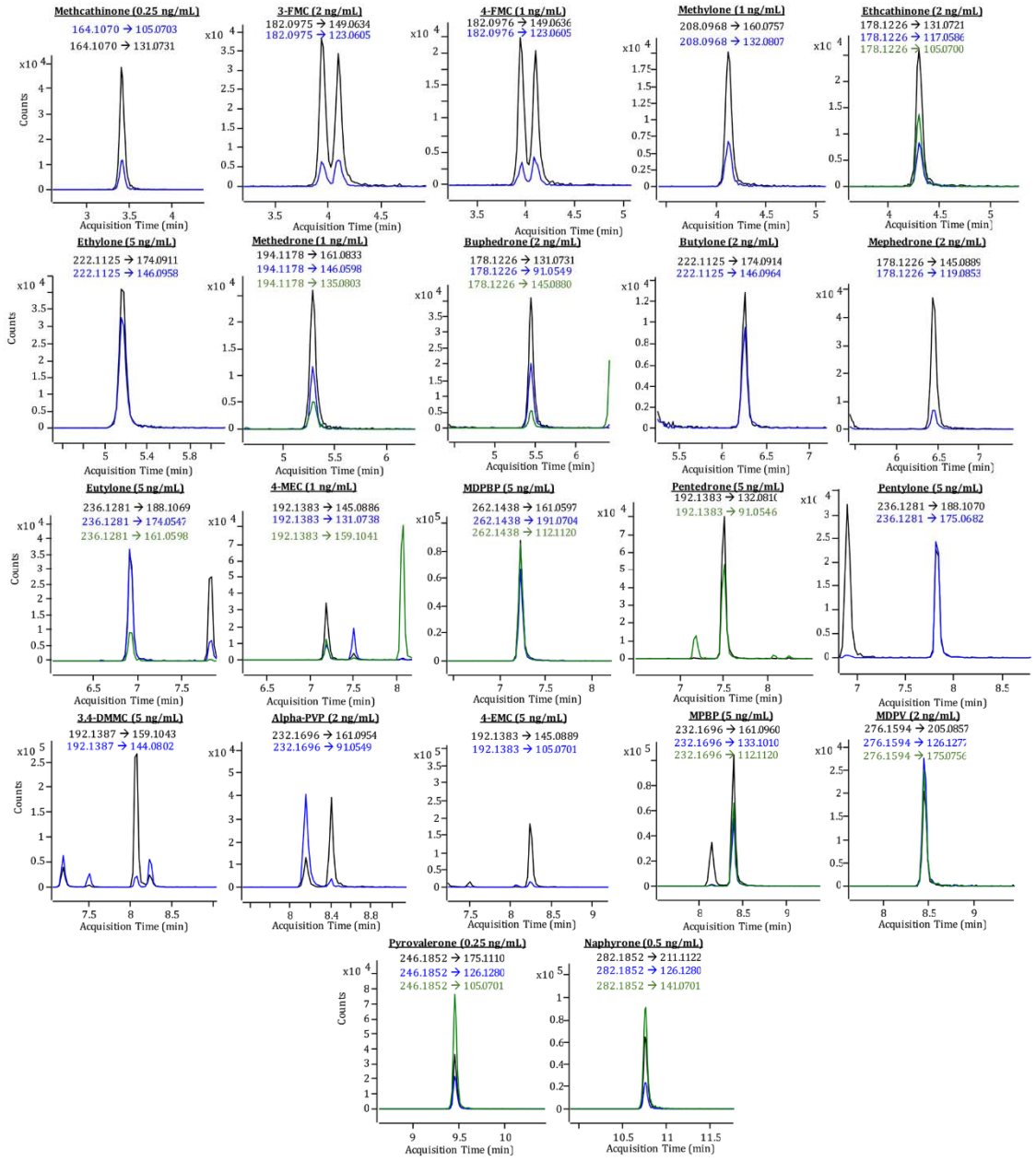

Figure 2.5. Extracted ion chromatograms in urine at the limit of quantitation.

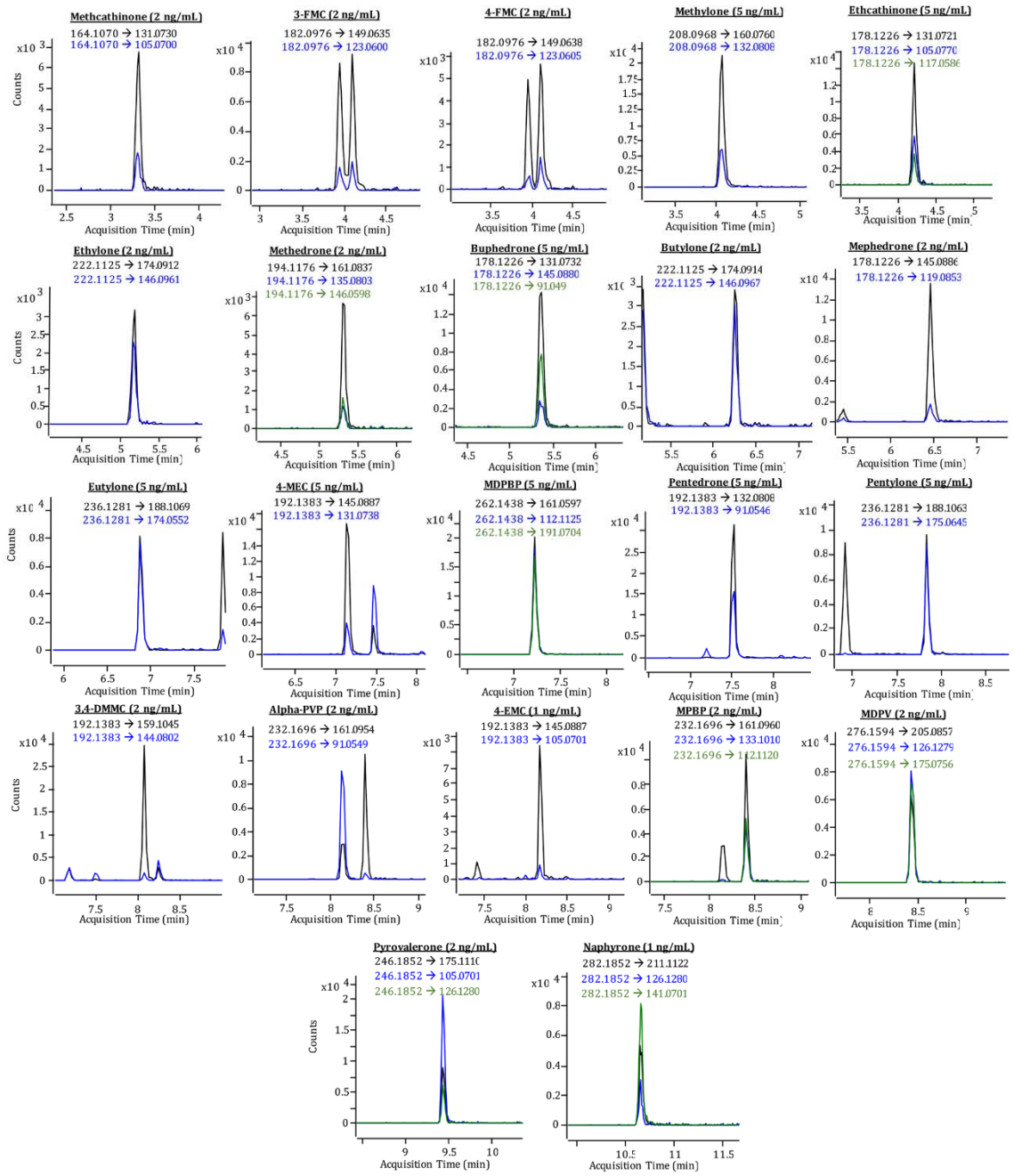

Figure 2.6. Extracted ion chromatograms in blood at the limit of quantitation.

Precision and bias were evaluated at low, medium, and high concentrations in triplicate over five days. Intra-assay CVs were 0.5 – 10.8% (10 ng/mL); 0.2 – 7.3% (100 ng/mL); 0.2 – 8.6% (800 ng/mL) for urine and 0.2 – 17.0% (20 ng/mL); 0.2 – 8.7% (100

ng/mL); 0.8 – 13.8% (800 ng/mL) for blood. Inter-assay CVs over the same concentration ranges were 4.4 - 12.1%, 1.7 - 11.5% and 2.5 - 8.6% in urine (n=15) and 3.3 – 11.7%, 2.7 – 7.0%, and 3.4 – 10.1% in blood (n=15). Bias and precision at all concentrations tested were within acceptable ranges (±20%) (25) and are summarized in **Tables 2.8 and 2.9**.

Table 2.8. Precision and bias (n=15) in urine at low (10 ng/mL), medium (100 ng/mL) and high (800 ng/mL) concentrations.

Cathinone	**Intra-assay Precision (n=3, %CV)**			**Inter-assay Precision (n=15, %CV)**			**Bias (n=15, %)**		
	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL
3,4-DMMC	2.9-6.1	0.3-6.3	0.4-4.9	11.7	8.6	5.5	-1	-3	3
3-FMC	1.2-10.8	1.1-5.1	0.7-5.3	8.9	4.7	5.9	9	0	2
4-EMC	1.3-4.1	0.3-2.2	2.7-5.5	6.8	2.2	3.5	8	2	3
4-FMC	0.5-3.8	1.8-6.9	0.3-3.1	5.6	4.5	9.2	7	1	4
4-MEC	1.0-8.9	1.0-3.4	0.6-4.1	12.1	11.5	4.3	1	1	4
α-PVP	1.5-3.9	0.2-3.6	1.6-4.7	6.7	4.2	8.9	9	0	6
Buphedrone	0.8-5.6	0.9-4.1	0.7-5.3	8.3	2.8	4.7	10	2	6
Butylone	1.7-7.0	0.2-6.2	1.4-5.0	4.6	4.1	3.5	6	0	4
Ethcathinone	1.3-4.5	3.4-7.3	0.9-4.1	9.3	6.3	7.5	12	1	8
Ethylone	0.6-3.4	1.7-4.3	0.2-4.6	6.9	3.0	4.6	7	2	1
Eutylone	1.8-6.0	1.1-3.1	1.1-4.2	6.7	2.4	5.8	3	2	2
MDPBP	1.6-7.2	0.6-3.0	0.4-5.3	7.1	4.4	5.7	7	2	1
MDPV	0.8-6.8	1.5-3.4	1.1-4.6	6.1	5.0	5.1	7	2	1
Mephedrone	0.5-6.8	0.9-2.1	1.5-5.5	4.8	2.0	3.3	7	2	2
Methcathinone	0.9-6.4	0.9-3.9	1.4-5.8	7.0	3.0	3.5	8	1	5
Methedrone	3.6-7.2	0.4-1.4	2.0-8.6	4.7	1.7	6.4	8	1	2
Methylone	0.8-5.7	0.6-2.3	3.2-7.4	4.4	2.4	2.5	6	1	2
MPBP	2.4-4.5	2.5-4.2	0.4-4.7	9.4	4.3	3.2	6	2	5

(continued)

Cathinone	Intra-assay Precision (n=3, %CV)			Inter-assay Precision (n=15, %CV)			Bias (n=15, %)		
	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL
Naphyrone	3.9-7.2	0.5-2.5	0.4-4.2	6.0	1.8	3.3	8	3	3
Pentedrone	1.0-3.2	1.2-4.6	1.9-7.3	7.8	3.6	4.1	8	1	5
Pentylone	2.9-8.5	1.3-3.8	1.1-3.9	11.6	3.6	5.8	3	4	3
Pyrovalerone	1.6-3.9	1.3-2.5	0.7-3.5	8.7	2.3	3.4	7	2	3

Table 2.9. Precision and bias (n=15) in blood at low (20 ng/mL), medium (100 ng/mL) and high (800 ng/mL) concentrations.

Cathinone	Intra-assay Precision (n=3, %CV)			Inter-assay Precision (n=15, %CV)			Bias (n=15, %)		
	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL
3,4-DMMC	2.3-9.5	1.2-7.2	2.1-13.8	6.9	4.9	10.1	-3	1	2
3-FMC	1.1-17.0	0.3-6.2	1.8-9.8	9.1	5.6	8.1	0	11	4
4-EMC	1.5-5.8	0.9-7.2	4.6-5.6	4.9	5.0	8.3	-1	-2	-2
4-FMC	0.8-11.7	1.3-5.8	5.1-8.5	6.0	3.8	7.6	2	11	2
4-MEC	0.7-5.4	1.3-4.0	2.1-7.3	5.7	3.7	7.7	3	1	2
α-PVP	3.1-8.8	0.8-8.7	7.0-10.8	7.2	5.4	7.6	-3	4	-4
Buphedrone	1.3-9.7	0.8-5.5	2.1-8.7	6.8	5.6	7.4	4	9	4
Butylone	0.9-8.1	2.4-3.8	3.8-5.2	4.6	4.0	5.5	-6	1	-1
Ethcathinone	1.6-14.0	0.5-7.4	5.5-9.4	8.8	6.0	7.2	6	11	4

(continued)

Cathinone	Intra-assay Precision (n=3, %CV)			Inter-assay Precision (n=15, %CV)			Bias (n=15, %)		
	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL	20 ng/mL	100 ng/mL	800 ng/mL
Ethylone	1.1-7.5	1.5-2.6	1.7-6.3	3.3	4.3	3.6	0	-1	0
Eutylone	1.3-5.6	2.2-6.0	6.3-10.0	6.6	5.9	5.1	-1	3	1
MDPBP	1.1-5.5	1.5-5.4	1.0-6.7	5.7	5.1	3.9	9	-2	1
MDPV	1.2-5.8	0.2-6.1	1.5-6.4	3.6	4.1	4.9	-7	5	3
Mephedrone	1.3-6.6	1.0-2.2	4.2-4.6	3.6	3.7	6.2	-5	3	1
Methcathinone	0.6-8.6	0.8-3.9	1.7-5.5	6.2	4.6	8.0	3	6	3
Methedrone	0.2-10.7	1.1-3.9	2.3-5.7	6.6	2.9	6.3	-2	-6	-4
Methylone	1.4-5.5	0.5-3.7	2.4-4.8	3.9	3.7	3.4	-6	1	1
MPBP	0.2-12.6	0.9-6.2	0.8-6.5	11.7	4.9	9.4	3	3	1
Naphyrone	1.1-9.1	0.7-2.4	1.4-3.0	6.8	2.7	3.8	-1	1	0
Pentedrone	1.9-11.5	2.0-3.7	2.5-5.9	6.3	4.8	7.2	4	7	6
Pentylone	0.7-6.2	0.9-6.7	8.4-8.4	6.6	7.0	8.3	-5	-2	1
Pyrovalerone	0.3-10.6	0.6-4.0	3.7-7.3	5.3	3.9	6.8	7	5	3

Interferences from matrix, isotopically labeled internal standards, and other drugs were systematically evaluated. Ten drug-free urine matrices from independent sources did not reveal interferences and there were no interfering ion contributions associated with the deuterated analogs. Furthermore, there were no qualitative interferences from more than fifty other compounds, including common drugs, amphetamine-like drugs, designer drugs, or therapeutically used cathinones, and putrefactive amines (**Table 2.2**). Negative and positive controls (10 ng/mL and 100 ng/mL) were analyzed in the presence of a 10- and 100-fold higher concentration of potential interferents (1,000 ng/mL). No qualitative interferences were present for any of the compounds tested.

The potential for ion suppression or enhancement was evaluated using ten independently sourced blood and urine samples. Matrix effects were evaluated quantitatively using the post-extraction addition technique for all twenty-two analytes and nine internal standards. Ionization suppression in urine was -17 to -1% at 20 ng/mL and -21 to -4% at 200 ng/mL. Corresponding CVs were 2.4-13.7% and 3.5-7.5%, respectively. Ionization suppression in blood was -15 to 7% at 50 ng/mL and -3 to 3% at 500 ng/mL. Corresponding CVs in blood were 2.5-7.6% and 0.9–3.2%, respectively (**Table 2.10**). Although some ion suppression was evident, matrix effects were well-within tolerable limits (±20%) and CVs were <15% (25).

Table 2.10. Matrix effect (%) and associated CVs in urine (20 and 200 ng/mL) and blood (50 and 500 ng/mL) (n=10).

Cathinone	**Urine**				**Blood**			
	CV (%) n=10		**Matrix Effect (%)**		**CV (%) n=10**		**Matrix Effect (%)**	
	20 ng/mL	200 ng/mL	20 ng/mL	200 ng/mL	50 ng/mL	500 ng/mL	50 ng/mL	500 ng/mL
3,4-DMMC	10.5	6.0	-17	-18	7.0	1.0	5	2
3-FMC	13.2	3.5	-15	-21	4.0	3.2	-5	0
4-EMC	9.8	5.2	-14	-5	7.6	1.3	4	1
4-FMC	7.6	7.5	-2	-16	3.1	0.9	2	2
4-MEC	4.0	4.9	-10	-16	5.9	1.8	4	-2
α-PVP	8.0	6.9	-1	-10	4.6	1.6	-9	-1
Buphedrone	6.9	7.2	-6	-7	5.1	1.7	3	3
Butylone	4.0	6.9	-10	-18	5.2	1.9	6	3
Ethcathinone	5.5	8.9	-5	-9	3.9	1.2	-5	-3
Ethylone	4.7	6.1	-5	-13	4.0	1.5	4	1
Eutylone	8.7	5.5	-14	-9	4.7	2.2	1	2
MDPBP	4.9	4.8	-8	-12	3.3	1.5	-2	2
MDPV	8.6	4.4	-6	-7	5.3	1.5	-11	2
Methcathinone	9.9	5.8	-13	-14	5.4	1.5	5	2
Methedrone	7.0	5.7	-12	-9	3.4	1.4	-3	0
Methylone	5.4	5.7	-6	-4	4.9	1.2	7	2
Mephedrone	3.8	6.6	-12	-15	4.6	1.2	-2	0
MPBP	6.6	4.1	-9	-10	2.5	1.0	-8	0
Naphyrone	2.4	4.8	-8	-11	3.3	1.4	-15	1
Pentedrone	5.9	5.9	-5	-9	3.7	2.4	-1	0
Pentylone	13.7	5.7	-8	-11	4.4	1.5	0	3
Pyrovalerone	3.5	4.6	-4	-10	2.7	0.9	-10	2
α-PVP-D8	5.4	3.8	-4	-2	3.6	2.6	-8	0
Butylone-D3	3.6	3.3	-7	-10	4.9	2.6	8	-1
Ethylone-D5	4.8	2.9	-7	-6	3.7	2.3	4	1
Eutylone-D5	11.0	3.0	-6	-7	4.8	4.6	2	1

(continued)

Cathinone	Urine				Blood			
	CV (%) n=10		Matrix Effect (%)		CV (%) n=10		Matrix Effect (%)	
	20 ng/mL	200 ng/mL	20 ng/mL	200 ng/mL	50 ng/mL	500 ng/mL	50 ng/mL	500 ng/mL
MDPV-D8	8.0	4.3	-22	-6	2.2	2.4	-12	2
Methylone-D3	5.1	3.2	-16	-6	5.3	3.3	9	-1
Mephedrone-D3	3.6	2.5	-8	-7	2.8	3.1	4	0
Naphyrone-D5	2.3	4.2	-14	-10	3.2	1.9	-25	-1
Pentylone-D3	13.2	3.5	-6	-6	6.2	3.9	-1	1

Dilution integrity was evaluated using two and four-fold dilutions of matrix at 1,000 ng/mL. All quantitative measurements were within 20% of the expected value. No carryover was present at 1,000 or 2,500 ng/mL for any of the analytes. However, at 5,000 ng/mL, carryover was observed for naphyrone in both matrices. Finally, processed samples were stable for 48 hours at 25 and 350 ng/mL.

Although not yet widespread in routine forensic toxicology laboratories, interest in the use of high resolution mass spectrometry (HRMS) is increasing. HRMS methods for the detection of cathinones in blood or urine are still relatively limited and this report offers some distinct advantages over previously published work. The quantitative assay was suitable for use with both blood and urine, produced significantly lower LODs and LOQs in blood (20), separated twenty-two cathinones in a shorter run time (21, 22) and was also capable of separating challenging isobaric compounds (3- and 4-FMC) using traditional reversed phase chromatography.

This novel HRMS detection method was further utilized using authentic urine samples provided by Redwood Toxicology Laboratory (RTL) (Santa Rosa, CA). **Table**

2.11 summarizes the original (RTL) results with quantitative analyses following ten months of refrigerated storage and **Figure 2.7** depicts a representative extract.

Table 2.11. Comparison of quantitative analyses of authentic urine samples (n=20) from cathinone users following ten months of refrigerated storage.

Sample	Reference Laboratory Result	Reanalysis by LC-Q-TOF	Remaining*
A	Ethylone (379 ng/mL) Methylone (87 ng/mL) Pentedrone (Present)	Ethylone (257 ng/mL) Methylone (54 ng/mL) Pentedrone (<LOQ)	Ethylone (68%) Methylone (62%)
B	Ethylone (98 ng/mL) Methylone (32 ng/mL) Pentedrone (Present)	Ethylone (69 ng/mL) Methylone (24 ng/mL) Pentedrone (<LOQ)	Ethylone (70%) Methylone (75%)
C	4-MEC (present)	4-MEC (113 ng/mL)	--
D	α-PVP (100 ng/mL) Methylone (75 ng/mL)	α-PVP (110 ng/mL) Methylone (7 ng/mL)	α-PVP (110%) Methylone (9%)
E	α-PVP (394 ng/mL)	α-PVP (310 ng/mL)	α-PVP (79%)
F	Ethylone (36 ng/mL)	Ethylone (24 ng/mL)	Ethylone (67%)
G	4-FMC and/or metabolite	4-FMC (<LOQ)	--
H	4-FMC and/or metabolite	4-FMC not detected	--
I	MDPV (1,301 ng/mL)	MDPV (1,095 ng/mL)	MDPV (84%)
J	Pentylone (585 ng/mL)	Pentylone (433 ng/mL)	Pentylone (74%)
K	Methylone (175 ng/mL)	Methylone (109 ng/mL)	Methylone (62%)
L	α-PVP (105 ng/mL) Methylone (108 ng/mL)	α-PVP (104 ng/mL) Methylone (8 ng/mL)	α-PVP (99%) Methylone (7%)
M	Butylone (177 ng/mL)	Butylone (12 ng/mL)	Butylone (7%)
N	Methylone (1,535 ng/mL) Pentedrone (Present)	Methylone (923 ng/mL) Pentedrone (<LOQ)	Methylone (60%)
O	Ethylone (189 ng/mL) Methylone (246 ng/mL)	Ethylone (152 ng/mL) Methylone (190 ng/mL)	Ethylone (80%) Methylone (77%)
P	α-PVP (101 ng/mL)	α-PVP (96 ng/mL)	α-PVP (95%)
Q	Methylone (84 ng/mL)	Methylone (13 ng/mL)	Methylone (16%)
R	α-PVP (87 ng/mL) Methylone (56 ng/mL)	α-PVP (93 ng/mL) Methylone (10 ng/mL)	α-PVP (107%) Methylone (18%)

(continued)

Sample	Reference Laboratory Result	Reanalysis by LC-Q-TOF	Remaining*
S	MDPV (271 ng/mL)	MDPV (212 ng/mL)	MDPV (78%)
T	MDPV (257 ng/mL)	MDPV (180 ng/mL)	MDPV (70%)

*Remaining (%) was calculated as follows: (Reanalysis by LC-Q-TOF/Initial Reference Laboratory Result)

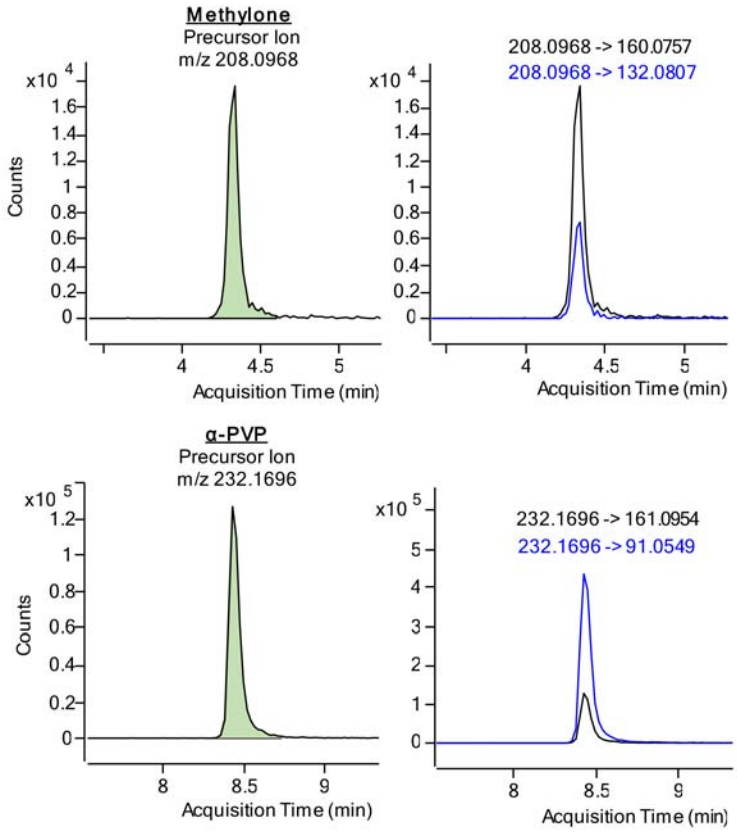

Figure 2.7. Extracted ion chromatogram of urine from a cathinone user (Sample D) containing 9 ng/mL methylone (4.34 min) and 110 ng/mL α-PVP (8.43 min).

These results highlight structurally dependent differences in stability between the synthetic cathinones. While tertiary or pyrrolidine-type cathinones exhibited the greatest stability, some of the ring-substituted secondary amines were notably unstable. In two instances, 4–FMC was either below the LOQ or not detected (Samples G and H). Although

results using α-PVP were in excellent agreement (79-110%), methylone was highly variable, with 7-77% of the drug remaining after storage. Structure-dependent stability was particularly evident in samples D, L and R (which contained both a secondary and tertiary amine). In these samples the α-PVP results were within 20% of their original value (99-110%), while only 7-18% of the methylone remained. These comparisons highlight issues associated with cathinone stability, first reported by Morad (26) and later described by Paul (27). Instability in biological samples has been noted for a small number of cathinones, but has not received widespread attention (13, 21, 28-30). Tsujiikawa (13) was the first to suggest that fluorinated cathinones may be particularly unstable and dependent on the position of the fluorine on the benzene ring. The influence of chemical structure, matrix, temperature, and pH on cathinone stability require additional study and are currently being systematically evaluated in our laboratory.

Conclusions

High resolution mass spectrometry techniques are gaining momentum in forensic toxicology. Accurate mass measurements can improve assay specificity and the ability to perform data dependent or targeted acquisition allows for considerable flexibility and potential for post analysis data acquisition. In this report, liquid chromatography-quadrupole/time of flight mass spectrometry was used to identify twenty-two cathinones, including regioisomers of flephedrone from urine and blood. The method was validated in accordance with published guidelines (25) and provided the necessary sensitivity and specificity for use with authentic casework samples.

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References

1. Capriola M. (2013) Synthetic cathinone abuse. Clinical Pharmacology, **5**, 109-115.
2. Coppola M., Mondola R. (2012) Synthetic cathinones: Chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as "bath salts" or "plant food". *Toxicology Letters*, **211**, 144-149.
3. Zawilska J.B., Wojcieszak J. (2013) Designer cathinones—An emerging class of novel recreational drugs. Forensic Science International, **231**, 42-53.
4. U.S. Drug Enforcement Administration. (2011) Schedules of controlled substances: temporary placement of three synthetic cathinones in Schedule I: Final Order. *Federal Register*, **76**, 65371-65375.
5. U.S. Drug Enforcement Administration. (2014) Schedules of controlled substances: temporary placement of 10 synthetic cathinones into Schedule I: Final order. *Federal Register*, **79**, 12938-12943.
6. National Conference of State Legislatures (2015) Synthetic Drug Threats. Available from: http://www.ncsl.org/research/civil-and-criminal-justice/synthetic-drug-threats.aspx.
7. de Castro A., Lendoiro E., Fernández-Vega H., Steinmeyer S., López-Rivadulla M., Cruz A. (2014) Liquid chromatography tandem mass spectrometry determination of selected synthetic cathinones and two piperazines in oral fluid. Cross reactivity study with an on-site immunoassay device. *Journal of Chromatography A*, **1374**, 93-101.

8. Regester L.E., Chmiel J.D., Holler J.M., Vorce S.P., Levine B., Bosy T.Z. (2015) Determination of designer drug cross-reactivity on five commercial immunoassay screening kits. *Journal of Analytical Toxicology,* **39**, 144-151.

9. Meyer M.R., Wilhelm J., Peters F.T., Maurer H.H. (2010) Beta-keto amphetamines: studies on the metabolism of the designer drug mephedrone and toxicological detection of mephedrone, butylone, and methylone in urine using gas chromatography–mass spectrometry. *Analytical and Bioanalytical Chemistry*, **397**, 1225-1233.

10. O'Byrne P.M., Kavanagh P.V., McNamara S.M., Stokes S.M. (2013) Screening of stimulants including designer drugs in urine using a liquid chromatography tandem mass spectrometry system. *Journal of Analytical Toxicology*, **37**, 64-73.

11. DeRuiter J., Hayes L., Valaer A., Clark C.R., Noggle F.T. (1994) Methcathinone and Designer Analogues: Synthesis, Stereochemical Analysis, and Analytical Properties. *Journal of Chromatographic Science*, **32**, 552-564.

12. Archer R.P. (2009) Fluoromethcathinone, a new substance of abuse. *Forensic Science International*, **185**, 10-20.

13. Tsujikawa K., Mikuma T., Kuwayama K., Miyaguchi H., Kanamori T., Iwata Y.T. (2012) Degradation pathways of 4-methylmethcathinone in alkaline solution and stability of methcathinone analogs in various pH solutions. *Forensic Science International,* **220**, 103-110.

14. Kerrigan S., Savage M., Cavazos C., Bella P. (2015) Thermal Degradation of Synthetic Cathinones: Implications for Forensic Toxicology. *Journal of Analytical Toxicology*, **40**, 1-11 .

15. Zuba D. (2012) Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods. *Trac-Trends in Analytical Chemistry*, **32**, 15-30.

16. Matsuta S., Katagi M., Nishioka H., Kamata H., Sasaki K., Shima N. (2014) Structural characterization of cathinone-type designer drugs by EI mass spectrometry. *Japanese Journal of Forensic Science Technology*, **19**, 77-89.

17. Ojanperä I.A., Heikman P.K., Rasanen I.J. (2011) Urine analysis of 3, 4-methylenedioxypyrovalerone in opioid-dependent patients by gas chromatography–mass spectrometry. *Therapeutic Drug Monitoring*, **33**, 257-263.

18. Power J.D., McDermott S.D., Talbot B., O'Brien J.E., Kavanagh P. (2012) The analysis of amphetamine-like cathinone derivatives using positive electrospray ionization with in-source collision-induced dissociation. *Rapid Communications in Mass Spectrometry*, **26**, 2601-2611.

19. Ammann D., McLaren J.M., Gerostamoulos D., Beyer J. (2012) Detection and quantification of new designer drugs in human blood: part 2 – designer cathinones. *Journal of Analytical Toxicology*, **36**, 381-389.

20. Pasin D., Bidny S., Fu S. (2015) Analysis of new designer drugs in post-mortem blood using high-resolution mass spectrometry. *Journal of Analytical Toxicology*, **39**, 163-171.

21. Concheiro M., Anizan S., Ellefsen K., Huestis M.A. (2013) Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. *Analytical and Bioanalytical Chemistry*, **405**, 9437-9448.

22. Concheiro M., Castaneto M., Kronstrand R., Huestis M.A. (2015) Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography–high resolution mass spectrometry and library matching. *Journal of Chromatography A*, **1397**, 32-42.

23. Paul M., Ippisch J., Herrmann C., Guber S., Schultis W. (2014) Analysis of new designer drugs and common drugs of abuse in urine by a combined targeted and untargeted LC-HR-QTOFMS approach. *Analytical and Bioanalytical Chemistry*, **406**, 4425-4441.

24. Li X., Uboh C.E., Soma L.R., Liu Y., Guan F., Aurand C.R. (2014) Sensitive hydrophilic interaction liquid chromatography/tandem mass spectrometry method for rapid detection, quantification and confirmation of cathinone-derived designer drugs for doping control in equine plasma. *Rapid Communications in Mass Spectrometry*, **28**, 217-229.

25. Scientific Working Group for Forensic Toxicology (SWGTOX). (2013) Standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*, **37**, 452-474..

26. Morad A., Al-Meshal I., Nasir M., El-Feraly F. (1989) High-performance liquid chromatographic determination of (−)-cathinone in plasma. *Chromatographia*, **27**, 201-204.

27. Paul B.D., Cole K.A. (2001) Cathinone (khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. *Journal of Analytical Toxicology*, **25**, 525-530.

28. Johnson R.D., Botch-Jones S.R. (2013) The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *Journal of Analytical Toxicology*, **37**, 51-55.

29. Soh Y.N.A., Elliott S. (2014) An investigation of the stability of emerging new psychoactive substances. *Drug Testing and Analysis*, **6**, 696-704.

30. Sorensen 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**, 727-736.

CHAPTER III

STABILITY OF SYNTHETIC CATHINONES IN BLOOD[1]

This dissertation follows the style and format of *The Journal of Analytical Toxicology*.

[1]Glicksberg L., Kerrigan S. (2017). Journal of Analytical Toxicology, https://doi.org/10.1093/jat/bkx071
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Abstract

The synthetic cathinones are powerful psychostimulants that have been associated with impairment, intoxication and fatal overdose. Forensic laboratories must be able to identify these new drugs as part of antemortem and postmortem toxicology investigations. Preliminary reports have indicated that some of the synthetic cathinones are unstable in biological matrices. It is important to understand drug stability in biological evidence so that analytical findings can be interpreted appropriately. The objective of this study was to systematically evaluate the concentration, temperature and analyte dependent stability of synthetic cathinones in preserved blood using liquid-chromatography/quadrupole-time of flight-mass spectrometry (LC/Q-TOF-MS). Cathinone stability was investigated at frozen, refrigerated, ambient and elevated temperature (-20°C, 4°C, 20°C and 32°C).

Although no concentration dependent differences in stability were observed, cathinone stability was highly temperature and analyte dependent. Substituents on the aromatic ring and nitrogen profoundly influenced stability. Tertiary amines (pyrrolidinyl analogs) were significantly more stable than their *N*-alkylated (secondary amine) counterparts. Furthermore, the methylenedioxy group also exerted a significant stabilizing effect, for both secondary and tertiary amines. The unsubstituted and ring substituted secondary amines were the least stable, most notably 3-fluoromethcathinone (3-FMC). Under some conditions, significant losses were observed within hours of storage. Half-lives ranged from a little as 8 hours (3-FMC) to 21 days (3,4-methylenedioxy-α-pyrrolidinobutiophenone, MDPBP) at elevated temperature (32°C). In contrast, half-lives ranged from 0.4 to >10 months when refrigerated and demonstrated even greater stability when frozen.

Biological evidence may be subjected to a variety of environmental conditions prior to, and during transport to the laboratory. These findings highlight the need to consider the potential for both temperature and analyte dependent differences. Due to the inherent instability of certain drugs within the class, quantitative drug findings in toxicological investigations must be interpreted with caution, and within the context of specimen storage and integrity.

Keywords: Synthetic cathinones, Stability, LC/Q-TOF-MS, Blood

STABILITY OF SYNTHETIC CATHINONES IN BLOOD

Introduction

The synthetic cathinones are powerful amphetamine-like psychostimulants that have increased in popularity in the United States since 2009 (1). According to the Drug Enforcement Administration, cathinones were reported in 48 of the 50 states in 2015, with the highest number of reports occurring in the South and Midwest regions. In 2010 the most common cathinones in the US were mephedrone, methylenedioxypyrovalerone (MDPV) and methylone (2). From 2013-2015 however, methylone, α-pyrrolidinopentiophenone (α-PVP) and ethylone accounted for 91% of all reports. In addition to increased drug seizures, illicit drug manufacturers produce new cathinones as part of their ongoing attempt to circumvent drug laws and evade judicial consequences. This is evidenced by the fact that the number of synthetic cathinones encountered in the National Forensic Laboratory Information System increased from five in 2009 to thirty-five in 2015. The sought-after effects of these drugs include increased energy, empathy, openness and libido. However, cardiac, psychiatric and neurological effects are common among users that require medical intervention. Over the past decade the federal government has taken numerous steps to ban specific synthetic cathinones and the majority of states have enacted legislation, often in the form of general class bans, in an effort to curb their appeal.

These compounds present a challenge to the forensic toxicology community due to the number of structurally related analogs and regioisomers that currently exist. The cathinones are β-keto amphetamines (2-aminopropiophenones) that can be categorized into *N*-alkylamines (secondary amines) and pyrrolidines (tertiary amines). The chemical

properties of these arylaminoketones are dominated by two functional groups: the ketone and the amine. The cathinones are either ring substituted (R_1 and R_2), formed by the variation of the α-carbon substituent (R_3), or *N*-alkylated (R_4 and R_5) (**Figure 3.1**). At the inception of this study, a total of twenty-two synthetic cathinones were commercially available and the individual structures of these compounds are shown in **Figure 3.2.**

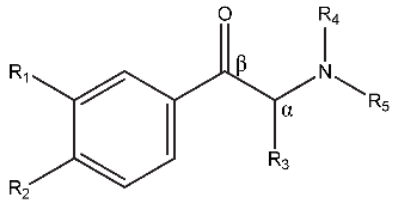

Figure 3.1. General cathinone structure.

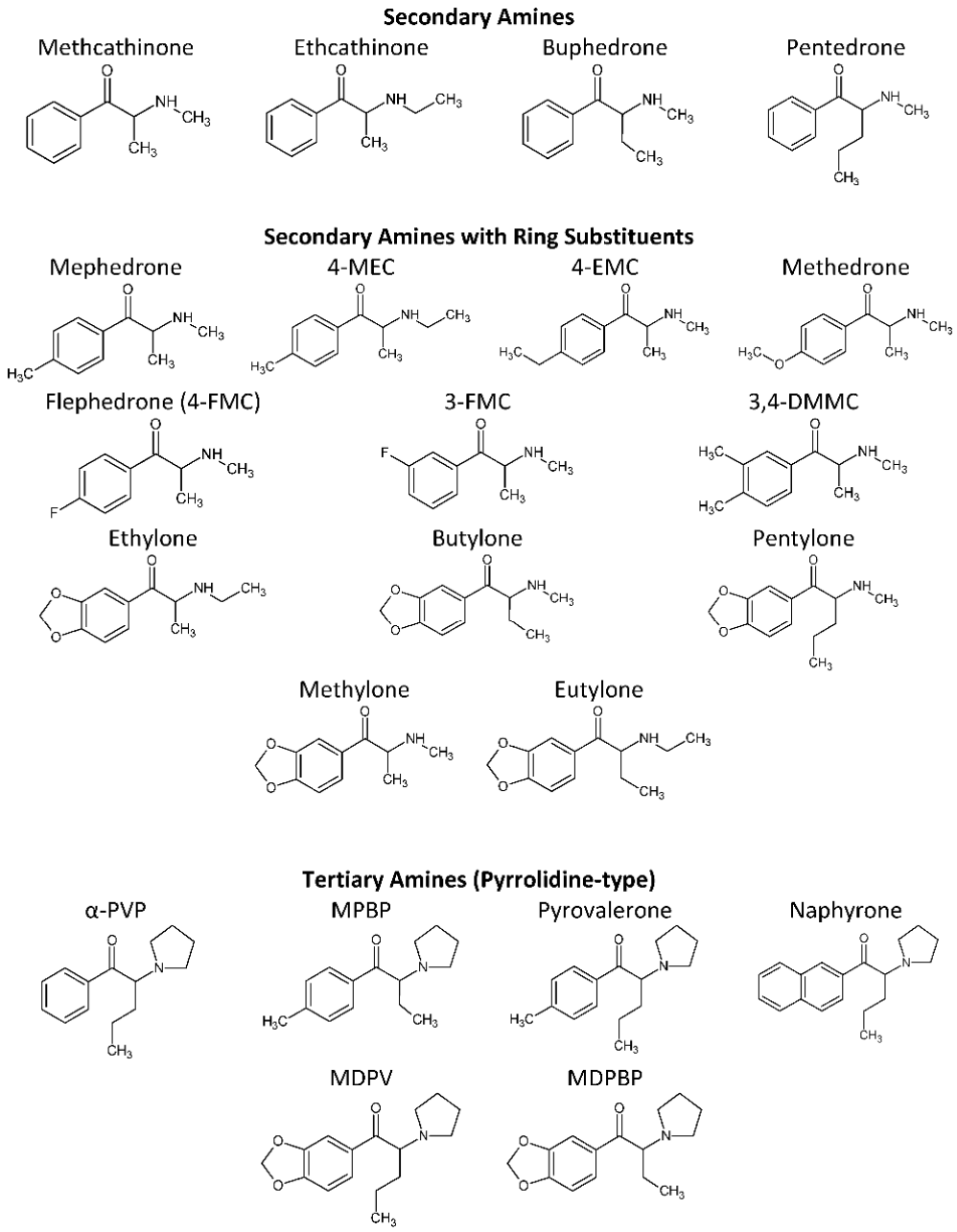

Figure 3.2. Structures of synthetic cathinones included in this study.

Drug stability must be carefully considered when interpreting toxicological results (3). Pre-analytical conditions, including specimen transport, storage and handling may cause the drug concentration to change. The stability and intrinsic chemical properties of many illicit and pharmaceutical drugs are widely known and understood, but this

information is relatively limited for the synthetic cathinones. An increased understanding of cathinone stability is needed due to the prevalence of these drugs in criminal and death investigations (4-9). Forensic toxicology laboratories go to considerable lengths to ensure the precision and accuracy of their quantitative results. However, in order to reliably interpret those results, drug stability and pre-analytical changes in drug concentration should be carefully considered.

While the proliferation of cathinone compounds over the past decade has renewed interest in the stability of synthetic cathinones, the instability of cathinone (its natural precursor) has been understood for decades. Cathinone was first identified in the 1970s as the principle pharmacologically active compound in "khat". Although its degradation product (cathine) had been identified years earlier, the delay in the identification of cathinone was largely due to its instability in the plant material (10-13). Cathinone was also reported to be unstable in basic conditions (11, 14). More recently, Tsujikawa, et al. investigated the stability of five synthetic cathinones in aqueous solutions over a range of pH. They concluded that drug stability increased with decreasing pH and that the rate of decomposition was likely dependent on the chemical structure (14). Following decomposition four degradation products were identified, one resulting from acetylation. The remaining three were produced following oxidative deamination of mephedrone **(Figure 3.3)** (14). Through analysis of synthetic cathinone powders exposed to air, Tsujikawa, et. al., identified two additional breakdown products for pyrrolidine-type cathinones: *N*-oxide and 2"-oxo **(Figure 3.3)** (13). Cathinone instability was also observed during gas chromatography-mass spectrometry (GC-MS) analysis. Under some conditions

cathinones can thermally degrade, resulting in the formation of oxidative breakdown products (15-17).

There are a relatively small number of studies that have addressed synthetic cathinone stability in blood or plasma. Morad *et. al.* was the first to report that cathinone was unstable in plasma in 1989 (18). More recently, issues associated with quantitative reproducibility and stability of the newer designer cathinones have emerged. Marinetti and Antonides described the lack of reproducibility of methylone and methedrone in toxicological samples in a series of published case reports (19). Soon thereafter, Johnson and Botch-Jones investigated the stability of MDPV and mephedrone at 1,000 ng/mL in blood, plasma and urine over 14 days of storage (20). Mephedrone was considerably less stable than MDPV, demonstrating complete loss (100%) after 7 days of storage at room temperature in blood. Both drugs were stable under frozen storage conditions for the entire two-week period. Based on the considerable difference between mephedrone and MDPV, the authors emphasized the need for additional research and the need to consider chemical instability when interpreting results.

Li *et. al.* investigated the stability of eleven synthetic cathinones in equine plasma over various time intervals. Samples were stored at 25°C for 24 hours, 4°C for 7 days, -20°C for 4 weeks, and -70°C for 24 weeks (6 months). The authors concluded that the eleven cathinones were stable for 30 days at -20°C, and 6 months in -70°C. Most were stable at room temperature for 24 hours, with the exception of 4-fluoromethcathinone (4-FMC), 3-fluoromethcathinone (3-FMC), and 3-methyoxymethcathinone (21). Busardò investigated the stability of mephedrone in antemortem and postmortem blood over six months, concluding that preserved blood should be stored at -20ºC to prevent significant

loss (23). Mephedrone was undetectable after 30 days at 20°C, 90 days at 4°C and stable at -20°C for the duration of the 6-month study. Soh and Elliott also described the stability of 4-methylethcathinone (4-MEC) in blood and plasma at ambient temperature (22). 4-MEC, originally fortified at 2,000 ng/mL was undetectable within 14 days, although plasma was reported to have greater stability. The degradation of 4-MEC in blood produced an unknown peak, which was identified as a degradation product, dihydro-4-MEC resulting from keto-reduction **(Figure 3.3)**.

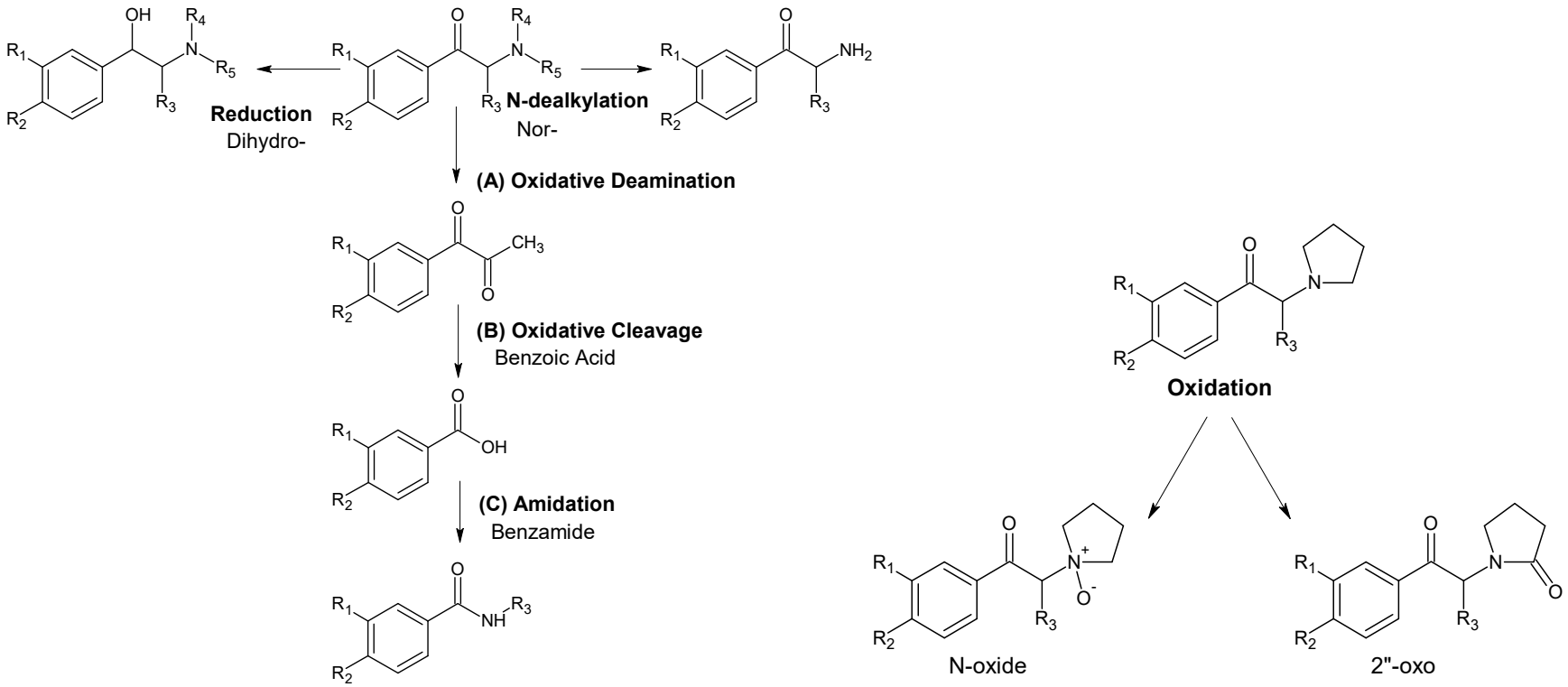

Figure 3.3. Proposed degradation pathway for synthetic cathinones (13, 14, 22).

In this report we describe the systematic evaluation of twenty-two synthetic cathinones in preserved blood to evaluate concentration, analyte and temperature dependent differences in stability. Over a period of six months, stability was evaluated at four temperatures, selected to represent frozen (-20°C) and refrigerated (4°C) long- and short-term storage temperatures at the laboratory; exposure to ambient (20°C) or room temperature during routine processing and handling; and finally, potential exposure to elevated temperatures, which might be experienced during shipping and transport to the laboratory (32°C). Tentative identification of breakdown products was also investigated where possible.

Materials and Methods

Chemicals and reagents

Methcathinone, 3-FMC, 4-FMC (flephedrone), methylone, ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-MEC, MDPBP, pentedrone, pentylone, 3,4-dimethylmethcathinone (3,4-DMMC), α-PVP, 4-ethylmethcathinone (4-EMC), 4-methyl-α-pyrrolidinobutiophenone (MPBP), MDPV, pyrovalerone, and naphyrone were purchased from Cerilliant Corporation (Round Rock, TX, USA) in 1.0 mg/mL methanolic solutions. Internal standards methylone-D3, ethylone-D5, buytlone-D3, mephedrone-D3, eutylone-D5, pentylone-D3, alpha-PVP-D8, MDPV-D8, and naphyrone-D5 were also purchased from Cerilliant Corporation in 0.1 mg/mL methanolic solutions. Bovine blood containing 1% (w/v) sodium fluoride and 0.2% (w/v) potassium oxalate was purchased from Quad Five (Ryegate, Montana, USA). Commercial evacuated glass tubes without additional preservative (BD Vacutainer™ red-top tubes, 10 mL, 16 x 100 mm) were purchased from VWR (Radnor, PA, USA).

Dichloromethane, isopropyl alcohol, and glacial acetic acid were purchased from Mallinckrodt Chemicals (St. Louis, MO, USA). LC-MS grade methanol, concentrated hydrochloric acid, LC-MS grade acetonitrile, and dibasic sodium phosphate were purchased from J.T. Baker (Center Valley, MA, USA). Optima® Hexane and HPLC grade ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (>99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and monobasic sodium phosphate was purchased from VWR (Radnor, PA, USA). Deionized water was purified in-house using a Millipore Direct-Q® UV Water Purification system (Billerica, MA, USA). PolyChrom ClinII 3 cc (35 mg) solid phase extraction (SPE) columns were purchased from SPEware (Baldwin Park, CA, USA). Phosphate buffer (pH 6, 0.1 M) was prepared from 0.1 M solutions of mono and dibasic sodium phosphate, and acidic methanol consisted of concentrated hydrochloric acid diluted in methanol (2%, v/v). The elution solvent (prepared daily) consisted of 2% concentrated ammonium hydroxide in 95:5 dichloromethane/isopropyl alcohol (v/v).

Instrumental analysis

An Agilent Technologies 6530 LC-Q/TOF-MS (Santa Clara, CA, USA) equipped with an Agilent 1290 Infinity autosampler and a Series 1200 LC system was used for analysis. The LC system was equipped with an Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm particle size) and a Poroshell 120 EC-C18 guard column (2.1 x 5 mm, 2.7 µm particle size) located in a thermostatically controlled column compartment (35°C). Nitrogen was generated using a Genius 3040 Nitrogen Generator (Peak Scientific, Billerica, MA, USA). Solid Phase Extraction (SPE) was performed using a JT Baker vacuum manifold and extracts were evaporated to dryness under nitrogen using a

TurboVap LV® concentration workstation (Caliper Life Sciences, Hopkinton, MA, USA). Mobile Phase A consisted of 0.1% formic acid in deionized water and Mobile Phase B consisted of 0.1% formic acid in acetonitrile. The LC-Q/TOF procedure used for the quantification of cathinones in blood was previously published and validated in accordance with generally accepted guidelines (24, 25).

Preparation and sampling of blood

Preserved drug-free blood (1 L, pH 7.35-7.45) was fortified with twenty-two synthetic cathinones to achieve a final concentration of 100 ng/mL and 1,000 ng/mL. Blood was immediately aliquoted into glass Vacutainer™ tubes and stored at the appropriate temperature for the duration of the study (-20°C, 4°C, 20°C and 32°C). The experimental design is summarized in **Figure 3.4**.

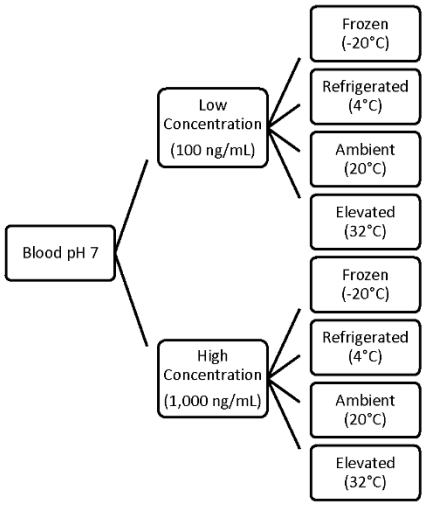

Figure 3.4. Experimental design.

Aliquots at each concentration were immediately removed and analyzed to establish T_0 (0% loss). At each sampling interval a single Vacutainer™ tube was removed from each temperature and aliquots (n=2) were removed and analyzed using the validated procedure.

After aliquots were removed, Vacutainer™ tubes were returned to the appropriate storage temperature. Sampling frequency was variable throughout the study. During the initial 48 hours, quantitative analysis was performed every 2-6 hours. Sampling remained frequent (4 assays/week) throughout the initial month, decreasing thereafter. Calibrators (10, 25, 100, 250, 350, 500 ng/mL) and controls (0, 100 ng/mL) were included in each run. Where appropriate, analysis of variance (ANOVA) was used to determine statistical significance (α=0.05). The drug was considered unstable when the concentration decreased by more than 20% of the original (T_0) concentration.

Isolation of cathinones from blood

Internal standard solution (0.5 µg/mL) was added to 2.0 mL blood to achieve a final concentration of 25 ng/mL. A protein precipitation was performed using 4 mL of cold acetonitrile. The samples were then centrifuged at 4,000 RPM for 5 minutes. The organic layer was decanted and diluted with 6 mL of pH 6 phosphate buffer (0.1 M) and briefly vortexed. Samples were transferred to PolyChrom ClinII SPE columns and allowed to flow through under gravity. Columns were rinsed with 1 mL deionized water followed by 1 mL of 1 M acetic acid. Columns were dried under full vacuum for 5 minutes and washed successively, with 1 mL of hexane, ethyl acetate, methanol, and dichloromethane. Synthetic cathinones were eluted using two 0.5 mL aliquots of elution solvent (2% conc. ammonium hydroxide in 95:5 dichloromethane: isopropyl alcohol). Acidic methanol (30 µL) was added to each extract prior to evaporation under nitrogen at 50°C. Extracts were reconstituted in 25 µL of 50:50 mixture of Mobile Phase A:B and 1 µL was injected onto the LC-Q/TOF-MS for analysis. Extraction efficiencies using this previously published procedure were 81-93% and limits of quantitation (LOQ) were 1-5 ng/mL (24).

Degradation product identification

Structural elucidation of degradation products was attempted using modified acquisition parameters. Targeted analysis was replaced with auto (full scan acquisition), insert mass range etc. Chromatographic and ionization conditions were unchanged. In order to improve the quality of MS/MS spectra, a preferred list (*m/z*) was used, based on predicted transformations (oxidative deamination, benzoic acid, amidation, *N*-oxide, 2”-oxo, and keto-reduction) **(Table 3.1)**. Agilent Technologies Qualitative Analysis B.07.00 was used to identify potential synthetic cathinone breakdown products in blood. When possible, breakdown products were monitored over the six months to determine relationship between synthetic cathinone degradation and breakdown product formation.

Table 3.1. Molecular formula and accurate mass for predicted synthetic cathinone degradation products.

Synthetic Cathinone	**Precursor (Parent)**		**Reduction**		**Oxidative Deamination**		**Benzoic Acid**		**Amidation**	
Secondary Amines	Formula	M+H	Formula	M+H	Formula	M+H	Formula	M+H	Formula	M+H
Methcathinone	$C_{10}H_{13}NO$	164.1069	$C_{10}H_{15}NO$	166.1226	$C_9H_8O_2$	149.0597	$C_7H_6O_2$	123.0441	C_8H_9NO	136.0757
Ethcathinone	$C_{11}H_{15}NO$	178.1226	$C_{11}H_{17}NO$	180.1383	$C_9H_8O_2$	149.0597	$C_7H_6O_2$	123.0441	C_8H_9NO	136.0757
Buphedrone	$C_{11}H_{15}NO$	178.1226	$C_{11}H_{17}NO$	180.1383	$C_9H_8O_2$	149.0597	$C_7H_6O_2$	123.0441	C_8H_9NO	136.0757
Pentedrone	$C_{12}H_{17}NO$	192.1383	$C_{12}H_{19}NO$	194.1539	$C_9H_8O_2$	149.0597	$C_7H_6O_2$	123.0441	C_8H_9NO	136.0757
3-FMC	$C_{10}H_{12}FNO$	182.0976	$C_{10}H_{14}FNO$	184.1132	$C_9H_7FO_2$	167.0503	$C_7H_5FO_2$	141.0346	C_8H_8FNO	154.0663
4-FMC	$C_{10}H_{12}FNO$	182.0976	$C_{10}H_{14}FNO$	184.1132	$C_9H_7FO_2$	167.0503	$C_7H_5FO_2$	141.0346	C_8H_8FNO	154.0663
Methedrone	$C_{11}H_{15}NO_2$	194.1176	$C_{11}H_{17}NO_2$	196.1332	$C_{10}H_{10}O_3$	179.0703	$C_8H_8O_3$	153.0546	$C_9H_{11}NO_2$	166.0863
Mephedrone	$C_{11}H_{15}NO$	178.1226	$C_{11}H_{17}NO$	180.1383	$C_{10}H_{10}O_2$	163.0754	$C_8H_8O_2$	137.0597	$C_9H_{11}NO$	150.0913
4-MEC	$C_{12}H_{17}NO$	192.1383	$C_{12}H_{18}NO$	194.1539	$C_{10}H_{10}O_2$	163.0753	$C_8H_8O_2$	137.0597	$C_9H_{11}NO$	150.0913
3,4-DMMC	$C_{12}H_{17}NO$	192.1383	$C_{12}H_{19}NO$	194.1539	$C_{11}H_{12}O_2$	177.091	$C_9H_{10}O_2$	151.0735	$C_{10}H_{13}NO$	164.1069
4-EMC	$C_{12}H_{17}NO$	192.1383	$C_{12}H_{19}NO$	194.1539	$C_{11}H_{12}O_2$	177.091	$C_9H_{10}O_2$	151.0735	$C_{10}H_{13}NO$	164.1069
Methylone	$C_{11}H_{13}NO_3$	208.0968	$C_{11}H_{15}NO_3$	210.1123	$C_{10}H_8O_4$	193.0495	$C_8H_6O_4$	167.0339	$C_9H_9NO_3$	180.0655
Ethylone	$C_{12}H_{15}NO_3$	222.1125	$C_{12}H_{17}NO_3$	224.1281	$C_{10}H_8O_4$	193.0495	$C_8H_6O_4$	167.0339	$C_9H_9NO_3$	180.0655
Butylone	$C_{12}H_{15}NO_3$	222.1125	$C_{12}H_{17}NO_3$	224.1281	$C_{10}H_8O_4$	193.0495	$C_8H_6O_4$	167.0339	$C_9H_9NO_3$	180.0655
Pentylone	$C_{13}H_{17}NO_3$	236.1281	$C_{13}H_{19}NO_3$	238.1438	$C_{12}H_{12}O_4$	221.0808	$C_8H_6O_4$	167.0339	$C_9H_9NO_3$	180.0655
Eutylone	$C_{13}H_{17}NO_3$	236.1281	$C_{13}H_{19}NO_3$	238.1348	$C_{11}H_{10}O_4$	207.0652	$C_8H_6O_4$	167.0339	$C_9H_9NO_3$	180.0655
Tertiary Amines	**Precursor (Parent)**		**Reduction**		***N*-oxide**		**2"-oxo**			
	Formula	M+H	Formula	M+H	Formula	M+H	Formula	M+H		
α-PVP	$C_{15}H_{21}NO$	232.1694	$C_{15}H_{23}NO$	234.1852	$C_{15}H_{21}NO_2$	248.1645	$C_{15}H_{19}NO_2$	246.1489		
MPBP	$C_{15}H_{21}NO$	232.1694	$C_{15}H_{23}NO$	234.1852	$C_{15}H_{21}NO_2$	248.1645	$C_{15}H_{19}NO_2$	246.1489		
Pyrovalerone	$C_{16}H_{23}NO$	246.1852	$C_{16}H_{25}NO$	248.2008	$C_{16}H_{23}NO_2$	262.1802	$C_{16}H_{21}NO_2$	260.1645		
Naphyrone	$C_{19}H_{23}NO$	282.1852	$C_{19}H_{25}NO$	284.2008	$C_{19}H_{23}NO_2$	298.1802	$C_{19}H_{21}NO_2$	296.1645		
MDPBP	$C_{15}H_{21}NO_3$	262.1438	$C_{15}H_{21}NO_3$	264.1594	$C_{15}H_{19}NO_4$	278.1387	$C_{15}H_{17}NO_4$	276.1230		
MDPV	$C_{16}H_{21}NO_3$	276.1594	$C_{16}H_{23}NO_3$	278.1751	$C_{16}H_{21}NO_4$	292.1543	$C_{16}H_{19}NO_4$	290.1397		

Data analysis

One-way ANOVA was used to statistically evaluate concentration, temperature, and analyte dependence. Concentration dependence was assessed by comparing the percentage of drug remaining (% target) at 100 ng/mL and 1,000 ng/mL to normalize the data. Absolute concentrations (ng/mL) were used to evaluate temperature and analyte dependence. To properly evaluate analyte dependence, ANOVA was used to examine variances *within* a population prior to comparisons *between* populations to ensure show that differences *within* a group were not significant ($F < F^{CV}$ or F-crit). No significance testing was performed when drug concentrations were within 20% of the initial (T_0) concentration for the entire duration of the study. Representative statistical values will be stated within the Results and Discussion; a complete list of statistical analysis can be found in **Appendix D**. Half-lives ($T_{1/2}$) for each drug were determined by estimating rate constants (k), assuming first order decay, based upon duplicate measurements at each time interval ($T_{1/2}$=Ln2/k). Rate plots were only generated if a significant decrease in concentration (>20%) was evident over three consecutive measurements. Due to the large number of drugs, graphical representations of the data were color-coded to facilitate interpretation. Cathinones (2° amines) without aromatic substituents (R_1 and R_2 = H) were identified in green, 2° amines with aromatic substituents (R_1 or $R_2 \neq$ H) were yellow, cathinones with methylenedioxy (MD) groups were indicated with a magenta line (for both 2° and 3° amines), and 3° amines (pyrrolidine-type) cathinones were shown in purple.

Results and Discussion

Half-life estimation

To estimate half-lives, rate plots were generated and are summarized in **Figure 3.5**.

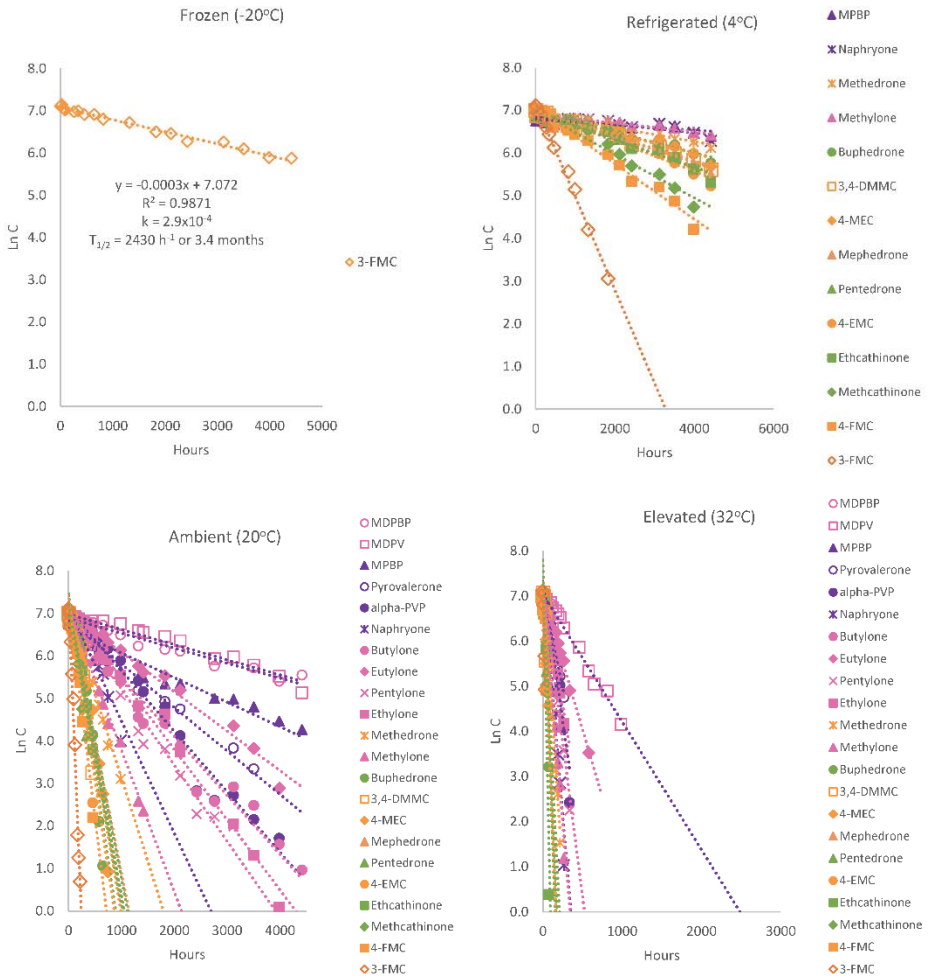

Figure 3.5. Rate plots and estimation of $T_{1/2}$ in blood.

In whole blood, half-lives were estimated for all drugs at elevated and ambient temperatures. In contrast, with the exception of 3-FMC, all drugs were stable for the entire six-month period when frozen. Cathinone half-lives in blood ranged from 8 hours (3-FMC) to 21 days (MDPBP) at elevated (32°C) temperature and 22 hours to almost three months at ambient (20°C) temperature. At refrigerated and frozen temperatures, synthetic

cathinones were considerably more stable and half-lives were measured for some but not all of the drugs (**Table 3.2**). These findings highlight the significant temperature and analyte dependent differences in stability, which are discussed in more detail below.

Table 3.2. Estimated $T_{1/2}$ of cathinones in blood in months (m), days (d), and hours (h).

Cathinone	Frozen (-20°C)	Refrigerated (4°C)	Ambient (20°C)	Elevated (32°C)
Secondary Amines				
3-FMC	2.6 m	13 d	22 h	8 h
3,4-DMMC	-	3.4 m	4.0 d	22 h
4-EMC	-	2.7 m	3.4 d	21 h
4-FMC	-	1.5 m	2.8 d	16 h
4-MEC	-	4.1 m	4.1 d	13 h
Buphedrone	-	4.0 m	3.4 d	14 h
Ethcathinone	-	2.9 m	4.5 d	8 h
Mephedrone	-	3.3 m	4.6 d	29 h
Methcathinone	-	1.9 m	4.2 d	17 h
Methedrone	-	5.9 m	7.3 d	28 h
Pentedrone	-	3.0 m	4.3 d	20 h
Secondary Amines - Methylenedioxy-Substituted				
Butylone	-	-	21 d	4.1 d
Ethylone	-	-	18 d	2.8 d
Eutylone	-	-	31 d	4.8 d
Methylone	-	9.6 m	8.6 d	1.4 d
Pentylone	-	-	16 d	2.1 d
Tertiary Amines				
MPBP	-	15 m	1.7 m	8.2 d
Naphyrone	-	10 m	11 d	1.4 d
α-PVP	-	-	20 d	2.4 d
Pyrovalerone	-	-	28 d	3.3 d
Tertiary Amines - Methylenedioxy-Substituted				
MDPBP	-	-	2.7 m	21 d
MDPV	-	-	2.7 m	10 d

Concentration dependence

No concentration dependent differences in stability were observed for any of the drugs. **Figure 3.6** depicts representative stability data for refrigerated blood at both concentrations tested (100 and 1,000 ng/mL). One-way ANOVA analysis confirmed lack of significance for unstable drugs (methcathinone, 32°C, $F(1,58)=0.004$, $p=0.95$) and stable drugs (pyrovalerone, 32°C, $F(1,54)=0.392$, $p=0.534$) alike. Due to the absence of concentration dependent stability for any of the cathinones, all subsequent statistical evaluations of temperature and analyte dependence were routinely undertaken at 1,000 ng/mL unless otherwise stated.

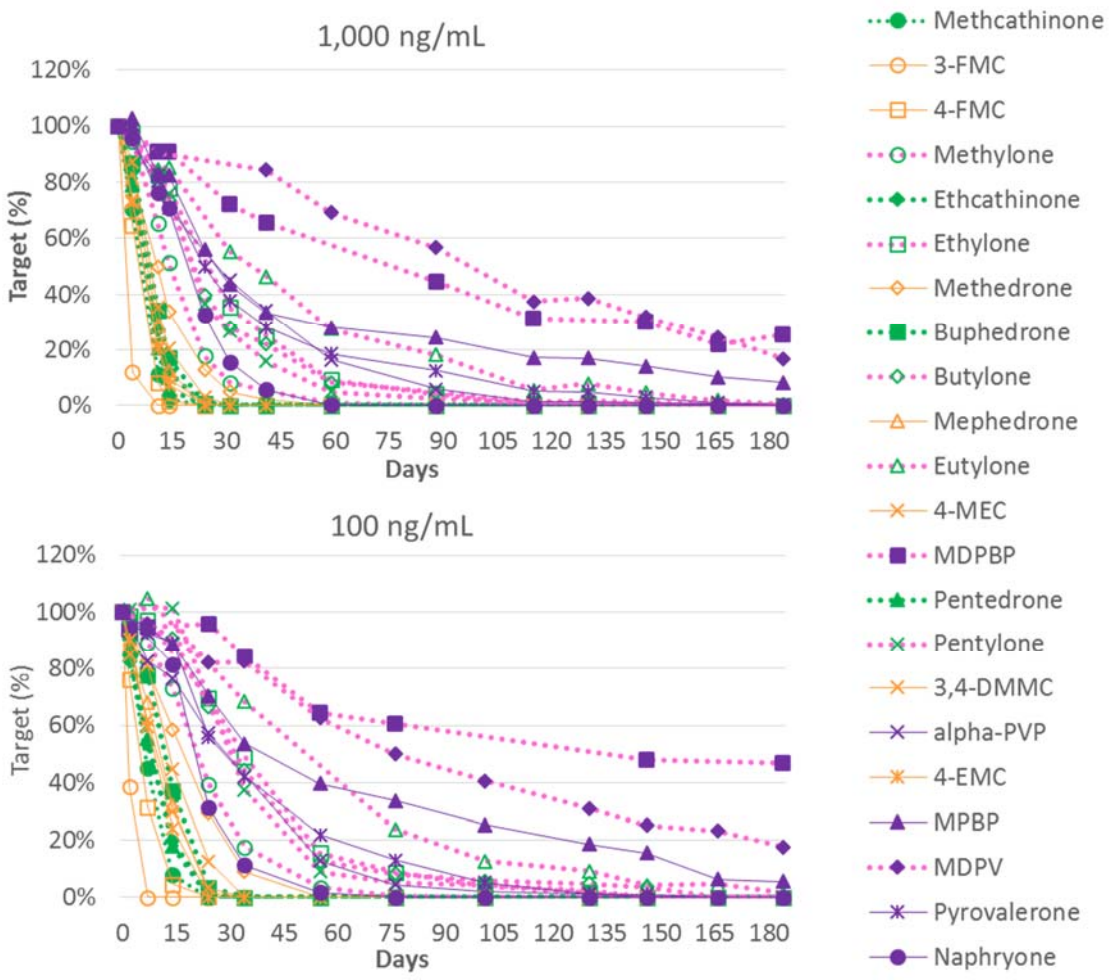

Figure 3.6. Cathinone stability in refrigerated blood at 100 and 1,000 ng/mL.

Analyte dependence

In order to evaluate the significance of analyte-dependence, ANOVA was used to examine the variances *within* and *between* populations. For example, before determining the significance of the MD group, it was necessary to show that differences *within* the group were not significant. The plots presented in **Figure 3.7** help visualize *within* and *between* sub-population significance. Due to notable differences in stability for some of the fluorinated cathinones (clearly illustrated in **Figure 3.7** at 20 and -20°C) 3- and 4-FMC were excluded for some of the statistical comparisons.

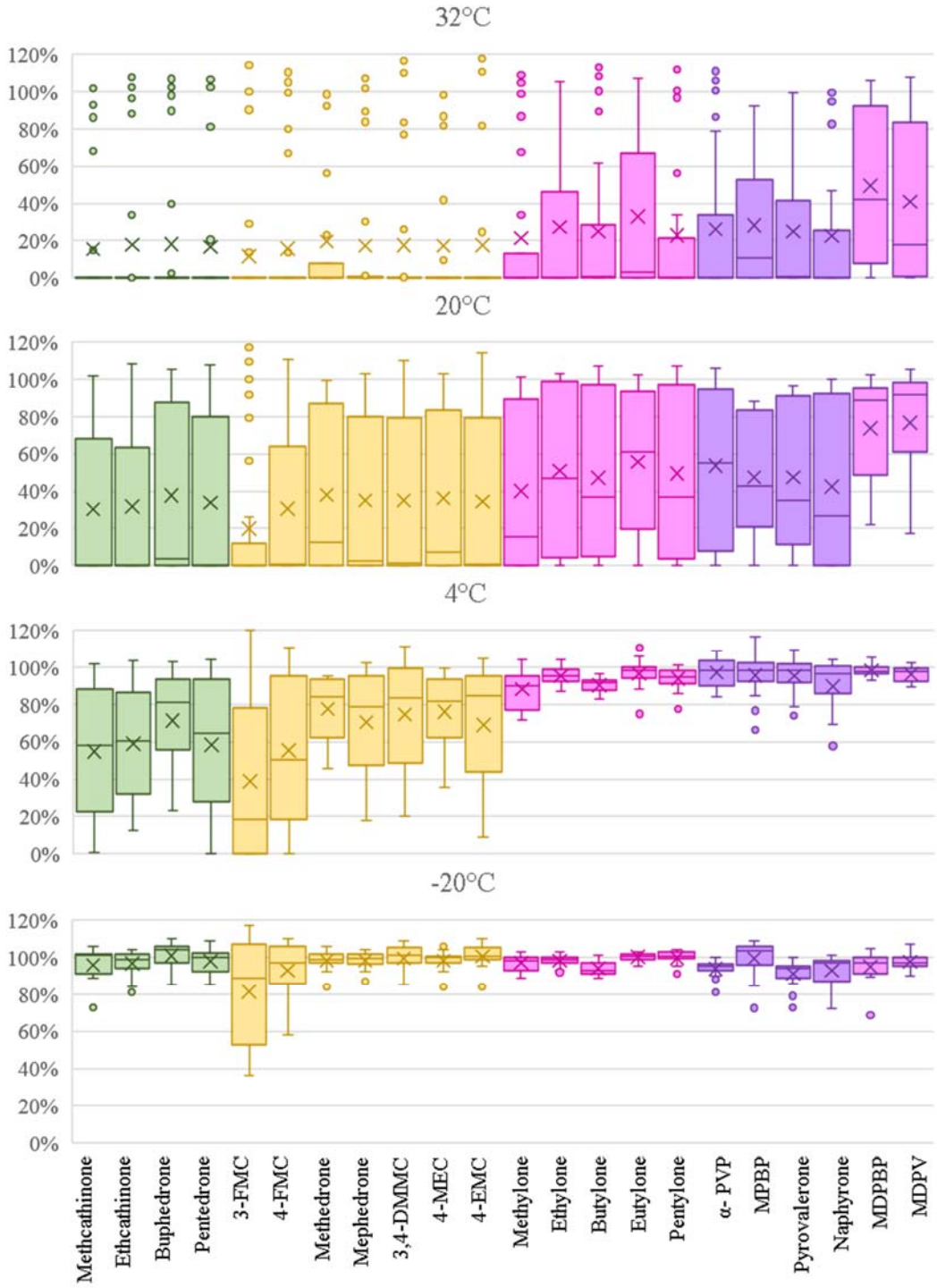

Figure 3.7. Box plots depicting within and between sub-populations of synthetic cathinones. Sub-populations include secondary amine, unsubstituted (green); secondary amine, ring substituted (yellow); secondary amine, MD (magenta); tertiary amine, non-MD (purple); tertiary amine, MD (magenta/purple). Mean ('X') and median (line) concentration for each drug over the six month study also identified.

Among the secondary amines, there were no significant differences in stability between unsubstituted and ring substituted cathinones at elevated and ambient temperatures in blood. This was attributed to their very rapid degradation under these conditions. No significant differences were observed in refrigerated or frozen blood when 3-FMC and 4-FMC were excluded. Addition of the MD group had a significant stabilizing effect. The MD-substituted secondary amines (ethylone, butylone, pentylone, methylone, eutylone) were considerably more stable than their unsubstituted counterparts (methcathinone, ethcathinone, buphedrone, pentedrone). The stabilizing effect of the MD group was also evident for the tertiary amines (pyrrolidines). MD-substituted pyrrolidines (MDPBP, MDPV) were generally observed to be more stable that their non-MD substituted counterparts (α-PVP, MPBP, pyrovalerone, naphyrone), although in all but a few instances, the differences were not statistically significant. Comparisons between these groups were not always possible due to within group variability among the pyrrolidinyl analogs, notably naphyrone (the least stable of the tertiary amines). The stabilizing effect of the MD group was evident throughout, most notably between the MD-substituted tertiary amines and the unsubstituted secondary amines ($F(5,125)=4.7$, $p<0.0001$) and the substituted secondary amines ($F(8,252)=3.1$, $p=0.002$) in refrigerated blood.

The nitrogen substituent exerted an even greater stabilizing influence. Tertiary amines were consistently more stable than their secondary amine counterparts. This stabilizing effect was even evident when comparing the most stable MD-substituted secondary amines with their pyrrolidinyl counterparts. Additionally, MD-substituted tertiary amines were more stable than MD-substituted secondary amines at refrigerated temperature ($F(6,165)=2.9$, $p<0.01$).

Cathinone stability was highly analyte dependent. Pyrrolidinyl-type cathinones with tertiary amines were notably more stable than their secondary amine counterparts. The inability of the tertiary amines to undergo oxidative deamination is a likely explanation. Although significant differences between unsubstituted and ring substituted secondary amines were not observed, substitution with a MD group had a notable stabilizing effect for all drugs. Among the twenty-two drugs tested, MD-substituted pyrrolidinyl cathinones were the most stable (i.e. MDPBP, MDPV), followed by tertiary amines (α-PVP, MPBP, pyrovalerone, naphyrone) and MD-substituted secondary amines (ethylone, butylone, pentylone, methylone, eutylone). Unsubstituted (methcathinone, ethcathinone, buphedrone, pentedrone) and ring-substituted cathinones (mephedrone, 4-MEC, 4-EMC, methedrone, 3,4-DMMC, 3-FMC, 4-FMC) were considerably less stable, with 3-FMC exhibiting the greatest instability.

Temperature dependence

Cathinone stability was also highly temperature dependent (**Figure 3.8**).

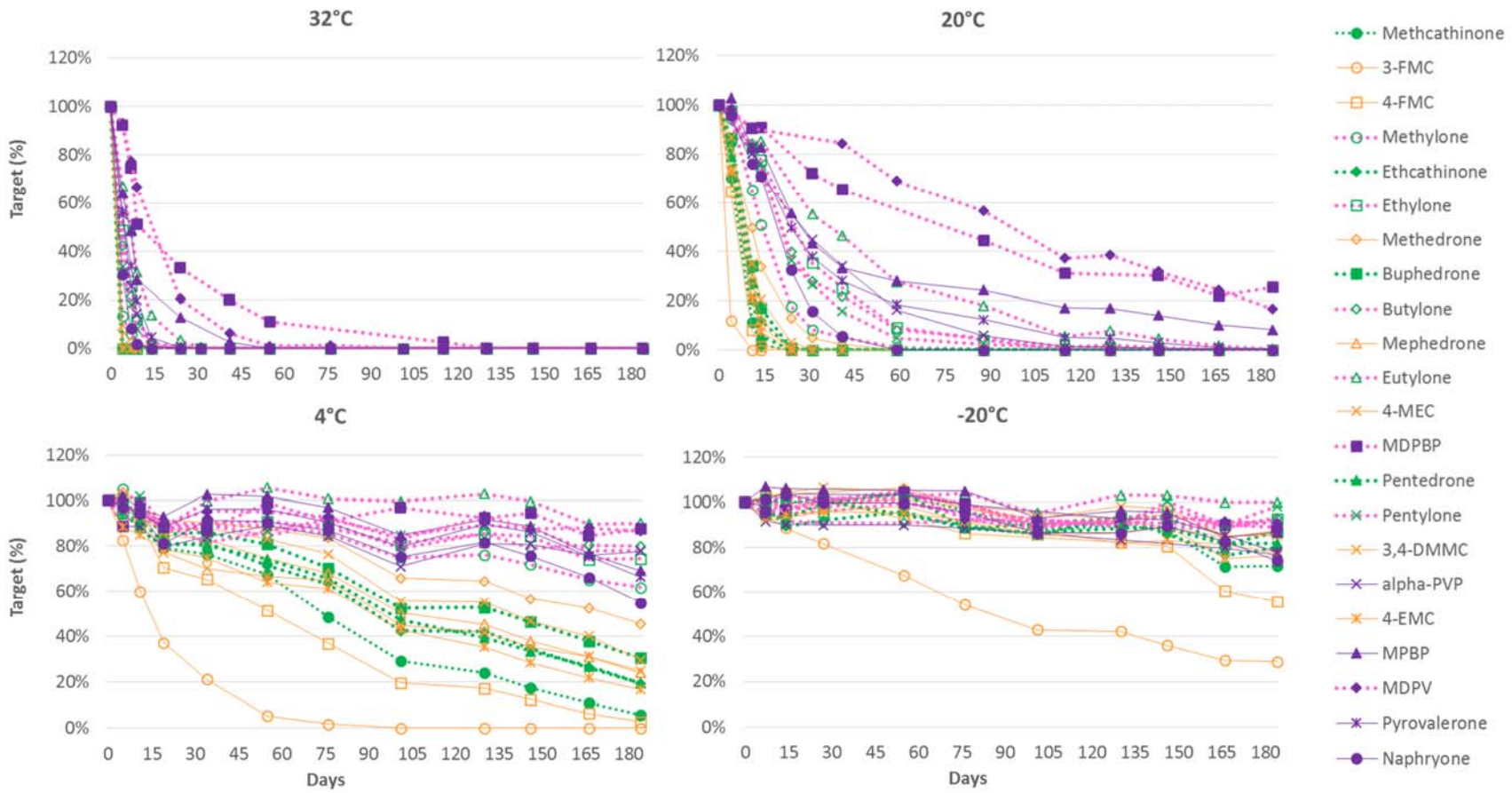

Figure 3.8. Cathinone stability in blood (1,000 ng/mL) at elevated, ambient, refrigerated, and frozen temperatures.

Temperature dependent differences were significant (p<0.001) for all twenty-two cathinones at both 100 ng/mL and 1,000 ng/mL. For the most unstable drug (3-FMC) half-lives ranged from 8 hours at elevated temperature to almost three months when frozen. These results highlight how low storage temperatures can significantly reduce degradation, even for the most unstable cathinone species. The dramatic influence of temperature on half-life (**Table 3.1**) is shown graphically in **Figure 3.9**.

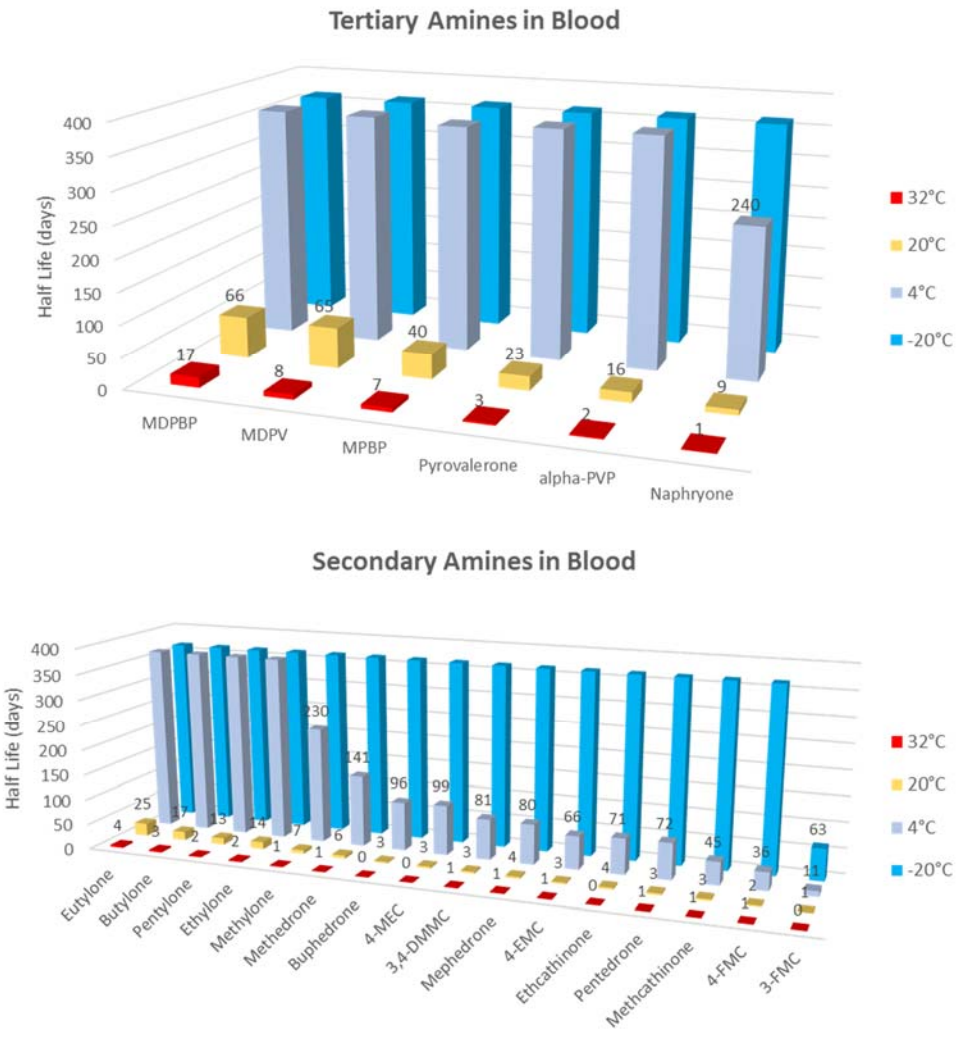

Figure 3.9. Temperature dependent stability of cathinones in blood. Unlabeled data indicates a half-life of >365 days or no measureable half-life due to stability.

Table 3.3 summarizes the number of days to significant (20%) or complete (100%) loss of drug in blood originally fortified at 100 ng/mL. This data not only highlights the importance of storage temperature on cathinone stability, but also the analyte dependent variables discussed earlier. With the exception of 3-FMC, all of the cathinones were relatively stable or exhibited only moderate losses (<40%) in blood after 30 days of refrigerated storage and significant losses were not observed until 2.5 months of frozen storage. At elevated temperature (32ºC), significant losses were observed for unsubstituted and ring substituted cathinones within 24 hours, compared with days or weeks for the pyrrolidines. Unless specifically packaged to protect from heat, it is not uncommon for specimens to be exposed to elevated temperatures during shipping and transport to the laboratory, particularly during summer months. At ambient temperature (20°C), all twenty-two were unstable by day 27, with the exception of MDPBP and MDPV (MD-substituted tertiary amines), which were unstable by day 55. In contrast the secondary amines with and without ring substituents experienced significant losses (>20%) within one to eight days. The ranges presented in Table II by chemical structure summarize days until significant (>20%) loss and 100% loss in blood (originally fortified at 100 ng/mL). This data also highlights that despite significant changes in concentration that might take place for some drugs under certain conditions, all the cathinones included in the study were detectable in blood for extended periods of time when refrigerated or frozen. It should be noted however that actual detection times are influenced by many factors, including dose of the drug (initial concentration) and the sensitivity of the assay.

Table 3.3. Number of days to significant (20%) and complete (100%) loss of drug (100 ng/mL in blood).

Cathinone	LOD (ng/mL)	LOQ (ng/mL)	Elevated (32°C)		Ambient (20°C)		Refrigerated (4°C)		Frozen (-20°C)	
			20% Loss	100% Loss	20% Loss	100% Loss	20% Loss	100% Loss	20% Loss	100% Loss
Secondary Amines										
3-FMC	1	2	5.5h	2	1	7	4	88	14	--
3,4-DMMC	5	5	1	4	4	76	19	--	101	--
4-EMC	2	5	1	4	4	24	55	--	130	--
4-FMC	1	1	1	3	2	24	19	166	76	--
4-MEC	1	1	2	7	8	34	41	--	--	--
Buphedrone	2	2	2	7	7	31	41	--	146	--
Ethcathinone	1	2	1.5	4	3	27	27	--	88	--
Mephedrone	2	2	2	11	4	27	55	--	130	--
Methcathinone	0.25	0.25	1.5	4	3	19	19	184	76	--
Methedrone	1	1	2	7	8	55	101	--	--	--
Pentedrone	5	5	1	4	4	24	41	--	130	--
Range	0.25 - 5	0.25 - 5	5.5h - 2d	2 - 11	1 - 8	7 - 76	4 - 101	88 - >6m	14 - >6m	>6m
Secondary Amines - Methylenedioxy-Substituted										
Butylone	1	2	4	27	19	166	--	--	--	--
Ethylone	1	5	4	24	19	146	--	--	--	--
Eutylone	5	5	4	41	27	--	--	--	--	--
Methylone	0.25	1	2	19	11	101	101	--	--	--
Pentylone	1	5	4	24	19	146	--	--	--	--
Range	0.25 - 5	1 - 5	2 - 4	19 - 41	11 - 27	101 - >6m	101 - >6m	>6m	>6m	>6m

(continued)

Cathinone	LOD (ng/mL)	LOQ (ng/mL)	Elevated (32°C)		Ambient (20°C)		Refrigerated (4°C)		Frozen (-20°C)	
			20% Loss	100% Loss	20% Loss	100% Loss	20% Loss	100% Loss	20% Loss	100% Loss
Tertiary Amines										
MPBP	1	5	7	59	24	--	--	--	--	--
Naphyrone	0.5	0.5	3	14	14	59	130	--	146	--
α-PVP	2	2	4	27	14	146	130	--	101	--
Pyrovalerone	0.25	0.25	3	24	19	166	130	--	184	--
Range	0.25 - 2	0.25 - 5	3 - 7	14 - 59	14 - 24	59 - >6m	130 - >6m	>6m	101 - >6m	>6m
Tertiary Amines - Methylenedioxy-Substituted										
MDPBP	0.5	5	14	115	55	--	--	--	--	--
MDPV	1	2	9	41	55	--	--	--	--	--
Range	0.5 - 1	2 - 5	9 - 14	41 - 115	55	>6m	>6m	>6m	>6m	>6m

Synthetic cathinone degradation products

Synthetic cathinone degradation product identification was extremely challenging due to twenty-two cathinones in a single sample, each potentially having multiple degradation products and intermediate species. This significantly hindered identification efforts. Furthermore, cathinones bearing a secondary amine may degrade into acidic products (figure and ref), which are not expected to extract from blood using the mixed-mode (cation exchange) SPE. While degradation product identification for the secondary amine cathinones was limited, the *N*-oxide degradation product was identified for all tertiary amines, except MPBP due to suspected interference from pyrovalerone. The 2”-oxo degradation product was also identified, albeit in very low abundance. Representative MS/MS spectra of *N*-oxide and 2”-oxo degradation products are shown for MDPV in **Figure 3.10**.

While MDPV, MDPV-*N*-oxide, and 2”-oxo-MDPV share many fragments, the MS/MS spectra for *N*-oxide and 2”-oxo had characteristic *m/z* of 86.0596 and 84.0803, respectively. The two ions correspond to the dissociation of γ-lactam (2”-oxo, C_4H_6NO) and *N*-oxidized pyrrolidine group (C_4H_8NO) from the phenethylamine pharmacophore. These characteristic ions were observed in all degradation product spectra for tertiary amine cathinones (**Appendix E**). The mass accuracy of precursor and fragment *m/z* were <14 ppm **(Table 3.4)**. Although mass accuracies of 5 ppm or less are ideal, biological samples had in many cases been subjected to highly unfavorable conditions for extended periods.

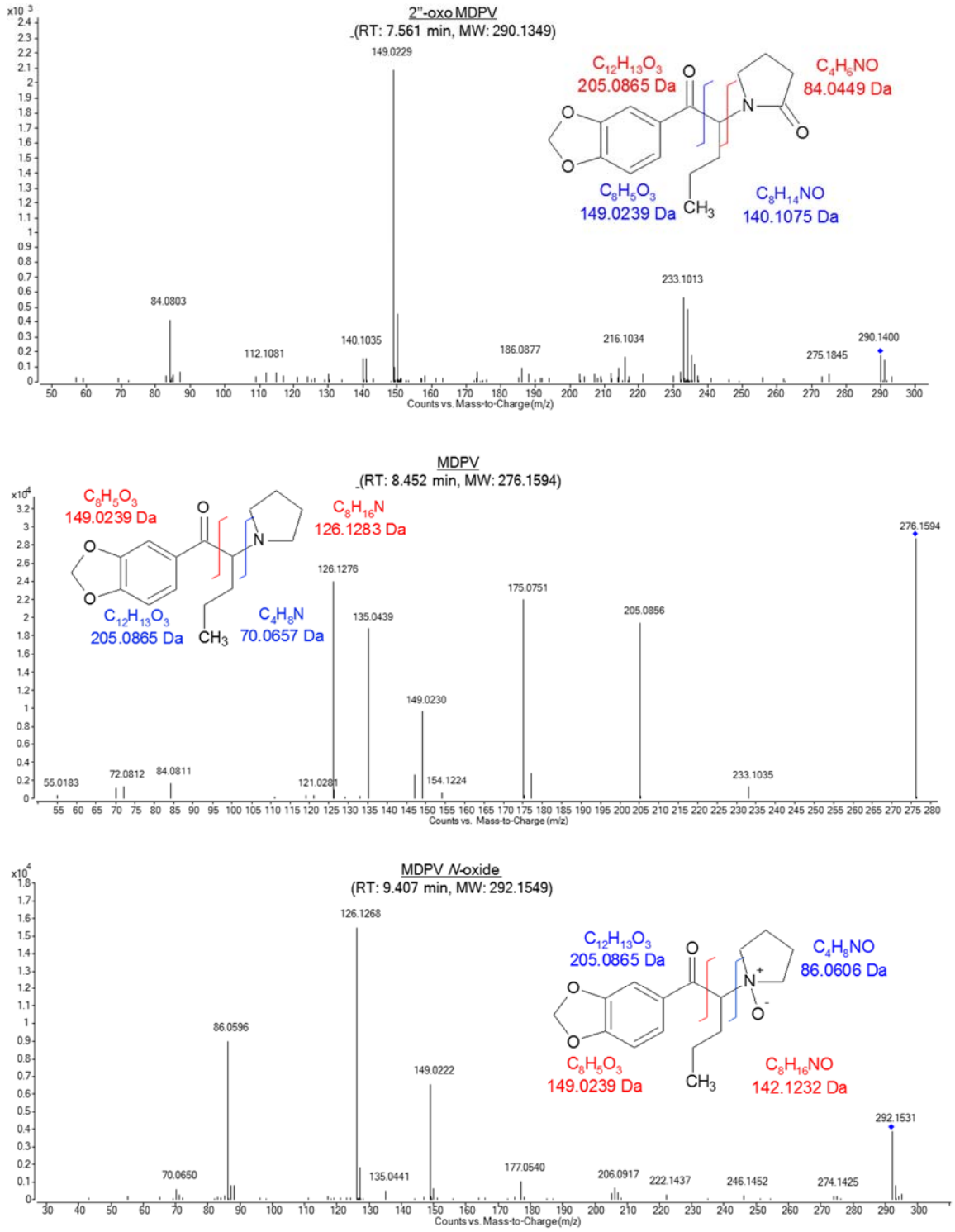

Figure 3.10. LC-Q/TOF MS MS/MS spectra for 2"-oxo-MDPV (top), MDPV (middle), and MDPV-*N*-oxide (bottom).

Table 3.4. Mass accuracy for *N*-oxide fragments for tertiary amine cathinones.

Fragment	Theoretical Mass	Experimental	Difference (PPM)
α-PVP			
$C_{15}H_{21}NO_2$	248.1645	248.1621	-9.7
$C_8H_{16}N$	126.1283	126.1268	-11.9
C_8H_7O	119.0491	119.0478	-10.9
$C_7H_{14}N$	112.1121	112.1115	-5.4
C_4H_8NO	86.0600	86.0598	-2.3
MDPBP			
$C_{15}H_{20}NO_4$	278.1387	278.1379	-2.9
$C_8H_5O_3$	149.0239	149.0221	-12.1
$C_7H_{14}N$	112.1121	112.1114	-6.2
C_4H_8NO	86.0600	86.0600	0.0
C_4H_8N	70.0651	70.0645	-8.6
MDPV			
$C_{16}H_{22}NO_4$	292.1543	292.1531	-4.1
$C_{10}H_9O_3$	177.0546	177.0540	-3.4
$C_8H_5O_3$	149.0233	149.0222	-7.4
$C_8H_{16}N$	126.1283	126.1268	-11.9
C_4H_8NO	86.0600	86.0596	-4.6
C_4H_8N	70.0651	70.0650	-1.4
Pyrovalerone			
$C_{16}H_{24}NO_2$	262.1802	262.1771	-11.8
$C_8H_{16}N$	126.1283	126.1266	-13.5
C_8H_7O	119.0491	119.0485	-5.0
$C_7H_{14}N$	112.1121	112.1108	-11.6
C_4H_8NO	86.0600	86.0598	-2.3
Naphyrone			
$C_{19}H_{24}NO_2$	298.1802	298.1831	9.7
$C_{11}H_7O$	155.0491	155.0479	-7.7
$C_8H_{16}N$	126.1283	126.1267	-12.7
C_4H_8NO	86.0600	86.0598	-2.3

Conclusions

Cathinone use is an ongoing drug problem, evidenced by increased drug seizures, adverse effects and fatalities. Cathinone stability was investigated to determine concentration, temperature and analyte dependence. Although no concentration dependence was observed, cathinone stability was greatly influenced by temperature and analyte-dependent variables. **Figure 3.11** summarizes overall analyte-dependent differences in stability among the twenty-two cathinones evaluated. Nitrogen substituents and MD ring-substitutions had the greatest impact. With the exception of 3-FMC, ring substituents had limited influence on stability among the secondary amines; differences in stability between ring substituted secondary amines were of sufficient magnitude to preclude any statistically significant difference between ring-substituted and unsubstituted cathinones.

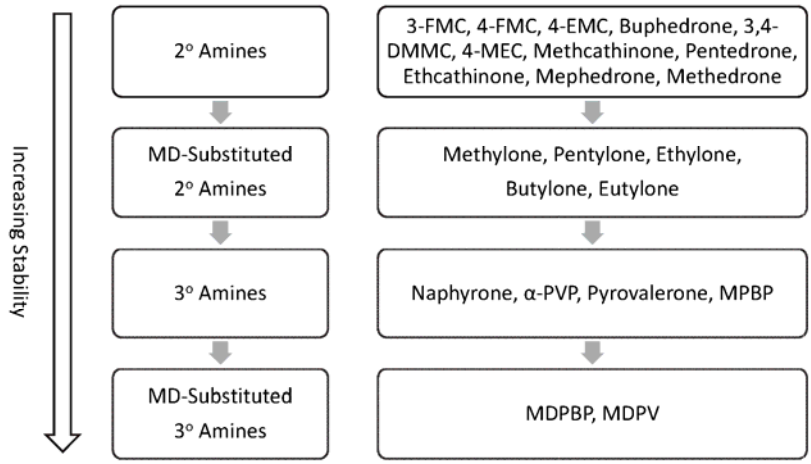

Figure 3.11. Influence of chemical structure on synthetic cathinone stability.

Although a highly systematic approach was taken in this study to address concentration, analyte and temperature dependent effects, the results are in good agreement

with previously published studies. Johnson and Botch-Jones' study of MDPV and mephedrone noted considerable differences in stability between the tertiary and secondary amine and also recommended frozen storage to minimize losses (20). The study of mephedrone in antemortem and postmortem blood reported by Busardò concluded that mephedrone was undetectable after 30 days at 20°C (23). These results are in close agreement with our results, which showed that mephedrone was undetectable by 27 days.

Drug instability and the magnitude of the loss was heavily influenced by temperature and chemical characteristics. Therefore, concentrations at the time of testing may not always reflect those at the time of interest *e.g.*, time of death or time of driving. Although drugs may still be detectable, significant losses are possible. Given that biological evidence is sometimes exposed to unfavorable conditions in both postmortem and antemortem toxicology investigations during routine shipping and transport to the laboratory, toxicological findings should be interpreted cautiously and within the full context of evidence disposition. These finding are of value in forensic toxicology investigations involving these twenty-two cathinones. However, a greater understanding of analyte dependent variables (specifically functional groups that have stabilizing effects) has a much broader impact, because it may allow us predict the stability of future synthetic cathinones, as these designer drugs continue to evolve.

Acknowledgements

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References

1. U.S. Drug Enforcement Administration: Diversion Control Division (2016) Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2013-2015. Springfield, VA: U.S. Drug Enforcement Administration.
2. U.S. Drug Enforcement Administration: Diversion Control Division (2014) Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2010-2013. Springfield, VA: U.S. Drug Enforcement Administration.
3. Kerrigan S. (2013) Sampling, Storage and Stability. In: Negrusz, A. and Cooper, G. (eds.), Clarke's Analytical Forensic Toxicology, 2nd edition, Chapter 13. Pharmaceutical Press, London, U.K., pp. 335-357.
4. Adamowicz P., Gieron J., Gil D., Lechowicz W., Skulska A., Tokarczyk B. (2016) The prevalence of new psychoactive substances in biological material - a three-year review of casework in Poland. *Drug Testing and Analysis*, **8,** 63-70.
5. Adamowicz P., Tokarczyk B., Stanaszek R., Slopianka M. (2013) Fatal Mephedrone Intoxication—A Case Report. *Journal of Analytical Toxicology*, **37**, 37-42.
6. Gil D., Adamowicz P., Skulska A., Tokarczyk B., Stanaszek R. (2013) Analysis of 4-MEC in biological and non-biological material—Three case reports. *Forensic Science International*, **228**, e11-e5.
7. Dickson A.J., Vorce S.P., Levine B., Past M.R. (2010) Multiple-Drug Toxicity Caused by the Coadministration of 4-Methylmethcathinone (Mephedrone) and Heroin. *Journal of Analytical Toxicology*, **34**, 162-168.

8. Namera A., Urabe S., Saito T., Torikoshi-Hatano A., Shiraishi H., Arima Y., et al. (2013) A fatal case of 3,4-methylenedioxypyrovalerone poisoning: coexistence of alpha-pyrrolidinobutiophenone and alpha-pyrrolidinovalerophenone in blood and/or hair. *Forensic Toxicology*, **31**, 338-43.

9. Wright T.H., Harris C. (2016) Twenty-One Cases Involving Alpha-Pyrrolidinovalerophenone (α-PVP). *Journal of Analytical Toxicology*, **40**, 396-402.

10. Chappell J.S., Lee M.M. (2010) Cathinone preservation in khat evidence via drying. *Forensic Science International*, **195**, 108-120.

11. Szendrei K. (1980) The Chemistry of Khat. Bulletin on Narcotics, 5-36.

12. Berrang B.D., Lewin A.H., Carroll F.I. (1982) Enantiomeric .alpha.-aminopropiophenones (cathinone): preparation and investigation. *The Journal of Organic Chemistry*, **47**, 2643-2647.

13. Tsujikawa K., Yamamuro T., Kuwayama K., Kanamori T., Iwata Y.T., Inoue H. (2015) Instability of the hydrochloride salts of cathinone derivatives in air. *Forensic Science International*, **248**, 48-54.

14. Tsujikawa K., Mikuma T., Kuwayama K., Miyaguchi H., Kanamori T., Iwata Y.T., et al. (2012) Degradation pathways of 4-methylmethcathinone in alkaline solution and stability of methcathinone analogs in various pH solutions. *Forensic Science International*, **220**, 103-10.

15. Deruiter J., Hayes L., Valaer A., Clark C.R., Noggle F.T. (1994) Methcathinone and designer analogues: synthesis, stereochemical analysis, and analytical properities. *Journal of Chromatographic Science*, **32**, 552-564.

16. Tsujikawa K., Kuwayama K., Kanamori T., Iwata Y.T., Inoue H. (2013) Thermal degradation of alpha-pyrrolidinopentiophenone during injection in gas chromatography/mass spectrometry. *Forensic Science International*, **231**, 296-299.

17. Kerrigan S., Savage M., Cavazos C., Bella P. (2015) Thermal degradation of synthetic cathinones: implications for forensic toxicology. *Journal of Analytical Toxicology*, **40**, 1-11.

18. Morad A., Al-Meshal I., Nasir M., El-Feraly F. (1989) High-performance liquid chromatographic determination of (−)-cathinone in plasma. *Chromatographia*, **27**, 201-204.

19. Marinetti L.J., Antonides H.M. (2013) Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results. *Journal of Analytical Toxicology*, **37**, 135-146.

20. Johnson R.D., Botch-Jones S.R. (2013) The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *Journal of Analytical Toxicology*, **37**, 51-55.

21. Li X., Uboh C.E., Soma L.R., Liu Y., Guan F., Aurand C.R, et al. (2014) Sensitive hydrophilic interaction liquid chromatography/tandem mass spectrometry method for rapid detection, quantification and confirmation of cathinone-derived designer drugs for doping control in equine plasma. *Rapid Communications in Mass Spectrometry*, **28**, 217-229.

22. Soh Y.A., Elliott S. (2014) An investigation of the stability of emerging new psychoactive substances. *Drug Testing and Analysis*, **6**, 696-704.

23. Busardò F.P., Kyriakou C., Tittarelli R., Mannocchi G., Pantano F., Santurro A., et al. (2016) Assessment of the stability of mephedrone in ante-mortem and post-mortem blood specimens. *Forensic Science International*, **256**, 28-37.

24. Glicksberg L., Bryand K., Kerrigan S. (2016) Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. *Journal of Chromatography B*, **1035**, 91-103.

25. Scientific Working Group for Forensic Toxicology (SWGTOX). (2013) Standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*, **37**, 452-474.

CHAPTER IV

STABILITY OF SYNTHETIC CATHINONES IN URINE[1]

This dissertation follows the style and format of *The Journal of Analytical Toxicology*

[1]Glicksberg L., Kerrigan S. (2017) Stability of synthetic cathinones in urine. *Journal of Analytical Toxicology,* in press.
Submitted to Journal of Analytical Toxicology.

Abstract

In this report, we evaluate the concentration, pH, temperature, and analyte dependent effects on cathinone stability in preserved human urine. A total of twenty-two synthetic cathinones were evaluated at 100 ng/mL and 1,000 ng/mL in pH 4 and pH 8 urine over six months. Specimens were stored at -20, 4, 20 and 32°C. The stability of synthetic cathinones was highly dependent upon urine pH and storage temperature. Cathinones were considerably more stable in acidic urine (pH 4) at low temperature. In alkaline urine (pH 8) at 32°C, significant losses (>20%) were observed within hours for the majority of drugs. In contrast, all drugs were stable in frozen and refrigerated urine at pH 4 for the duration of the study. These results highlight the importance of sample storage and the potential for pre-analytical changes in concentration during routine shipping and handling of specimens. Significant structural influence was also observed. Cathinones bearing a tertiary amine (pyrrolidine group) were significantly more stable than their secondary amine counterparts. The methylenedioxy group also exerted a significant stabilizing effect on both the tertiary and secondary amines. In the absence of the methylenedioxy group, no significant differences in stability were observed between the unsubstituted and ring substituted secondary amines. Half-lives at ambient temperature in pH 8 urine ranged from 9 hours (3-fluoromethcathinone, 3-FMC) to 4.3 months (methylenedioxypyrovalerone, (MDPV) and 3,4-methylenedioxy-α-pyrrolidinobutiophenone, MDPBP), demonstrating the importance of analyte dependence, and the dual stabilizing effect of both the pyrrolidine and methylenedioxy groups. Biological evidence may be subjected to a variety of environmental conditions prior to, and during transport to the forensic laboratory. These findings demonstrate the inherent instability of certain cathinone species in biological

evidence under some conditions. Moreover, this study highlights the need for quantitative drug findings in toxicological investigations to be interpreted cautiously, and within the context of specimen storage and integrity.

Keywords: Designer drugs, Cathinones, Stability, Urine

STABILITY OF SYNTHETIC CATHINONES IN URINE

Introduction

The ongoing proliferation of designer drugs and novel psychoactive substances (NPSs) continues to present a variety of public health and safety challenges (1, 2). Synthetic cathinones are an important class of designer drugs, capable of producing euphoric, empathogenic and central nervous system stimulant effects similar to methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") (3, 4). Their harmful and addictive effects, resulting in acute toxicity and death, have led to increased vigilance as they relate to forensic toxicology investigations.

Synthetic cathinones and other NPSs are constantly evolving, as part of a strategic effort to circumvent and undermine drug legislation. According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), a total of six hundred and twenty new substances are currently being monitored (2). Synthetic cathinones were the most frequently seized NPSs in 2015, accounting for one third of the total number of seizures. The emergence of new drugs each year, including cathinones, presents a formidable challenge to the forensic toxicology community. In Europe, the EMCDDA reported fourteen new cathinones in 2016 and in the United States, the Drug Enforcement Administration's National Forensic Laboratory Information System (NFLIS) reported a total of thirty five synthetic cathinones in 2015 (1, 2). Substitutions on the aromatic ring, side chain and nitrogen influence the pharmacological and physicochemical properties of the drug. Although many of the drugs share similar functionality, the proliferation and variety of cathinone analogs makes it an ongoing challenge to keep pace with the chemistry, toxicology and stability of these arylaminoketones.

Drug stability and in particular, pre-analytical changes in drug concentration, must be considered when interpreting toxicological results (5). The stability and properties of common illicit drugs are widely understood, but relatively little is known regarding the synthetic cathinones. However, there is real need to investigate their stability in biological evidence, due to their increasing prevalence in ante-mortem and post-mortem investigations (6-15). Reports of synthetic cathinone stability in biological evidence are still relatively limited and have been described for a relatively small number of cathinones. We recently described a systematic approach to determine cathinone stability in blood using twenty-two cathinones (16). In this report, we describe a similar approach to evaluate the concentration, analyte, matrix, and temperature dependent stability of cathinones in urine.

To date, there have been a limited number of reports that describe cathinone stability in urine (17-20). Paul and Cole (17) were the first in the early 2000s to investigate the stability of methcathinone and cathinone in urine over three months at -18°C and 4°C. Both drugs were stable for two months when frozen, and for three days when refrigerated. As much as 79% was lost within three months at 4°C. Al-Saffar (19) and Johnson (18) were among the first to draw attention to the analyte dependent differences in cathinone stability. MDPV and mephedrone were evaluated in urine at -20°C, 4°C, and 22°C over 14 days at 1,000 ng/mL. MDPV was stable in all matrices at all temperatures. In contrast, mephedrone was considerably less stable, demonstrating significant losses within 7 days at room temperature (18). Al-Saffar (19) evaluated the stability of ten cathinones in urine (1,000 ng/mL) over 3 months at 22, 6 and -20ºC. Significant losses were observed within one day at room temperature for methcathinone, buphedrone, mephedrone, 3-FMC, 4-

fluoromethcathinone (flephedrone, 4-FMC), and pentylone. Although MDPV, naphyrone, butylone and methedrone exhibited greater stability, all but MDPV were undetectable within three months at room temperature. Concheiro (20) reported losses as high as 68% for some cathinones in urine when stored at room temperature for 24 hours. Urinary pH and preservatives were not specified in any of the published studies to date.

Tsujikawa (21) investigated the stability of five synthetic cathinones in aqueous buffers at various pHs (4, 7, 10 and 12). They noted the influence of chemical structure and pH on rates of decomposition. Cathinone stability was highly pH dependent, demonstrating significant alkaline lability. Maskell (22) investigated the stability of three secondary amines, including mephedrone, in various formalin concentration solutions (5, 10, and 20%) at three pHs (3.5, 7, and 9.5). pH-Dependent degradation was independent of formalin concentration, with mephedrone having significant degradation within 1-3 days of storage at pH 9.5 and 7 in all formalin solutions. At a pH of 3.5 however, mephedrone was considerably more stable. The findings of Tsujikawa and Maskell are particularly significant for biological specimens such as urine, because the pH range can be quite variable. Although normal urinary pH is typically 4-8, it is influenced by diet, renal function, disease state, metabolic acidosis, diabetic ketoacidosis and other factors. Storage time and temperature can also play a role (23). Although preliminary studies suggest analyte, temperature and pH dependent effects, a comprehensive approach is needed. Such an approach may provide insight into new cathinones, yet to emerge. In this report we describe a systematic approach to evaluate the concentration, analyte, pH, and temperature-dependent stability of cathinones in urine, including a variety of substituted and

unsubstituted secondary and tertiary (pyrrolidine) analogs over a period of six months (**Figure 4.1**).

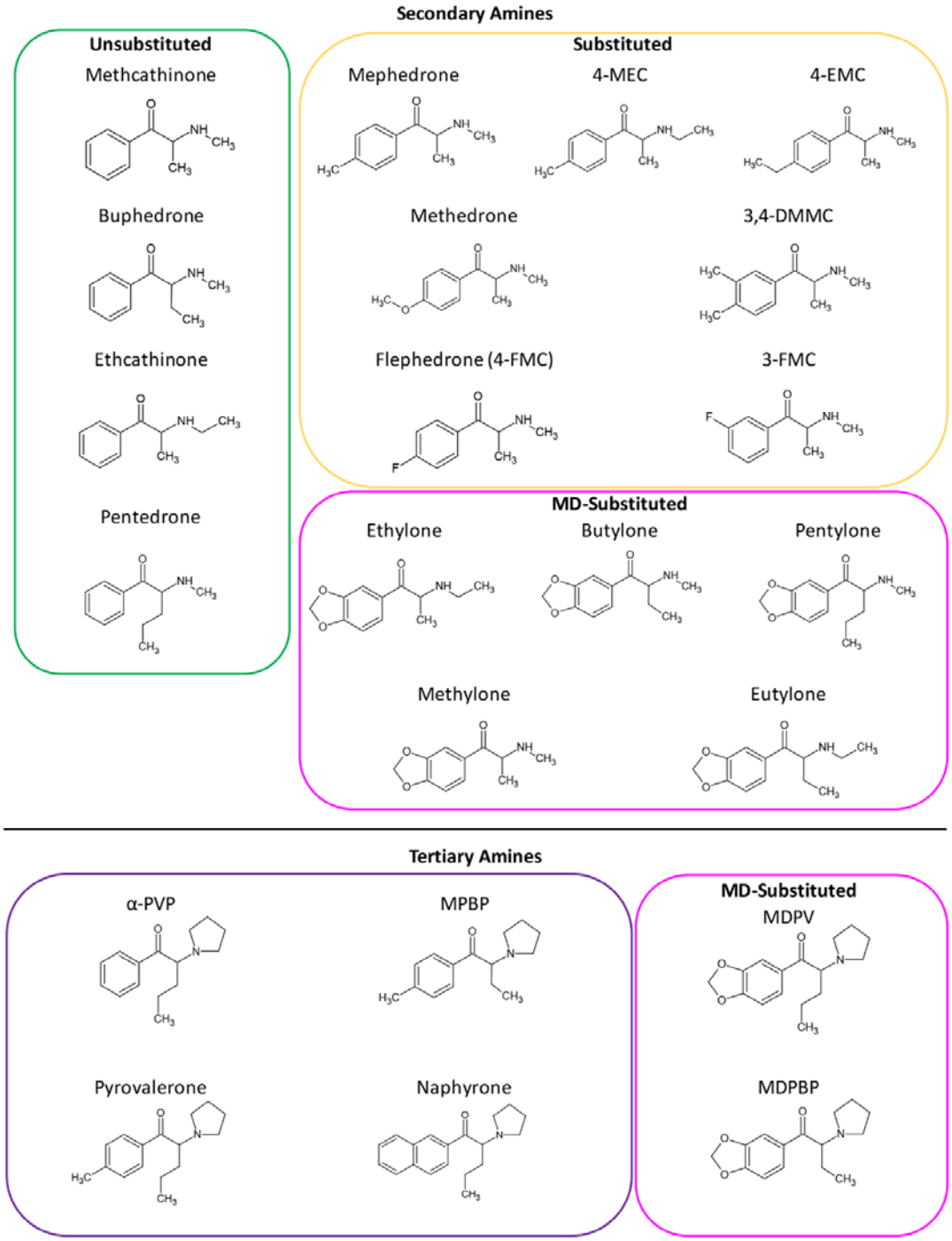


Figure 4.1. Cathinone structures, indicating secondary and tertiary amines, unsubstituted, ring-substituted and methylenedioxy (MD)-substituted drugs.

Materials and Methods

Chemicals and reagents

Methcathinone, 3-FMC, 4-FMC, methylone, ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-methylethcathinone (4-MEC), 3,4-methylenedioxy-α-pyrrolidinobutiophenone (MDPBP), pentedrone, pentylone, 3,4-dimethylmethcathinone (3,4-DMMC), α-pyrrolidinopentiophenone (α-PVP), 4-ethylmethcathinone (4-EMC), 4-methyl-α-pyrrolidinobutiophenone (MPBP), methylenedioxypyrovalerone (MDPV), pyrovalerone, and naphyrone were purchased from Cerilliant Corporation (Round Rock, TX, USA) in 1.0 mg/mL methanolic solutions. Internal standards methylone-D3, ethylone-D5, butylone-D3, mephedrone-D3, eutylone-D5, pentylone-D3, alpha-PVP-D8, MDPV-D8, and naphyrone-D5 were also purchased from Cerilliant Corporation in 0.1 mg/mL methanolic solutions. The internal standard working solution consisted of all nine deuterated internal standards at a concentration of 0.25 ng/μL in methanol. Pooled drug-free urine, preserved with 1% sodium fluoride, was purchased from Utak Laboratories (Valencia, CA, USA).

LC-MS grade dichloromethane and isopropyl alcohol, and ACS grade glacial acetic acid were purchased from Mallinckrodt Chemicals (St. Louis, MO, USA). LC-MS grade methanol, concentrated hydrochloric acid, concentrated ammonium hydroxide, LCMS grade acetonitrile, and dibasic sodium phosphate were purchased from J.T. Baker (Center Valley, MA, USA). Optima® Hexane and HPLC grade ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). LC-MS grade formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Monobasic sodium phosphate was purchased from VWR (Radnor, PA, USA). Deionized water was purified in-house using a Millipore Direct-

Q® UV Water Purification system (Billerica, MA, USA). PolyChrom ClinII 3 cc (35 mg) solid phase extraction (SPE) columns were purchased from SPEware (Baldwin Park, CA, USA). Non-sterile polypropylene urine specimen cups (100 mL) were purchased from Starplex Scientific Corp. (Cleveland, TN).

Instrumentation

Sample analysis was performed using an Agilent Technologies 6530 LC-Q/TOF-MS (Santa Clara, CA, USA) equipped with an Agilent 1290 Infinity autosampler and a Series 1200 LC system. The LC system was equipped with a Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm particle size) and an Agilent Poroshell 120 EC-C18 guard column (2.1 x 5 mm, 2.7 µm particle size) located in a thermostatically controlled column compartment (35°C). The mobile phase consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in acetonitrile (B). A flow rate of 0.4 mL/min was maintained using a gradient elution profile as follows: 96% A and 4% B (0 to 0.5 mins); 10% B (0.5 to 5 mins); 40% B (5 to 11 mins); 100% B (12 mins). The LC-Q/TOF MS was equipped with an electrospray ionization (ESI) source (positive mode) was operated under the following conditions: drying gas (N_2), 13 L/min; drying gas temperature, 200ºC; nebulizer, 20 psi; sheath gas temperature, 250ºC; nitrogen sheath gas flow, 12 L/min; capillary voltage, 4000 V; nozzle voltage, 0 V; fragmentor, 150 V; skimmer, 65V. Agilent MassHunter software was used for acquisition, qualitative, and quantitative analysis. Nitrogen was generated using a Genius 3040 Nitrogen Generator (Peak Scientific, Billerica, MA, USA). Solid Phase Extraction (SPE) was performed using a JT Baker vacuum manifold and extracts were evaporated to dryness under nitrogen using a TurboVap LV® concentration workstation (Caliper Life Sciences, Hopkinton, MA, USA).

The LC-Q/TOF procedure was described previously (24, 25) and was validated in accordance with previously published guidelines (24, 25).

Sample preparation

Preserved, drug-free urine (1 L) was adjusted to pH 8.0 and pH 4.0 using concentrated ammonium hydroxide and concentrated hydrochloric acid, respectively. Urine was fortified with twenty-two synthetic cathinones to achieve a final concentration of 100 ng/mL and 1,000 ng/mL. Cathinone-positive urine, which was prepared in bulk, was then aliquoted into non-sterile polypropylene specimen cups to allow for sampling throughout the duration of the six month study. The experimental design is summarized in Figure 2. Exposure to light was not evaulated. Specimens stored at elevated and frozen temperatures were stored predominantly in the dark, while ambient and refrigerated samples were periodically exposed to light. All urine samples were subjected to light during initial preparation, routine sampling, and analysis. Aliquots of urine at each concentration (100 and 1,000 ng/mL) and each pH were immediately analyzed to establish T_0 (0% loss). Samples were analyzed in duplicate using the validated procedure described previously (24). Sampling frequency varied throughout the study to allow for sufficient data collection during periods where significant concentration changes were taking place. Sampling occured every 2-4 hours during the intial 72 hours and remained frequent (4 assays/week) during the first month of storage. Sampling generally decreased to bimonthly and then to monthly over the remaining months. Calibrators (10, 25, 100, 250, 350, and 500 ng/mL) and controls (0, 100 ng/mL) included in each batch were prepared daily using independently fortified standards. Appropriate dilutions (up to four-fold) were applied initially at the high concentration (1,000 ng/mL) to ensure quantitative results were within

the calibration range of the assay. The drug was considered unstable when the concentration decreased by more than 20% of the original (T_0) concentration. Statistical tests, including analysis of variance (ANOVA), were used to determine statistical significance (p=0.05).

Isolation of cathinones from urine

Internal standard solution (100 µL) was added to 1 mL urine to achieve a final concentration of 25 ng/mL. The urine was diluted with 2 mL of pH 6 phosphate buffer (0.1 M) and briefly vortexed. Samples were transferred to PolyChrom ClinII SPE columns and allowed to flow through under gravity. Columns were rinsed with 1 mL deionized water followed by 1 mL of 1 M acetic acid. Columns were dried under vacuum for 5 minutes and washed, successively, with 1 mL of hexane, ethyl acetate, and methanol. Synthetic cathinones were eluted using two 0.5 mL aliquots of elution solvent (2% conc. ammonium hydroxide in 95:5 dichloromethane: isopropyl alcohol). Acidic methanol (30 µL) was added to each extract prior to evaporation under nitrogen at 50°C. Extracts were reconstituted in 25 µL of 50:50 mixture of mobile phase A:B and 1 µL was injected onto the LC-Q/TOF-MS for analysis.

Results

The purpose of the study was to systematically evaluate the concentration, pH, temperature, and analyte dependent stability of synthetic cathinones. Each variable is considered below. Due to the large number of drugs, graphical representations of the data were color-coded to facilitate interpretation. Cathinones (secondary amines) without aromatic substituents are depicted in green; secondary amines with aromatic substituents are yellow, and tertiary amines (pyrrolidine-type) cathinones are purple. Finally,

cathinones with a methylenedioxy (MD) group are shown with a magenta line (for both secondary and tertiary amines).

Concentration dependence

Concentration dependence was assessed by comparing the percentage of drug remaining (% target) at 100 ng/mL and 1,000 ng/mL, in order to normalize the data. One-way ANOVA was used to compare the mean (% target at each sampling interval) at each temperature and pH in urine. Statistical significance was evaluated for each drug under all eight conditions (**Figure 4.2**). **Figure 4.3** depicts the changes in concentration over time in pH 4 urine at ambient temperature. No concentration dependent differences in stability were observed for any of the conditions (p=0.05). Therefore, all subsequent statistical evaluations of temperature, pH, matrix, and analyte dependence were undertaken at 1,000 ng/mL.

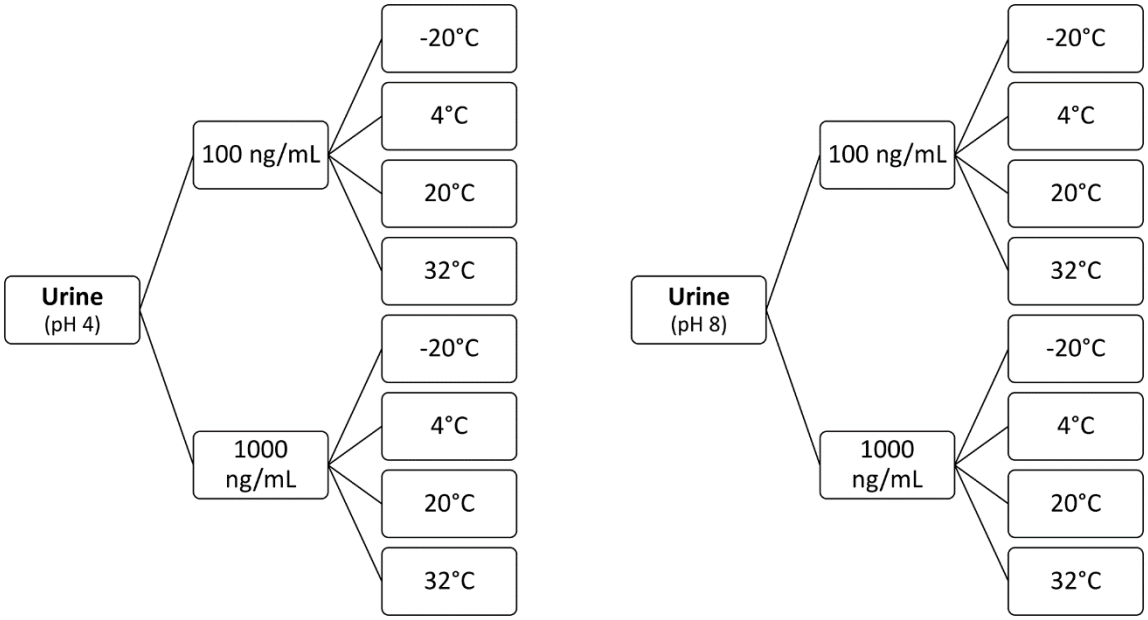


Figure 4.2. Summary of experimental design, indicating the sixteen conditions under which cathinone stability was evaluated.

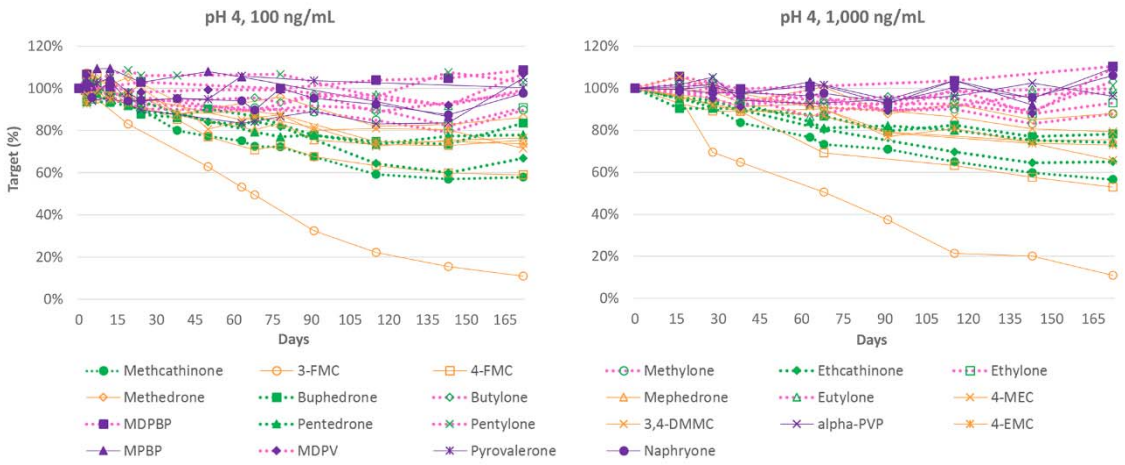


Figure 4.3. Degradation of synthetic cathinones (100 and 1,000 ng/mL) at 20ºC. Cathinone structure throughout can be differentiated as follows: unsubstituted secondary amines (green), ring-substituted secondary amines (yellow), tertiary amines (purple) and methylenedioxy (MD)-substituted cathinones (magenta line).

Half-life determination

Assuming first-order decay, rate constants (*k*) and half-lives ($T_{1/2}$) were estimated for each drug based upon duplicate measurements at each time interval ($T_{1/2}$=Ln2/*k*). Rate plots were only generated if a significant decrease in concentration (>20%) was evident over three consecutive measurements during the six month storage period (**Figure 4.4**).

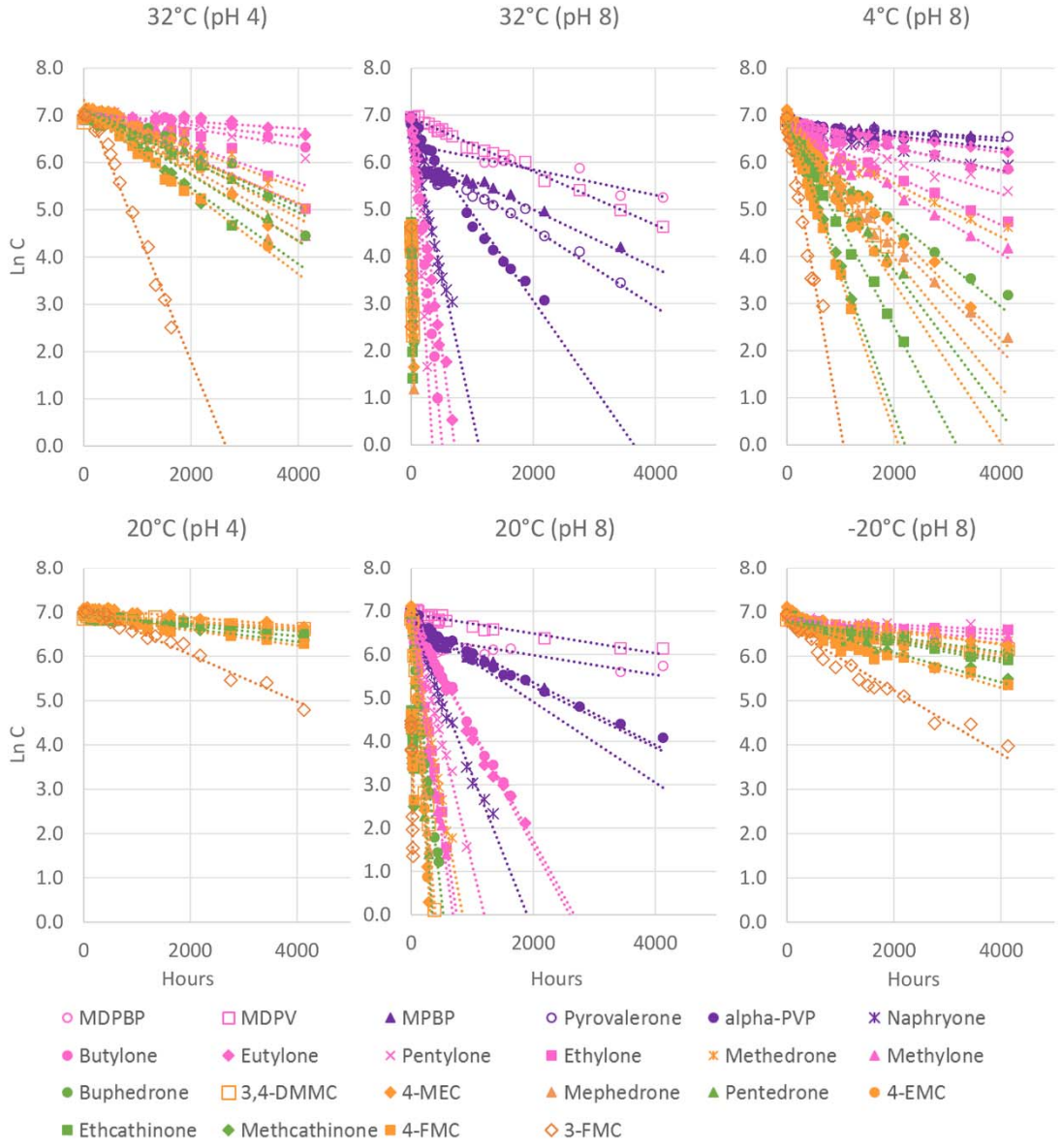


Figure 4.4. Decomposition of cathinones at pH 4 (elevated and ambient temperature) and pH 8 (all temperatures).

The synthetic cathinones were significantly more stable in acidic urine, therefore half-life estimation was only possible for some cathinones at ambient and elevated temperatures. Half-lives in acidic urine ranged from 10.4 days to 13.2 months, and 1.8 to 14.2 months at elevated and ambient temperature, respectively (**Table 4.1**). At pH 4, all cathinones were

stable over the six month period at refrigerated and frozen temperatures, precluding half-life determination.

Table 4.1. Half-lives of cathinones in urine (pH 4 and 8) in hours (h), days (d) and months (m) at elevated, ambient, refrigerated and frozen temperature.

Cathinone	**Frozen (-20ºC)**		**Refrigerated (4ºC)**		**Ambient (20ºC)**		**Elevated (32ºC)**	
	pH 4	**pH 8**	**pH 4**	**pH 8**	**pH 4**	**pH 8**	**pH 4**	**pH 8**
3,4-DMMC	--	5.3 m	--	21 d	14 m	1.7 d	2.0 m	11 h
4-EMC	--	4.4 m	--	17 d	11 m	1.3 d	1.3 m	9 h
3-FMC	--	1.3 m	--	4.5 d	1.8 m	9 h	10 d	2 h
4-FMC	--	2.7 m	--	8.8 d	5.8 m	20 h	1.1 m	5 h
Buphedrone	--	5.6 m	--	1 m	--	2.3 d	1.8 m	20 h
Butylone	--	--	--	3.8 m	--	8 d	7.4 m	2.1 d
Ethcathinone	--	4.5 m	--	14 d	8.2 m	23 h	1.4 m	8 h
Ethylone	--	13 m	--	1.8 m	--	3.2 d	2.5 m	19 h
Eutylone	--	--	--	6.2 m	--	11 d	13 m	3 d
MDPBP	--	--	--	--	--	4.3 m	--	3.6 m
MDPV	--	--	--	--	--	4.3 m	--	1.7 m
4-MEC	--	5.5 m	--	25 d	9.7 m	1.3 d	1.6 m	9 h
Mephedrone	--	4.7 m	--	25 d	14 m	1.5 d	1.5 m	10 h
Methcathinone	--	2.9 m	--	9.3 d	6.6 m	18 h	1.2 m	5 h
Methedrone	--	7.6 m	--	1.7 m	--	3.7 d	2.3 m	19 h
Methylone	--	8.7 m	--	1.4 m	--	3 d	1.9 m	1 d
MPBP	--	--	--	15 m	--	1.4 m	--	1.6 m
Naphyrone	--	--	--	3.9 m	--	11 d	--	4.8 d
Pentedrone	--	4.3 m	--	19 d	13 m	1.4 d	1.8 m	10 h
Pentylone	--	15 m	--	2.6 m	--	5 d	5.2 m	1.4 d
α-PVP	--	--	--	7.1 m	--	1.3 m	--	18 d
Pyrovalerone	--	--	--	11 m	--	1.0 m	--	1.2 m

In contrast, half-lives were estimated for all cathinones in alkaline urine at ambient and elevated temperature. The half-lives for all twenty-two cathinones ranged from 2 hours (3-FMC) to 3.6 months (MDPBP), and 9 hours (3-FMC) to 4.3 months (MDPBP and

MDPV) at elevated and ambient temperatures, respectively. At refrigerated and frozen temperatures, cathinones were considerably more stable. Half-lives in pH 8 urine were estimated for all but two cathinones (MDPV and MDPBP) at refrigerated temperature, and all but eight drugs (α-PVP, butylone, eutylone, MDPBP, MDPV, MPBP, naphyrone and pyrovalerone) at frozen temperature. When specimens were refrigerated, half-lives ranged from 4.5 days to 11 months, and when frozen, ranged from 1.3 to 15 months. Half-life estimates for these drug clearly demonstrate the significance of storage temperature, matrix pH and analyte dependent variables.

Analyte dependence

Analyte dependent differences in stability were readily observed from graphical representations of the data (**Figure 4.5 & 4.6**). Statistical comparisons were also made, wherever possible. The twenty-two cathinones (**Figure 4.1**) selected in the study included secondary and tertiary amines, with and without ring substituents, which allowed for the comparison of cathinone stability *within* a sub-population and *between* populations using one-way ANOVA. Statistical comparisons between structural groups were only made if the differences in stability within the group were not significant. 3-FMC was excluded from all statistical tests presented because it degraded much more extensively than its ring-substituted counterparts. As would be expected, analyte dependent differences in stability were more readily identified under unfavorable conditions (elevated pH and temperature). Statistical comparisons between groups were not made when overall stability was observed, which included the majority of conditions at pH 4 (**Figure 4.5**). As a result, conclusions pertaining to analyte dependent differences in stability are predominantly

drawn from results obtained in alkaline urine. A summary of statistical analysis for concentration, temperature, pH, and analyte dependence can be found in **Appendix F**.

Figure 4.5. Box plots depicting within and between sub-populations of synthetic cathinones. Sub-populations include secondary amine, unsubstituted (green); secondary amine, ring substituted (yellow); secondary amine, MD (magenta); tertiary amine, non-MD (purple); tertiary amine, MD (magenta/purple). Mean ('X') and median (line) concentration for each drug over the six month study also identified.

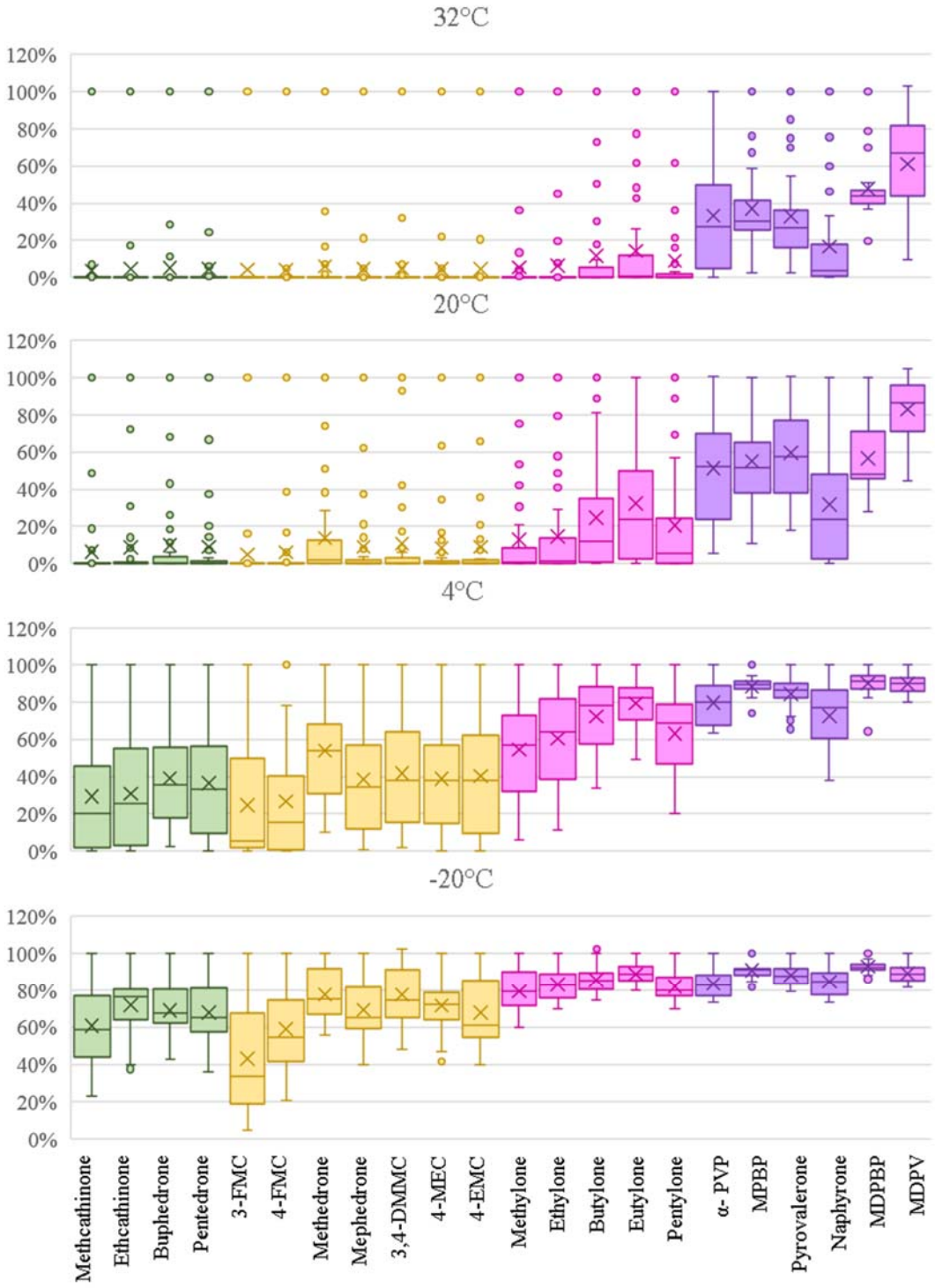


Figure 4.6. Box plots depicting within and between sub-populations of synthetic cathinones. Sub-populations include secondary amine, unsubstituted (green); secondary amine, ring substituted (yellow); secondary amine, MD (magenta); tertiary amine, non-MD (purple); tertiary amine, MD (magenta/purple). Mean ('X') and median (line) concentration for each drug over the six month study also identified.

In pH 8 urine, no significant differences in stability were observed within the unsubstituted secondary amines (methcathinone, buphedrone, ethcathinone, pentedrone), substituted secondary amines (mephedrone, 4-MEC, 4-EMC, methedrone, 3,4-DMMC, 4-FMC) or MD-substituted secondary amines (ethylone, butylone, pentylone, methylone, eutylone). Collectively however, significant differences were observed within all of the secondary amines. Tertiary amines produced similar results. Differences within the groups were highly significant for secondary ($F(14,354=7.71$, $p<0.0001$, 4ºC) and tertiary amines ($F(5,116)=8.22$, $p<0.0001$ at 4ºC), suggesting that cathinone stability is influenced by more than just the amine moiety.

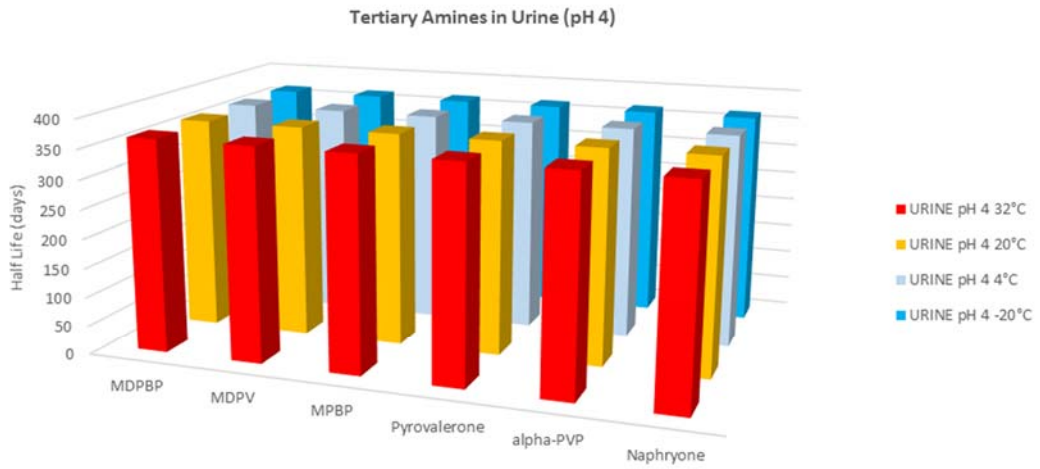
The methylenedioxy group exerted a significant stabilizing effect on both the secondary and tertiary amines (**Figure 4.5 & 4.6**). In refrigerated pH 4 urine, MD-substituted secondary amines were significantly more stable than their unsubstituted ($F(8,210)=11.67$, $p<0.0001$) or substituted counterparts ($F(11,273)=8.74$, $p<0.0001$). MD-substituted pyrrolidinyl analogs were also more stable than their non-MD substituted counterparts, further highlighting the stabilizing effect of the methylenedioxy group, independent of the amine.

Cathinones bearing a tertiary amine were generally more stable than the secondary amines (**Figure 4.5 & 4.6**). It was not possible to evaluate these differences statistically however, because of significant within group differences arising from ring substituents. Cathinones bearing a the tertiary amine and a methylenedioxy substituent were more stable than their secondary amine methylenedioxy-type counterparts ($F(6,147)=10.03$, $p<0.0001$, 4°C), making the MD-type pyrrolidines (MDPBP and MDPV) the most stable of the twenty-two drugs evaluated. The half-life estimation further highlights the stability of the

pyrrolidines, with half-lives on the order of days to months under many of the conditions tested, compared with hours to days for the secondary amines (**Table 4.2, Figure 4.7 & 4.8**). An understanding of the analyte dependent differences in stability, particularly the stabilizing effect of both the pyrrolidine and methylenedioxy groups, is particularly useful as laboratories encounter new and emerging cathinone derivatives in the future.

Table 4.2. Influence of chemical structure on cathinone half-life in hours (h), days (d) and months (m) at elevated, ambient, refrigerated and frozen temperature.

		Urine (pH 8)			
Amine	**Structure**	**32˚C**	**20˚C**	**4˚C**	**-20˚C**
Tertiary	MD-substituted	2 – 4 m	4 m	-	-
Tertiary	-	5 – 46 d	0.4 - 1.4 m	≥4 m	-
Secondary	MD-substituted	19 – 72 h	3 – 11 d	1.4 – 6 m	≥8 m
Secondary	-	2 – 20 h	0.4 – 4 d	4 – 25 d	≥2 m
		Urine (pH 4)			
Amine	**Structure**	**32˚C**	**20˚C**	**4˚C**	**-20˚C**
Tertiary	MD-substituted	-	-	-	-
Tertiary	-	-	-	-	-
Secondary	MD-substituted	≥2 m	-	-	-
Secondary	-	0.3 - 2.3 m	≥2 m	-	-



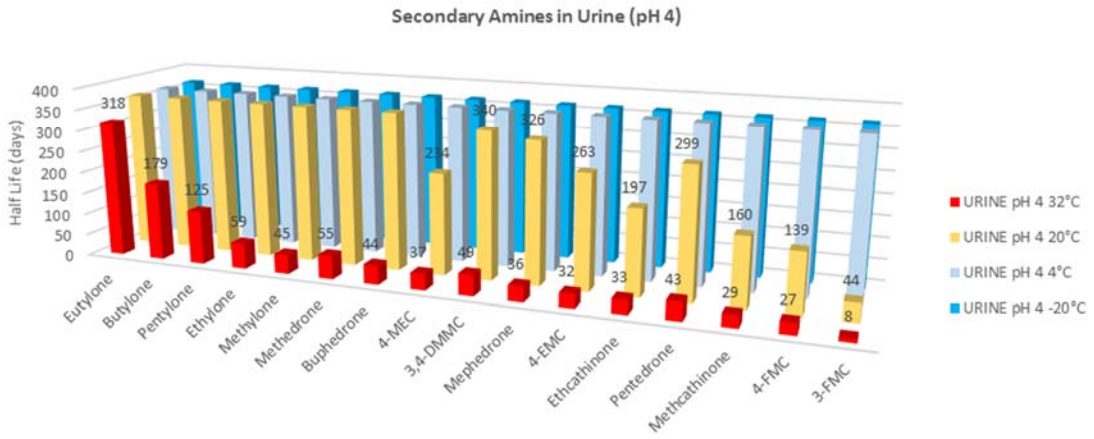


Figure 4.7. Analyte dependent stability of cathinones (tertiary amine to secondary amine) in acidic urine (pH 4). Unlabeled data indicates a half-life of >365 days or no measureable half-life due to stability.

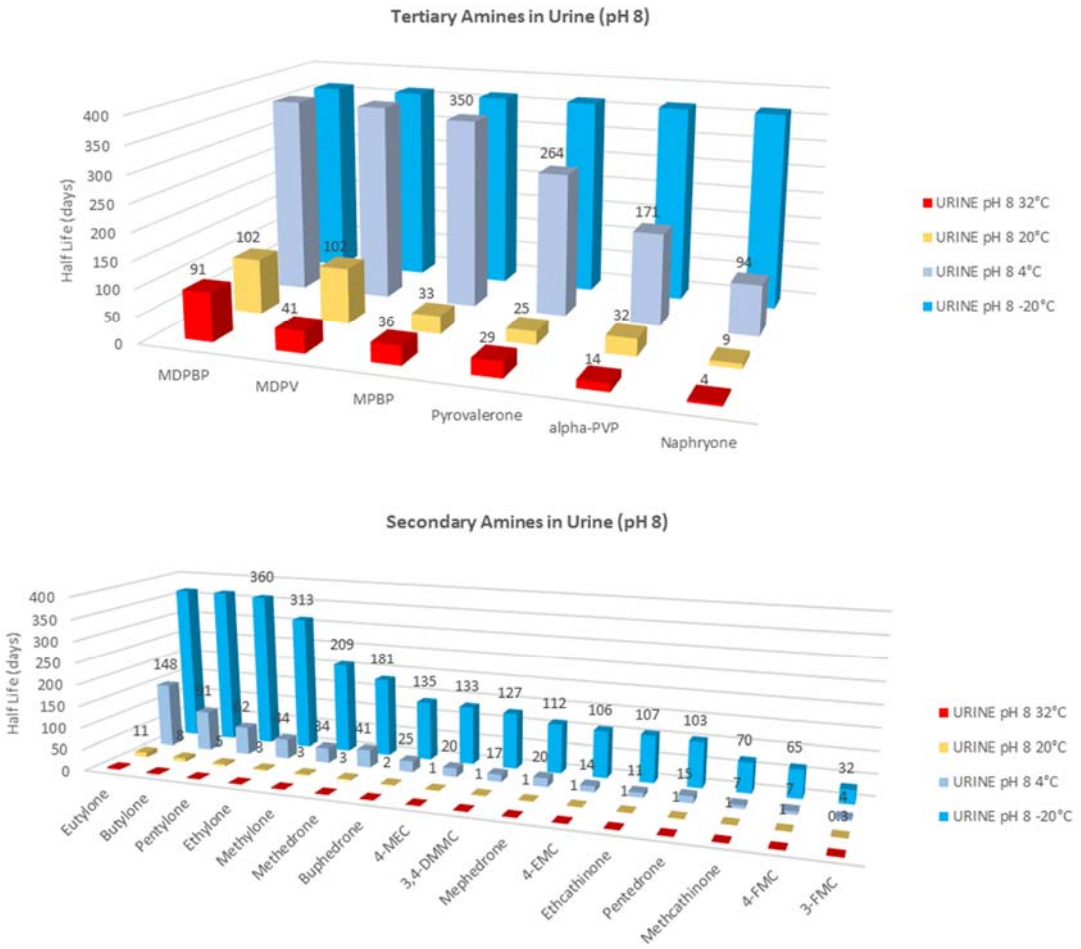


Figure 4.8. Analyte dependent stability of cathinones (tertiary amine to secondary amine) in alkaline urine (pH 8). Unlabeled data indicates a half-life of >365 days or no measureable half-life due to stability.

Matrix pH and temperature dependence

Not surprisingly, stability was significantly influenced by storage temperature, regardless of urine pH. All cathinones, from the least stable (3-FMC, $F(3,101)=9.83$, $p<0.0001$) to most stable (MDPV, $F(3,98)=18.76$, $p<0.00001$, pH 8; MDPBP, $F(3,78)=38.87$, $p<0.0001$, pH 8) were optimally preserved at decreased temperatures. Stability was also significantly dependent upon matrix pH, even for the most stable cathinones like MDPV ($F(1,53)=26.63$, $p<0.0001$, 4ºC) and MDPBP ($F(1,41)=23.56$, $p<0.0001$, 4ºC. Cathinones were significantly more stable in acidic urine, illustrated in **Figure 4.5 & 4.6 and 4.9**. Over the six month period, all twenty-two drugs were stable in pH 4 urine when refrigerated or frozen. Changes in pH during the course of the study were moderate (less than 0.5 pH units) for urine stored at frozen, refrigerated, and ambient temperatures over six months. Urine stored at elevated temperature was subject to a greater increase in pH (less than 2 pH units), consistent with previously published reports (23, 26). Due to the analyte dependent differences discussed earlier, the influence of temperature and pH are presented below within that context.

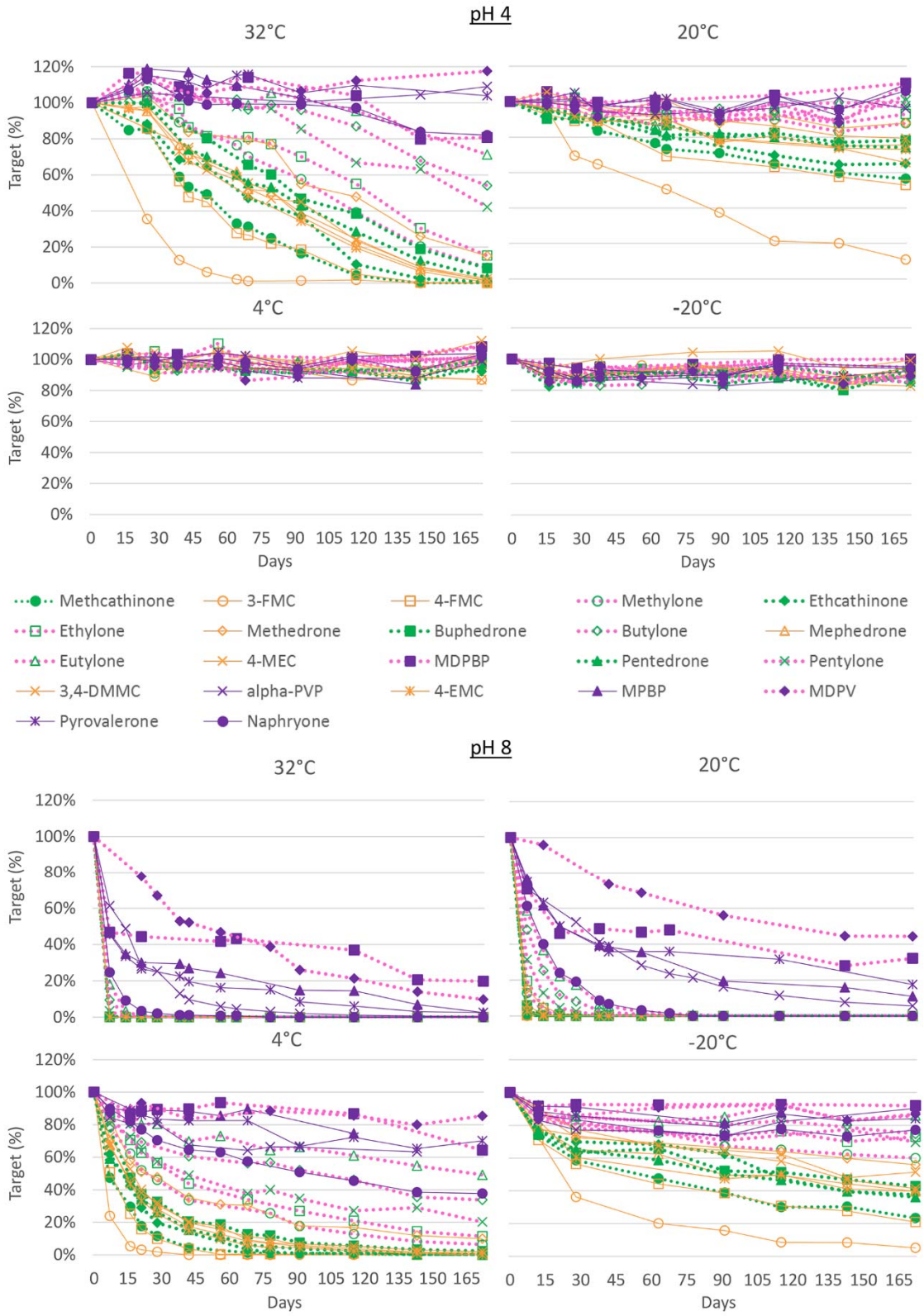


Figure 4.9. Cathinone stability in acidic (pH 4) and alkaline (pH 8) urine at 32°C, 20°C, 4°C, and -20°C.

Unsubstituted & substituted secondary amines

All secondary amines (without the methylenedioxy group) proved to be stable in acidic urine at elevated temperature for approximately 30 days, with the exception of 3-FMC (**Figure 4.9).** Methcathinone and 4-FMC were unstable at elevated temperature after 28 days. At ambient temperature, all were stable for at least two months (with the exception of 3-FMC), and when refrigerated or frozen no instability was observed over the six month period.

In contrast, instability was observed in alkaline urine at all temperatures within hours of storage at elevated and ambient temperatures, and within days at refrigerated and frozen temperatures **(Table 4.3).** This highlights the significant pH and temperature dependent stability of these drugs. For almost all secondary amines, instability occurred within 5 hours of storage at pH 8 at 32ºC, emphasizing the need to minimize exposure to elevated temperatures during shipping and transport. Losses of 100% were observed in pH 8 urine within days of exposure to elevated temperature (32˚C) (**Table 4.4**). In contrast, although significant losses were observed in pH 8 urine within days or weeks when frozen (**Table 4.3**), all drugs were detectable over the six month period (**Table 4.4**).

Methylenedioxy-type

With the exception of elevated temperature, all five methylenedioxy-type secondary amines were stable in pH 4 urine for the duration of the study (**Table 4.3**). In pH 8 urine, instability was observed under all conditions, with the exception of eutylone at -20°C. However, even under unfavorable alkaline conditions, the stabilizing effect of the methylenedioxy group was evident relative to other secondary amines. This is readily observed in refrigerated and frozen urine at pH 8 (**Figure 4.9**). At elevated temperature at

pH 8, all were unstable within one day, compared with 0.9 to greater than 6 months when frozen (**Table 4.3**). In addition to the dramatic effect of temperature, matrix pH also played a key role. Methylenedioxy-substituted secondary amines were stable for 5 to 21 days in pH 8 urine, compared with over 6 months of storage in pH 4 urine.

Pyrrolidine-type

The tertiary amines were the most stable group under all conditions tested (**Figure 4.9**). Degradation however, was still greatly influenced by temperature and pH. When refrigerated, non-MD substituted pyrrolidines were stable for 18 to 91 days at pH 8, compared with greater than 6 months at pH 4. At alkaline pH, this same group was stable for 1 to 3 days at elevated temperature, compared with 0.9 to over 6 months of storage when frozen. When refrigerated or frozen, MDPBP and MDPV were the only drugs to remain stable in urine for the duration of the study, regardless of pH. This again highlights the dual stabilizing effect of the tertiary amine and the methylenedioxy group.

Overall

The influence of storage temperature and matrix pH was highly significant. This is reflected in the extreme differences in the number of days to significant loss (20%) and complete loss (100%) depicted in **Tables 4.3 and 4.4**. Based on these findings, positive cathinone toxicology results should be interpreted accordingly. Although the laboratory can ensure that biological samples are stored appropriately upon receipt, exposure to elevated temperatures during transport and individual specimen pH are factors largely beyond their control. Not only can these factors decrease drug concentrations, it could potentially render some of the most unstable drugs undetectable.

Table 4.3. Time to significant (20%) loss of drug at 1,000 ng/mL. Time is expressed in hours (h), days (d) and months (m). Data is also summarized by amine type and aromatic ring substitution. The limit of detection (LOD) is shown in parentheses.

Cathinone (LOD)	**Elevated**		**Ambient**		**Refrigerated**		**Frozen**	
	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4
Secondary Amine, Unsubstituted								
Buphedrone (2 ng/mL)	6 h	63 d	<1 d	91 d	3 d	--	5 d	--
Ethcathinone (1 ng/mL)	5 h	38 d	<1 d	63 d	2 d	--	12 d	--
Methcathinone (0.25 ng/mL)	4 h	28 d	6 h	63 d	1 d	--	12 d	--
Pentedrone (5 ng/mL)	5 h	42 d	<1 d	115 d	3 d	--	12 d	--
Secondary Amine, Substituted								
3,4-DMMC (5 ng/mL)	5 h	42 d	1 d	172 d	5 d	--	28 d	--
3-FMC (1 ng/mL)	2 h	9 d	4 h	21 d	<1 d	--	5 d	--
4-EMC (2 ng/mL)	5 h	38 d	1 d	91 d	5 d	--	11 d	--
4-FMC (1 ng/mL)	4 h	28 d	6 h	63 d	1 d	--	9 d	--
4-MEC (1 ng/mL)	5 h	42 d	<1 d	91 d	3 d	--	9 d	--
Mephedrone (2 ng/mL)	5 h	38 d	<1 d	91 d	3 d	--	12 d	--
Methedrone (1 ng/mL)	8 h	68 d	1 d	--	8 d	--	16 d	--
Secondary Amine, Methylenedioxy-Type								
Butylone (1 ng/mL)	1 d	143 d	3 d	--	18 d	--	38 d	--
Ethylone (1 ng/mL)	8 h	78 d	1 d	--	9 d	--	28 d	--
Eutylone (5 ng/mL)	1 d	172 d	4 d	--	21 d	--	--	--
Methylone (0.25 ng/mL)	6 h	63 d	1 d	--	5 d	--	38 d	--

(continued)

Cathinone (LOD)		Elevated		Ambient		Refrigerated		Frozen	
		pH 8	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4
Pentylone (1 ng/mL)		<1 d	115 d	2 d	--	7 d	--	38 d	--
Tertiary Amine (Pyrrolidine-Type)									
α-PVP (2 ng/mL)		3 d	--	3 d	--	18 d	--	--	--
MDPBP* (0.5 ng/mL)		1 d	143 d	4 d	--	143 d	--	--	--
MDPV* (1 ng/mL)		18 d	--	28 d	--	--	--	--	--
MPBP (1 ng/mL)		1 d	--	7 d	--	91 d	--	--	--
Naphyrone (0.5 ng/mL)		1 d	172 d	4 d	--	18 d	--	38	--
Pyrovalerone (0.25 ng/mL)		<2 d	--	6 d	--	91 d	--	--	--
Amine	**Structure**	**Time to Significant Loss**							
Tertiary	MD	1 – 18 d	4.7- >6 m	4-28 d	>6 m	>6 m	>6 m	>6 m	>6 m
Tertiary	-	1 – 3 d	5.7- >6 m	3-7 d	>6 m	18-91 d	>6 m	0.9- >6 m	>6 m
Secondary	MD	6 h – 1 d	2.1- 4.7 m	1-4 d	>6 m	5–21 d	>6 m	0.9- >6 m	>6 m
Secondary	-	2 – 8 h	9 – 68 d	4h-1 d	0.7-5.7 m	1 – 8 d	>6 m	9-28 d	>6 m

Table 4.4. Time to complete (100%) loss of drug at 1,000 ng/mL. Time is expressed in hours (h), days (d) and months (m). Data is also summarized by amine type and aromatic ring substitution. The limit of detection (LOD) is shown in parentheses.

Cathinone (LOD)	Elevated		Ambient		Refrigerated		Frozen	
	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4
Secondary Amine, Unsubstituted								
Buphedrone (2 ng/mL)	5 d	--	14 d	--	--	--	--	--
Ethcathinone (1 ng/mL)	3 d	--	9 d	--	143 d	--	--	--
Methcathinone (0.25 ng/mL)	<2 d	143 d	5 d	--	56 d	--	--	--
Pentedrone (5 ng/mL)	4 d	--	11 d	--	143 d	--	--	--
Secondary Amine, Substituted								
3,4-DMMC (5 ng/mL)	4 d	--	16 d	--	--	--	--	--
3-FMC (1 ng/mL)	<1 d	143 d	3 d	--	42 d	--	--	--
4-EMC (2 ng/mL)	3 d	172 d	11 d	--	143 d	--	--	--
4-FMC (1 ng/mL)	<3 d	143 d	7 d	--	56 d	--	--	--
4-MEC (1 ng/mL)	3 d	--	11 d	--	172 d	--	--	--
Mephedrone (2 ng/mL)	4 d	--	12 d	--	--	--	--	--
Methedrone (1 ng/mL)	7 d	--	38 d	--	--	--	--	--
Secondary Amine, Methylenedioxy-Type								
Butylone (1 ng/mL)	18 d	--	63 d	--	--	--	--	--
Ethylone (1 ng/mL)	7 d	--	24 d	--	--	--	--	--
Eutylone (5 ng/mL)	21 d	--	91 d	--	--	--	--	--
Methylone (0.25 ng/mL)	7 d	--	24 d	--	--	--	--	--

(continued)

Cathinone (LOD)		Elevated		Ambient		Refrigerated		Frozen	
		pH 8	**pH 4**	**pH 8**	**pH 4**	**pH 8**	**pH 4**	**pH 8**	**pH 4**
Pentylone (1 ng/mL)		11 d	--	38 d	--	--	--	--	--
Tertiary Amine (Pyrrolidine-Type)									
α-PVP (2 ng/mL)		172 d	--	--	--	--	--	--	--
MDPBP* (0.5 ng/mL)		--	--	--	--	--	--	--	--
MDPV* (1 ng/mL)		--	--	--	--	--	--	--	--
MPBP (1 ng/mL)		172 d	--	--	--	--	--	--	--
Naphyrone (0.5 ng/mL)		78 d	--	78 d	--	--	--	--	--
Pyrovalerone (0.25 ng/mL)		--	--	--	--	--	--	--	--
Amine	**Structure**	**Days to Complete Loss**							
Tertiary	MD	>6 m	>6 m	>6 m	>6 m	>6 m	>6 m	>6 m	>6 m
Tertiary	-	2.6 - >6 m	>6 m	2.6 - >6 m	>6 m	>6 m	>6 m	>6 m	>6 m
Secondary	MD	7 – 21 d	>6 m	24 – 91 d	>6 m	>6 m	>6 m	>6 m	>6 m
Secondary	-	<2 – 7 d	4.8 - >6 m	3 – 38 d	>6 m	1.4 - >6 m	>6 m	>6 m	>6 m

Discussion

This six month systematic stability study shows that cathinone stability is highly analyte, pH and temperature dependent. These findings are consistent with our previously reported results in whole blood (16). To reduce pre-analytical losses, biological samples should be protected from heat wherever possible. Specimen pH, which is not typically measured during routine human performance or postmortem toxicology investigations, might also be considered. Finally, analyte dependent differences in stability are influenced by multiple factors. The increased stability of the pyrrolidine analogs might be explained by their inability to undergo oxidative deamination. However, methylenedioxy substitution and ring position also plays an important role. Of the twenty-two drugs included in the study, 3-FMC was the least stable in urine, while MDPV and MDPBP (MD-type pyrrolidines) were the most stable.

Our findings are in consistent with previous published studies. Tsujikawa (21) assessed the stability of methcathinone, mephedrone, 3-FMC, 4-FMC, and ethcathinone in various aqueous buffer systems. They also observed increased stability with decreasing pH, in addition to structural influences. Although direct comparisons cannot be made between estimated half-lives in their study (pH 4, 7, 10, and 12) and ours (pH 4 and 8), the overall trends are in good agreement. Johnson and Botch-Jones' (18) study resulted in minimal loss of mephedrone in urine over two weeks, but significant losses in blood (pH of 7). While they did not specify the urinary pH or the use of preservative in their matrix, it is likely that it may have been more acidic, based on the findings of this research. Al-Saffar (19) evaluated eleven cathinones in urine after one day, one week, and three months of storage at -20, 6, and 22°C. Although the urinary pH was not specified and the sampling

frequency was limited, they concluded that MDPV and 3-FMC were the most and least stable drugs, respectively, within their scope of testing. The comprehensive study presented here supports previously published literature and offers greater insight into analyte dependent variables.

Commercial drug-free urine was used for this study, rather than authentic urine from cathinone users. Although this might be considered a limitation, it was necessary in order to include a sufficient number of structural analogs for statistical analysis, and to accommodate a sufficient quantity of urine for the experimental design. Drug stability was not evaluated in unpreserved urine. Although antioxidants such as ascorbic acid and sodium sulfite may inhibit the oxidative degradation of synthetic cathinones (21), these are not typically encountered during routine urine collections. Sodium fluoride (1%), which was utilized in this study, is the most common urinary additive for preservation purposes. Finally, increases in urinary pH that take place during storage are largely attributed to the chemical breakdown of nitrogenous analytes (23, 26). It should be noted that this natural phenomenon creates a less favorable environment for cathinone species, increasing their rate of decay and ultimately detection time.

Conclusion

Cathinone stability in preserved human urine can be summarized as follows:

- Cathinone stability is pH, temperature and analyte dependent.
- Cathinones are alkaline-labile drugs. Degradation rates increase with increasing pH and temperature.

- With the exception of 3-FMC, no significant differences in stability were observed between ring substituted and unsubstituted secondary amine cathinones.
- The position of the ring substitution can influence stability (*e.g.,* 3- and 4-FMC).
- Although cathinones bearing a tertiary amine (pyrrolidines) are generally more stable than their secondary amine counterparts, stability is influenced by more than just the nitrogen.
- The methylenedioxy group exerts significant stabilizing effect on both the secondary and tertiary amine cathinones.
- The most stable cathinones contain both the tertiary amine (pyrrolidine) and the methylenedioxy group.

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References

1. U.S. Drug Enforcement Administration: Diversion Control Devision (2016) Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2013-2015. Springfield, VA: U..S. Drug Enforcement Administration.

2. European Monitoring Centre for Drugs and Drug Addiction. (2017) European Drug Report 2017: Trends and Developments. Available at http://www.emcdda.europa.eu/.

3. Coppola, M.; Mondola, R. (2012) Synthetic cathinones: chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as "bath salts" or "plant food". *Toxicology Letters*, **211**, 144-149.

4. Katz, D.P.; Bhattacharya, D.; Bhattacharya, S.; Deruiter, J.; Clark, C.R.; Suppiramanian, V.; et al. (2014) Synthetic cathinones: "A khat and mouse game". *Toxicology Letters*, **229**, 349-356.

5. Kerrigan, S. (2013) Sampling, Storage and Stability, in: A. Negrusz, G. Cooper (Eds.), Clarke's Analytical Forensic Toxicology 2 Ed, Pharmaceutical Press, London, England, pp 335-347.

6. Belhadj-Tahar, H.; Sadeg, N. (2005) Methcathinone: a new postindustrial drug. *Forensic Science International*, **153**, 99-101.

7. Gil, D.; Adamowicz, P.; Skulska, A.; Tokarczyk, B.; Stanaszek, R. (2013) Analysis of 4-MEC in biological and non-biological material—three case reports. *Forensic Science International*, **228**, e11-e15.

8. Potocka-Banaś, B.; Janus, T.; Majdanik, S.; Banaś, T.; Dembińska, T.; Borowiak, K. (2017) Fatal intoxication with α-PVP, a synthetic cathinone derivative. *Journal of Forensic Science*, **62**, 553-556.

9. McIntyre, I.M.; Hamm, C.E.; Sherrard, J.L.; Gary, R.D.; Burton, C.G.; Mena, O. (2014) Acute 3,4-methylenedioxy-N-ethylcathinone (Ethylone) intoxication and related fatality: a case report with postmortem concentrations. *Journal of Analytical Toxicology*, **39**, 225-228.

10. McIntyre, I.M.; Hamm, C.E.; Aldridge, L.; Nelson, C.L. (2013) Acute methylone intoxication in an accidental drowning - a case report. *Forensic Science International*, **231**, e1-e3.

11. Murray, B.L.; Murphy, C.M.; Beuhler, M.C. (2012) Death following recreational use of designer drug "bath salts" containing 3, 4-methylenedioxypyrovalerone (MDPV). *Journal of Medical Toxicology*, **8**, 69-75.

12. Wright, T.H.; Cline-Parhamovich, K.; Lajoie, D.; Parsons, L.; Dunn, M.; Ferslew, K.E. (2013) Deaths involving methylenedioxypyrovalerone (MDPV) in upper east Tennessee. *Journal of Forensic Science*, **58**, 1558-1562.

13. Pearson, J.M.; Hargraves, T.L.; Hair, L.S.; Massucci, C.J.; Frazee, C.C.; Garg, U.; et al. (2012) Three fatal intoxications due to methylone. *Journal of Analytical Toxicology*, **36**, 444-451.

14. Gerace, E.; Petrarulo, M.; Bison, F.; Salomone, A.; Vincenti, M. (2014) Toxicological findings in a fatal multidrug intoxication involving mephedrone. *Forensic Science International*, **243**, 68-73.

15. Lusthof, K.J.; Oosting, R.; Maes, A.; Verschraagen, M.; Dijkhuizen, A.; Sprong, A.G.A. (2011) A case of extreme agitation and death after the use of mephedrone in The Netherlands. *Forensic Science International*, **206**, e93-e95.

16. Glicksberg, L.; Kerrigan, S. (2017) Stability of synthetic cathinones in blood. *Journal of Analytical Toxicology*, https://doi.org/10.1093/jat/bkx071.

17. Paul, B.D.; Cole, K.A. (2001) Cathinone (khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. *Journal of Analytical Toxicology*, **25**, 525-530.

18. Johnson, R.D.; Botch-Jones, S.R. (2013) The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *Journal of Analytical Toxicology*, **37**, 51-55.

19. Al-Saffar, Y.; Stephanson, N.N.; Beck, O. (2013) Multicomponent LC–MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine—experience from the Swedish population. *Journal of Chromatography B*, **930**, 112-120.

20. Concheiro, M.; Anizan, S.; Ellefsen, K.; Huestis, M.A. (2013) Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. *Analytical and Bioanalytical Chemistry*, **405**, 9437-9448.

21. Tsujikawa, K.; Mikuma, T.; Kuwayama, K.; Miyaguchi, H.; Kanamori, T.; Iwata, Y.T.; et al. (2012) Degradation pathways of 4-methylmethcathinone in alkaline solution and stability of methcathinone analogs in various pH solutions. *Forensic Science International*, **220**, 103-110.

22. Maskell, P.D.; De Paoli, G.; Seneviratne, C.; Pounder, D.J. (2011) Mephedrone (4-methylmethcathinone)-related deaths. *Journal of Analytical Toxicology*, **35**, 188-191.

23. Cook, J.D.; Strauss, K.A.; Caplan, Y.H.; LoDico, C.P.; Bush, D.M. (2007) Urine pH: the effects of time and temperature after collection. *Journal of Analytical Toxicology*, **31**, 486-496.

24. Glicksberg, L.; Bryand, K.; Kerrigan, S. (2016) Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. *Journal of Chromatography B*, **1035**, 91-103.

25. Scientific Working Group for Forensic Toxicology (SWGTOX). (2013) Standard Practices for Method Validation in Forensic Toxicology. *Journal of Analytical Toxicology*, **37**, 452-474.

26. Fura, A; Harper, T.W.; Zhang, H.; Fung, L.; Shyu, W.C. (2003) Shift in pH of biological fulids during storage and processing: effect on bio analysis. *Journal of Pharmaceutical and Biomedical Analysis*, **32**, 513-522.

CHAPTER V

CATHINONE STABILITY IN AUTHENTIC URINE SPECIMENS[1]

This dissertation follows the style and format of *The Journal of Analytical Toxicology*.

[1]Glicksberg L., Rana S., Kerrigan S. (2017).
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Abstract

Synthetic cathinones are encountered in a variety of antemortem and postmortem forensic toxicology investigations. Earlier experimental studies using fortified urine have evaluated analyte, temperature and pH-dependent variables associated with their stability. The purpose of this study was to compare experimental findings with those obtained using authentic urine from cathinone users.

In this report we compare cathinone concentrations in 180 authentic unpreserved urine specimens, following specified periods of refrigerated storage. These findings are compared with previously published experimental data using fortified drug-free urine. Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q/TOF-MS) was used to target twenty-two cathinones. Quantitative results were compared in urine specimens (pH 4.5–10) following 5 to 17 months of storage.

The 180 specimens resulted in 156 quantitative findings involving α-PVP, ethylone, methylone, MDPV and pentylone. Initial drug concentrations ranged from 25 ng/mL to over 100,000 ng/mL. Upon reanalysis, the percentage of drug remaining (0 – 119%) was correlated with storage time and specimen pH. The ability to reconfirm original results was not correlated with storage time. Instead, specimen pH was far more predictive. The relationship between initial and final drug concentration was highly pH-dependent, yielding significant correlations for α-PVP, ethylone and methylone, particularly under acidic conditions.

These results are in good agreement with experimental findings and highlight the critical importance of specimen pH, rather than conventional time dependent variables, when considering cathinone stability in biological samples. The potential for pre-

analytical changes in cathinone concentrations must be carefully considered when interpreting their results.

Key Words: Urine, Synthetic cathinones, Stability, pH

CATHINONE STABILITY IN AUTHENTIC URINE SPECIMENS

Introduction

The escalating use of new psychoactive substances (NPS) has significantly impacted the global landscape of recreational drug use. These increases have been documented through reports issued by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA), the National Forensic Laboratory Information System (NFLIS), poison control center reports, and both ante-mortem and post-mortem forensic toxicology casework (1-6). Synthetic cathinones still represent a significant portion of the NPS market, which is significantly fueled by the Internet (7). The epidemiology of their use is complicated by the rapid emergence of new analogs and derivatives.

As of 2016, the EMCDDA was monitoring as many as 103 synthetic cathinones (3). According to the 2017 European Drug Report, this number increased to 118, making it the second largest new substance group, having been identified in more than half of the participating European countries (8). NFLIS reported an increase from 5 cathinones in 2009 to 35 in 2015. The five cathinones reported in 2009 were mephedrone, MDPV, methylone, methcathinone, and 4-MEC, with mephedrone and methcathinone being the most prevalent (1). By 2015, methcathinone was no longer among the top twenty cathinones, having been largely replaced by methylone, ethylone, and α-PVP. Although NPS use in the US lags behind many of the European countries, they tend to follow the same overall trends.While some cathinones have remained popular, others have decreased (e.g. buphedrone), or have been largely replaced by newer analogs such as brephedrone (4-BMC) and other halogenated species. Despite their evolving nature, surveillance reports from both the United States and Europe confirm that recreational use of these novel psychostimulants continues to be a problem.

Synthetic cathinones are capable of producing stimulant and euphoric effects similar to methamphetamine and cocaine. Sought after effects may include increased sociability, energy, focus, and empathy (9-11). Synthetic cathinone toxicity has resulted in neurological, cardiovascular, and psychopathological symptoms including hyperthermia, paranoid psychosis, organ failure, and death (4, 10-13). Their physical and neurological effects are attributed to their interactions with the monoamine neurotransmitters dopamine, norepinephrine, and serotonin.

Synthetic cathinones have been associated with impairment, intoxication, and fatal overdose. Quantitative determinations have been reported throughout the scientific literature in a variety of biological matrices. In this report, we describe changes in urinary drug concentrations among a population of cathinone users. Cathinone concentrations in antemortem urine have been reported over a very wide range, from tens of nanograms per milliliter to several thousand (14-18). Therefore, analytical methods must have not only adequate specificity to identify structurally similar drugs and regioisomers, but also high sensitivity. The latter becomes critically important, particularly if pre-analytical changes in concentration take place due to instability or degradation of the drug.

We previously reported a comprehensive synthetic cathinone stability study in urine to address analyte, pH, temperature, concentration and time-dependent variables (19). Although no concentration dependence was observed, cathinone stability was significantly dependent upon urinary pH, storage temperature, and structural characteristics of the cathinone itself. A total of twenty-two cathinones were evaluated in pooled human urine (pH 4 and 8) at four temperatures (32, 20, 4, and -20°C) during six months of storage. Cathinones were less stable in alkaline urine, with significant changes observed within

hours for some drugs under certain conditions. In contrast, all drugs remained stable over the entire six month period in acidic urine when refrigerated or frozen. The pyrrolidine (tertiary amine) and methylenedioxy groups exerted significant stabilizing effects. Drugs containing both groups (e.g. methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxy-α-pyrrolidinobutiophenone (MDPBP)) were the most stable of the twenty-two drugs investigated. Unsubstituted and ring substituted cathinones were considerably less stable, with 3-fluoromethcathinone (3-FMC) exhibiting the greatest instability. Although the approach using fortified preserved urine affords a robust experimental design for the evaluation of long-term stability, authentic urine specimens from cathinone users can also provide valuable information. In this study, we compare cathinone concentrations in 180 authentic unpreserved urine specimens from cathinone users after specified periods of storage. These findings are compared with previously published experimental data using fortified drug-free urine.

Materials and Methods

Chemicals and reagents

Methcathinone, 3-FMC, 4-fluoromethcathinone (4-FMC, flephedrone), methylone, ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-methylethcathinone (4-MEC), 3,4-methylenedioxy-α-pyrrolidinobutiophenone (MDPBP), pentedrone, pentylone, 3,4-dimethylmethcathinone (3,4-DMMC), α-pyrrolidinopentiophenone (α-PVP), 4-ethylmethcathinone (4-EMC), 4-methyl-α-pyrrolidinobutiophenone (MPBP), methylenedioxypyrovalerone (MDPV), pyrovalerone, and naphyrone were purchased from Cerilliant Corporation (Round Rock, TX, USA) in 1.0 mg/mL methanolic solutions. Internal standards methylone-D3, ethylone-D5, butylone-

D3, mephedrone-D3, eutylone-D5, pentylone-D3, α-PVP -D8, MDPV-D8, and naphyrone-D5 were also purchased from Cerilliant Corporation in 0.1 mg/mL methanolic solutions. The internal standard solution consisted of all nine deuterated internal standards at a concentration of 0.25 µg/mL in methanol. Pooled drug-free urine, preserved with 1% sodium fluoride, was purchased from Utak Laboratories (Valencia, CA, USA).

Dichloromethane, isopropyl alcohol, and glacial acetic acid were purchased from Mallinckrodt Chemicals (St. Louis, MO, USA). LC-MS grade methanol, concentrated hydrochloric acid, LCMS grade acetonitrile, and dibasic sodium phosphate were purchased from J.T. Baker (Center Valley, MA, USA). Optima® hexane and HPLC grade ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (>99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Monobasic sodium phosphate was purchased from VWR (Radnor, PA, USA). Deionized water was purified in-house using a Millipore Direct-Q® UV Water Purification system (Billerica, MA, USA). PolyChrom ClinII 3 cc (35 mg) solid phase extraction (SPE) columns were purchased from SPEware (Baldwin Park, CA, USA).

Instrumentation

An Agilent Technologies 6530 LC-Q/TOF-MS (Santa Clara, CA, USA) equipped with an Agilent 1290 Infinity autosampler and a Series 1200 LC system was used for instrumental analysis. Cathinones were separated using an Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm particle size) and an Agilent Poroshell 120 EC-C18 guard column (2.1 x 5 mm, 2.7 µm particle size) maintained at 35°C. Mobile phase A and B comprised of 0.1% formic acid in deionized water and acetonitrile, respectively, and used the following gradient elution profile: 96% A and 4% B (0 – 0.5 mins); 90% A (0.5 – 5

mins); 60% A (5 – 11 mins); 0% A and 100% B (11 – 12 mins). Nitrogen was generated using a Genius 3040 Nitrogen Generator (Peak Scientific, Billerica, MA, USA). Solid phase extraction (SPE) was performed using a JT Baker vacuum manifold and extracts were evaporated to dryness under nitrogen using a TurboVap LV® concentration workstation (Caliper Life Sciences, Hopkinton, MA, USA). A FiveEasy™ FiveGo™ pH meter FE20/FG2 was used for pH measurement (Mettler Toledo, Columbus, OH, USA). The LC-Q/TOF-MS procedure used for quantification of cathinones in urine was based on a previously published method and was validated in accordance with generally accepted guidelines (20, 21).

Authentic urine samples

Urine specimens from cathinone users were obtained in accordance with an IRB-approved study. Samples were transported on ice and were reanalyzed after a specified period of refrigerated storage. A one milliliter aliquot of unpreserved urine was analyzed quantitatively and appropriate dilutions were performed for samples that exceeded the calibration range of the assay (500 ng/mL). Following SPE, samples were reconstituted in 25 µL of a 50:50 mixture of Mobile Phase A:B and 1 µL was injected onto the LC-Q/TOF-MS for analysis. Limits of quantitation for the twenty-two target compounds ranged from 0.25 to 5 ng/mL (20).

Extraction of cathinones from urine

Internal standard solution (100 µL) was added to 1 mL urine to achieve a final concentration of 25 ng/mL. Urine, diluted with 2 mL of pH 6 phosphate buffer (0.1 M), was transferred to PolyChrom ClinII SPE columns and allowed to flow through under gravity. Columns were rinsed successively with 1 mL deionized water and 1 M acetic acid.

SPE columns were dried for 5 minutes under full vacuum, followed by 1 mL hexane, ethyl acetate, and methanol washes. Drugs were eluted using two 0.5 mL aliquots of elution solvent (2% concentrated ammonium hydroxide in 95:5 dichloromethane: isopropyl alcohol). Extracts were salted out using acidic methanol (30 µL) prior to evaporation under nitrogen at 50°C. Samples were reconstituted in 25 µL Mobile Phase A:B (50:50) and 1 µL was injected onto the LC-Q/TOF-MS for analysis.

Data analysis

Quantitative measurements were compared and statistically evaluated for urine specimens stored for a period of less than 24 months. Where possible, storage time, analyte, and pH dependent differences in stability were addressed. Results using authentic urine specimens from cathinone users were compared with experimentally determined findings using fortified urine (19). Storage temperature was not assessed however, because all specimens were refrigerated throughout.

Results and Discussion

The 180 urine specimens yielded a total of 164 cathinone positive results for α-PVP (n=92), ethylone (n=55), methylone (n=8), MDPV (n=1), pentylone (n=1), 4-FMC (n=2), 4-MEC (n=2) and pentedrone (n=3). No quantitative comparisons were made if the original date of analysis was unknown, or if results were reported qualitatively during either assay. Correlations and statistical evaluations were performed only on urine specimens that had been stored for less than 24 months.

Of the 164 positive findings, it was possible to make 156 quantitative comparisons involving α-PVP (n=92), ethylone (n=55), methylone (n=8), MDPV (n=1) and pentylone (n=1). The quantitative comparisons are summarized in **Table 5.1** and the remaining

qualitative data is shown in **Table 5.2**. Results for individual cases can be found in **Appendix G**. Specimens were stored for a period of 5 to 17 months (median=14.5) at refrigerated temperature in the absence of preservative. Urinary pH upon reanalysis ranged from 4.5 to 10. Among all samples tested, the percentage of drug remaining was highly variable (0-119%). The median percentage of drug remaining for α-PVP, ethylone and methylone was 13, 2 and 61%. Synthetic cathinones bearing a pyrrolidinyl group are significantly more stable than their secondary amine counterparts (19, 22). However, both ethylone and methylone contain methylenedioxy groups, which are also known to have a stabilizing effect. Given the population size and the known differences in stability between these secondary and tertiary amines, these were further investigated.

No correlation was observed between the percentage of drug remaining and storage time (**Figure 5.1**). This is significant because it suggests that factors other than storage time may play a role. When initial and final concentrations were compared, specimen pH was also evaluated (**Figure 5.2**).

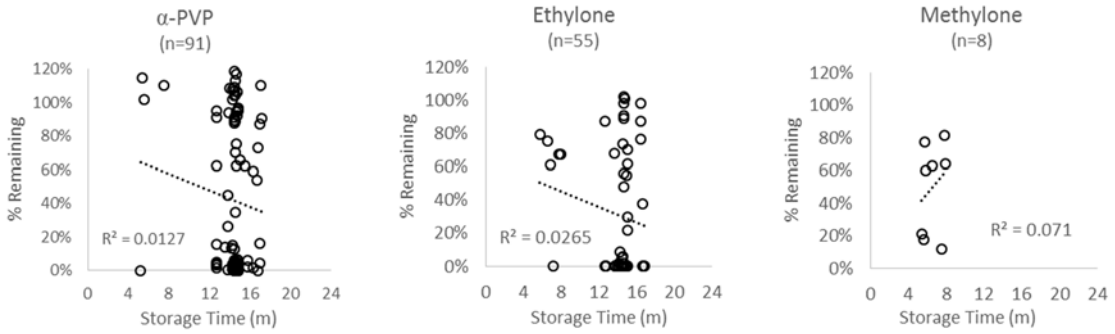

Figure 5.1. Percentage of cathinone remaining after specified periods of storage (months).

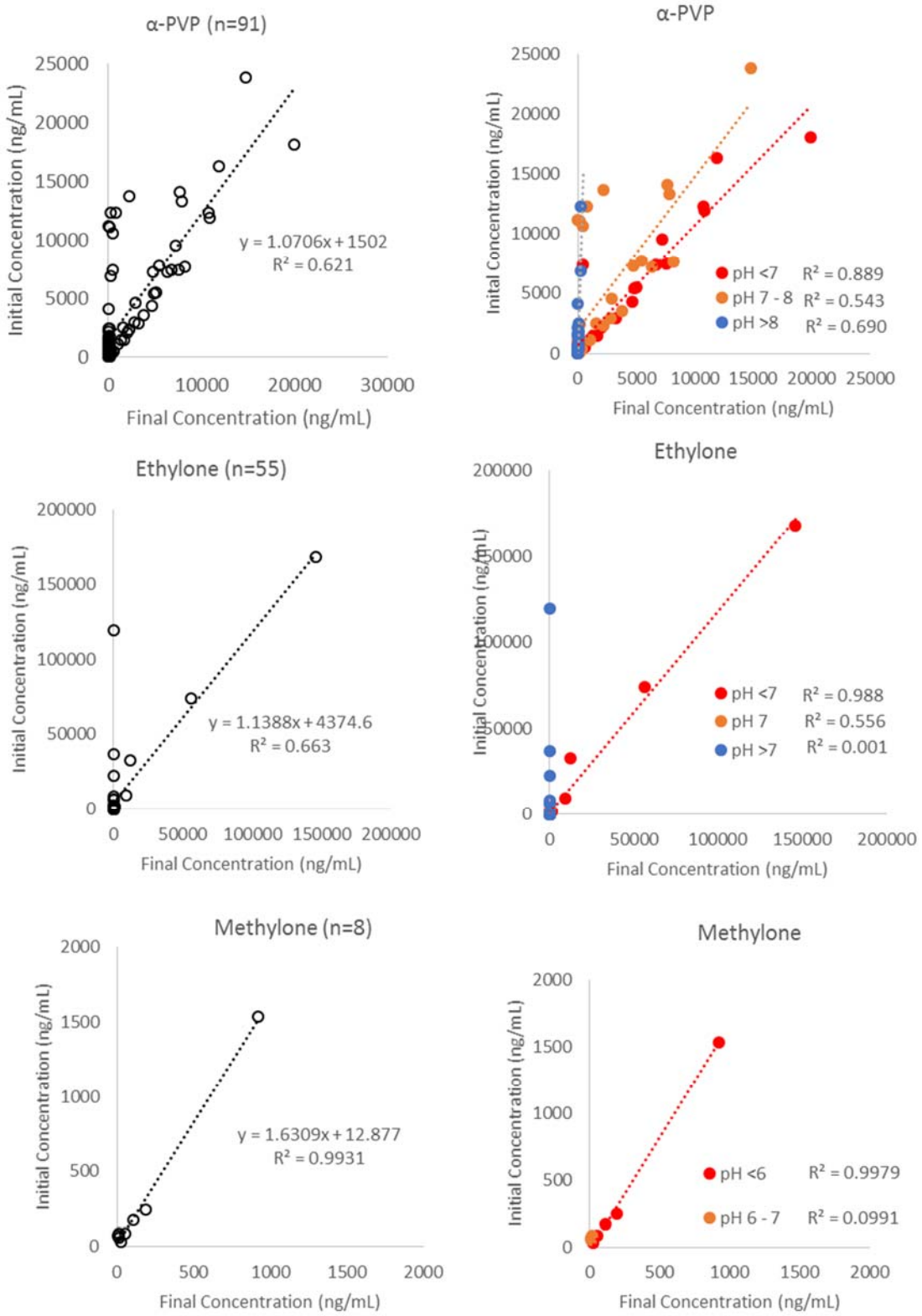

Figure 5.2. Correlation between initial and final cathinone concentrations (ng/mL)

Significance tests on correlation coefficients (R) were performed using the *t*-test. The strongest correlations were clearly observed at acidic pH. Coefficients of determination (R^2) for α-PVP in acidic urine (<pH 7) were 0.889, compared with 0.543 in alkaline urine (>pH 8). Correlations were significant for each pH range tested ($p<0.001$). Similar results were observed for ethylone, with R^2 values of 0.988 at pH <7, compared with 0.001 at pH >7. Correlations were significant in acidic and neutral urine, $p<0.001$ and $p=0.05$, respectively. Although the methylone positive population was limited in size and urinary pH (range 4.5-6.5), the correlation was highly significant at pH<6 ($p<0.001$).

Consistent with experimental studies, cathinone concentrations in acidic urine specimens were readily confirmed following periods of storage up to 24 months. In acidic urine, the relationship between initial and final α-PVP concentration was strong ($y = 0.998\ x + 732$). Strong relationships were also observed for ethylone ($y = 1.169\ x + 1078$) and methylone ($y = 1.683\ x + 24$), although the steeper gradients reflect the overall reduced stability of these drugs. Due to the profound effect of pH on cathinone stability, alkaline specimens often suffered dramatic losses. For example, a specimen with a pH of 8.5 containing >37,000 ng/mL ethylone was undetectable upon reanalysis (LOD 2 ng/mL). Although this specimen had been stored for 17 months, studies have shown that even when refrigerated, ethylone can undergo significant losses within 9 days of storage (19). Under the same conditions, significant losses were observed for 3-FMC in < 1 day, while no decrease in concentration was observed for MDPV over six months. These findings highlight the critical importance of specimen pH and analyte dependent differences in stability among these arylaminoketones.

Table 5.1. Summary of quantitative cathinone determinations in urine following periods of specified storage (< 24 months). The percentage of drug remaining and mean pH is shown in parentheses.

Cathinone	LOQ (ng/mL)	Original Concentration (ng/mL)			Final Concentration (ng/mL)			Storage Time (m)	Urine pH (Mean)
		Range	Median	Mean	Range	Median	Mean		
α-PVP (n=91)	2	25 - 104,111	1,100	3,560	0 - 19,926 (0 - 119%)	99 (13%)	1,923 (42%)	5 - 17	4.5 - 10 (8)
Ethylone (n=55)	1	30 - 167,973	206	9,119	0 - 146,124 (0 - 102%)	7 (2%)	4,166 (31%)	6 - 17	4.5 - 10 (7)
Methylone (n=8)	0.25	32 - 1,535	86	286	2-922 (12 - 81%)	41 (61%)	168 (50%)	5 - 8	4.5 - 6.5 (6)
MDPV (n=1)	1		6,626			479 (7%)		14	8.5
Pentylone (n=1)	1		585			434 (74%)		6	6.5

Table 5.2. Qualitative cathinone determinations (excluded from data analysis).

Cathinone	LOQ (ng/mL)	Unique ID	Original Analysis	Final Analysis	Urine pH	Storage Time (m)
Pentedrone	5 ng/mL	2R002	Positive	ND	5.5	7
		2R016	Positive	<LOQ	5	5
		2R001	Positive	ND	4.5	7
4-MEC	1 ng/mL	2R003	Positive	<LOQ	6	8
		2R019	Positive	ND	7	6
4-FMC	1 ng/mL	2R008	Positive	ND	4.5	7
		2R007	Positive	<LOQ	7	7
α-PVP	2 ng/mL	R042	Positive	7,947	7	15

ND: Not detected.

Among the α-PVP positive samples (n=91) specimen pH ranged from 4.5 to 10 and storage time ranged from 5 to 17 months. Original α-PVP concentrations in urine were 25 – 104,111 ng/mL, with mean and median concentrations of 3,560 and 1,100 ng/mL, respectively. Upon reanalysis α-PVP concentrations were in the range 0 – 19,926 ng/mL, with mean and median concentrations of 1,923 and 99 ng/mL, respectively. Quantitative reanalysis produced results between 0% and 119% of the original result. The profound effect of specimen pH on the percentage of drug remaining is shown in **Figure 5.3**. When considering the effect of pH, storage time was not considered. At a pH of 8.5 and above, amount of α-PVP that remained was significantly diminished. In contrast, most urine specimens with a pH of 6.5 suffered relatively minimal losses. Although not included in the correlation of quantitative results, no significant decrease in α-PVP was observed (94% remaining) in a urine specimen that was stored for as long as 40 months, attributed to the acidity of the specimen (pH 5.5).

Among the ethylone positive samples (n=55), the pH of the specimen ranged from 4.5 to 10 and storage time ranged from 6 to 17 months. Original ethylone concentrations

in urine were 30 – 167,973 ng/mL, with mean and median concentrations of 9,119 and 206 ng/mL respectively. Upon reanalysis ethylone concentrations were in the range 0 – 146,124 ng/mL, with mean and median concentrations of 4,166 and 7 ng/mL, respectively. Quantitative reanalysis produced results between 0% and 102% of the original results. Consistent with α-PVP, specimen pH exerted much greater influence than storage time (**Figure 5.3**). At a pH of 7.5 or above, virtually all of the ethylone was lost.

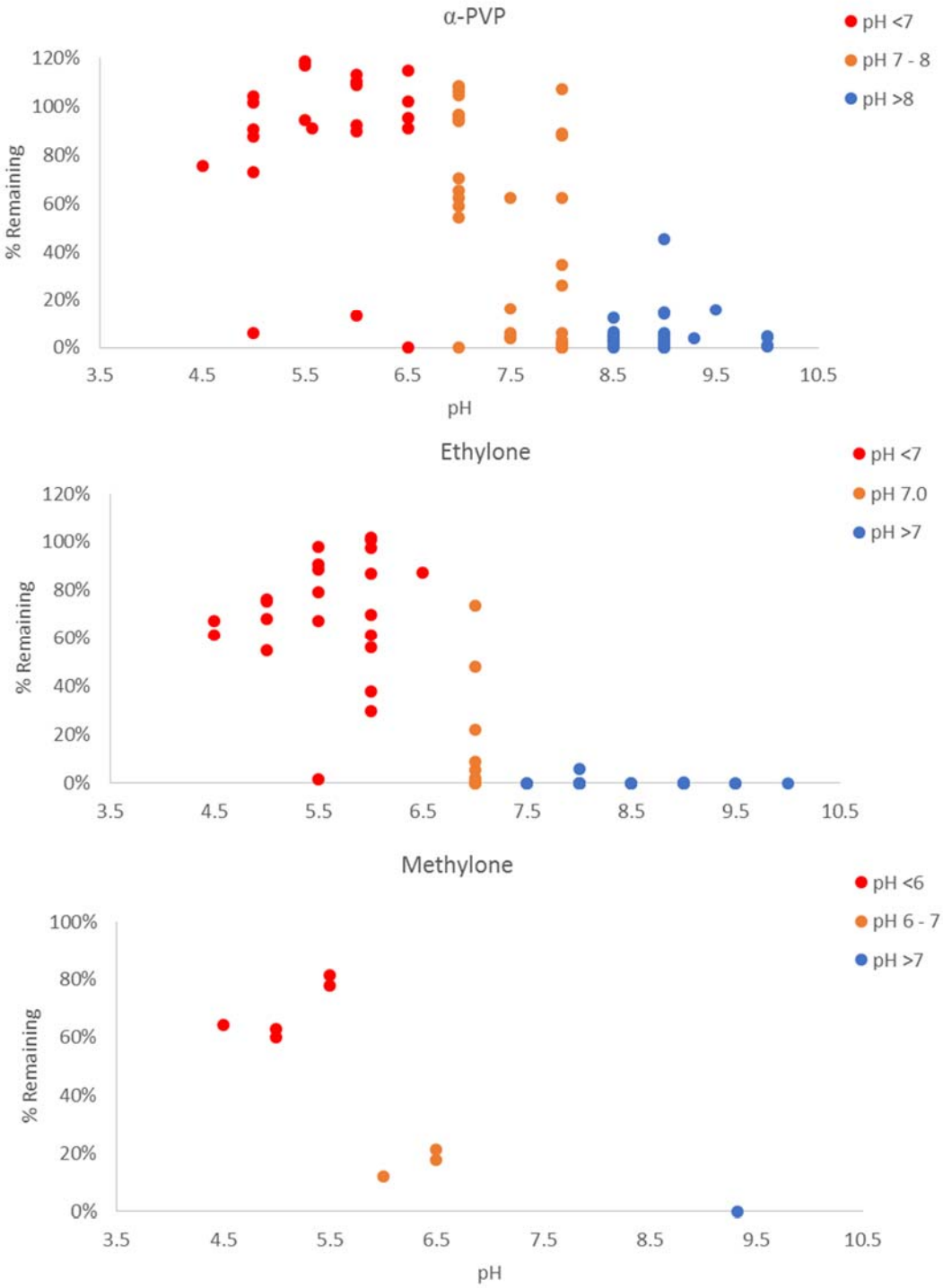

Figure 5.3. Influence of specimen pH on cathinone stability.

Methylone consisted of a relatively small population of samples (n=8) and specimen pH was more limited (4.5 to 6.5) for samples stored < 24 months. Original methylone concentrations in urine were 32 – 1,535 ng/mL, with mean and median concentrations of 286 and 86 ng/mL, respectively. Upon reanalysis methylone concentrations were in the range 2 – 922 ng/mL, with mean and median concentrations of 168 and 41 ng/mL, respectively. Quantitative reanalysis produced concentrations between 12% and 81% of the original results. Once again, **Figure 5.3** depicts the importance of pH rather than storage time, when considering cathinone stability. The ability to reconfirm original results became increasingly more difficult at a pH of 6 or above. These results for both ethylone and methylone are consistent with earlier experimental findings using fortified urine that suggest that the secondary amines are more susceptible to pH dependent degradation. This may be attributed to the inability of the pyrrolidine derivatives to undergo oxidative deamination.

Concerns related to cathinone stability have been reported for several biological matrices, including urine (23-26). Tsujikawa (27) and Maskell (28) were among the first to highlight the importance of pH in aqueous and formalin solutions. Half-lives for methylone, ethylone, and α-PVP in refrigerated pH 8 urine were 1.4, 1.8, and 7.1 months, respectively (19). In contrast, at pH 4, drugs were relatively stable when refrigerated. Experimental studies using drug-fortified urine are advantageous from an experimental design standpoint. They may allow for more extensive sampling over extended periods, multiple experimental conditions, and the simultaneous evaluation of a large number of substances. In contrast, when authentic urine specimens are used, the number of analytes is dependent upon drug user preference, and sample volumes may be limited. In the

previous reported study, cathinone stability in urine was evaluated at pH 4 and 8 [19]. In this report however, urine pH reflects a much broader range (pH 4.5 to 10).

Although normal urinary pH is generally in the range ~4 to 8, this varies with the time of day, diet, disease state, and many other factors (29, 30). Post-collection, urinary pH can increase over time. At elevated temperatures, increases of 2 pH units or more have been observed within 24 hours (29, 30). Although increases in pH are also possible in refrigerated urine, the magnitude of the increase is significantly diminished. Urine contains a variety nitrogenous and inorganic species including bicarbonate, phosphates, and ammonium salts. Elevations in urinary pH have been attributed to the chemical breakdown of nitrogenous analytes. Pre-analytical contamination of the sample with microorganisms during collection can also increase urinary pH, due to the bacterial decomposition of urea to ammonia (29, 30). While the authentic unpreserved urine samples presented here have some uncontrolled variables, the data can be used to complement the results from a controlled stability study, and are more likely to represent actual cases encountered in the laboratory. In this report, urinary pH values exceeded the normal physiological range. This may be attributed to the length of storage, possible exposure to elevated temperatures during initial shipping of the specimens, and the absence of preservative.

Conclusions

These results using authentic urine specimens from cathinone users support our previously reported experimental findings (19). This study reinforces the importance of analyte and pH-dependent considerations, when interpreting forensic toxicology results involving cathinones. While all cathinones should be considered alkaline-labile drugs, their susceptibility to pH-mediated degradation is analyte-dependent. Urinary pH values within

the normal physiological range can result in significant degradation, and pre-analytical increases in pH during storage may further exacerbate the issue. Results from this study suggest that although conventional time-dependent interpretation is often used when comparing forensic toxicology results, specimen pH is a more critical variable for the synthetic cathinones.

Acknowledgements

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References

1. U.S. Drug Enforcement Administration: Diversion Control Division (2014) National Forensic Laboratory Information System Special Report: Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2010-2013, Springfield, VA: U.S. Drug Enforcement Administration.
2. U.S. Drug Enforcement Administration: Diversion Control Division (2016) National Forensic Laboraotry Information System Special Report: Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2013-2015, Springfield, VA: U.S. Drug Enforcement Administration.
3. European Monitoring Centre for Drugs and Drug Addition, European Drug Report 2016: Trends and Developments. Available at http://www.emcdda.europa.eu/.
4. 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*, **49**, 499-505.
5. Warrick B.J., Hill M., Hekman K., Christensen R., Goetz R., Casavant M.J., Wahl M., Mowry J.B., Spiller H., Anderson D. (2013) A 9-state analysis of designer stimulant,“bath salt,” hospital visits reported to poison control centers, *Annals of Emergency Medicine*, **62**, 244-251.
6. Forrester M.B. (2012) Synthetic cathinone exposures reported to Texas poison centers. *American Journal of Drug and Alcohol Abuse*, **38**, 609-615.
7. Karila L., Megarbane B., Cottencin O., Lejoyeux M. (2015) Synthetic cathinones: a new public health problem. *Current Neuropharmacology*, **13**, 12-20.

8. European Monitoring Centre for Drugs and Drug Addition, European Drug Report 2017: Trends and Developments. Available at http://www.emcdda.europa.eu/.

9. Prosser J., Nelson L. (2012) The toxicology of bath salts: a review of synthetic cathinones. *Journal of Medical Toxicology*, **8**, 33-42.

10. Coppola M., Mondola R. (2012) Synthetic cathinones: Chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as "bath salts" or "plant food". *Toxicology Letters*, **211**, 144-149.

11. German C.L., Fleckenstein A.E., Hanson G.R. (2014) Bath salts and synthetic cathinones: An emerging designer drug phenomenon. *Life Science*, **97**, 2-8.

12. Capriola M. (2013) Synthetic cathinone abuse. *Clinical Pharmacology*, **5**, 109-115.

13. Simmler L.D., Buser T.A., Donzelli M., Schramm Y., Dieu L.H., Huwyler J., Chaboz S., Hoener M.C., Liechti M.E. (2013) Pharmacological characterization of designer cathinones in vitro. *British Journal of Pharmacology*, **168**, 458-470.

14. Belhadj-Tahar H., Sadeg N. (2005) Methcathinone: A new postindustrial drug. *Forensic Science International*, **153**, 99-101.

15. Thornton S.L., Gerona R.R., Tomaszewski C.A. (2012) Psychosis from a bath salt product containing flephedrone and MDPV with serum, urine, and product quantification. *Journal of Medical Toxicology*, **8**, 310-313.

16. Bertol E., Mari F., Boscolo Berto R., Mannaioni G., Vaiano F., Favretto D. (2014) A mixed MDPV and benzodiazepine intoxication in a chronic drug abuser:

Determination of MDPV metabolites by LC–HRMS and discussion of the case. *Forensic Science International*, **243**, 149-155.

17. Borek H.A., Holstege C.P. (2012) Hyperthermia and multiorgan failure after abuse of "bath salts" containing 3,4-methylenedioxypyrovalerone. *Annals of Emergency Medicine*, **60**, 103-105.

18. Pedersen A.J., Reitzel L.A., Johansen S.S., Linnet K. (2013) In vitro metabolism studies on mephedrone and analysis of forensic cases. *Drug Testing and Analysis*, **5**, 430-438.

19. Glicksberg L., Kerrigan S. (2017) Stability of synthetic cathinones in urine. Journal of Analytical Toxicology, https://doi.org/10.1093/jat/bkx071.

20. Glicksberg L., Bryand K., Kerrigan S. (2016) Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. *Journal of Chromatography B*, **1035**, 91-103.

21. Scientific Working Group for Forensic Toxicology (SWGTOX). (2013) Standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*, **37**, 452-474.

22. Glicksberg L., Kerrigan S. (2017) Synthetic cathinone stability in blood. *Journal of Analytical Toxicology*.

23. Paul B.D., Cole K.A. (2001) Cathinone (khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. *Journal of Analytical Toxicology*, **25**, 525-530.

24. Johnson R.D., Botch-Jones S.R. (2013) The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *Journal of Analytical Toxicology*, **37**, 51-55.

25. Al-Saffar Y., Stephanson N.N., Beck O. (2013) Multicomponent LC–MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine—experience from the Swedish population. *Journal of Chromatography B*, **930**, 112-120.

26. Concheiro M., Anizan S., Ellefsen K., Huestis M.A. (2013) Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. *Analytical and Bioanalytical Chemistry*, **405**, 9437-9448.

27. Tsujikawa K., Mikuma T., Kuwayama K., Miyaguchi H., Kanamori T., Iwata Y.T., Inoue H. (2012) Degradation pathways of 4-methylmethcathinone in alkaline solution and stability of methcathinone analogs in various pH solutions. *Forensic Science International*, **220**, 103-110.

28. Maskell P.D., Seetohul L.N., Livingstone A.C., Cockburn A.K., Preece J., Pounder D.J. (2013) Stability of 3,4-methylenedioxymethampetamine (MDMA), 4-methylmethcathinone (mephedrone) and 3-trifluromethylphenylpiperazine (3-TFMPP) in formalin solution. *Journal of Analytical Toxicology*, **37**, 440-446.

29. Cook J.D., Strauss K.A., Caplan Y.H., LoDico C.P., Bush D.M. (2007) Urine pH: the effects of time and temperature after collection. *Journal of Analytical Toxicology*, **31**, 486-496.

30. Fura A., Harper T.W., Zhang H., Fung L., Shyu W.C. (2003) Shift in pH of biological fluids during storage and processing: effect on bioanalysis. *Journal of Pharmaceutical Biomedicine*, **32**, 513-522.

CHAPTER VI

POSTMORTEM DISTRIBUTION AND REDISTRIBUTION OF SYNTHETIC CATHINONES[1]

This dissertation follows the style and format of *The Journal of Analytical Toxicology*.

[1]Glicksberg L., Winecker R., Miller C., Kerrigan S. (2017).
Submitted to *Forensic Toxicology*

Abstract

Synthetic cathinones are powerful psychostimulants that have been associated with fatal intoxications. Due to changes that take place following death, postmortem toxicology results require careful interpretation. The purpose of this study was to evaluate the distribution of synthetic cathinones in postmortem specimens in a series of fifty cathinone-positive fatalities.

Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q/TOF-MS) was used to quantitatively identify cathinones in central blood (n=51), peripheral blood (n=31), urine (n=33), liver (n=22), vitreous humor (n=1) and stomach contents (n=1). The distribution of cathinones and the potential for postmortem redistribution was assessed.

Among the fifty cases investigated, a total of nine synthetic cathinones (α-PVP, ethylone, methylone, butylone, MDPV, methedrone, pentylone, 4-MEC, and MDPBP) were identified in 139 specimens. The number of specimens per case ranged from one to six. In cases that included central blood or liver, together with a peripheral blood source, the C/P or L/P ratio was estimated (n=21 C/P; n=11 L/P). Methylone and ethylone appeared to exhibit the greatest potential for postmortem redistribution, producing C/P ratios of 4.0 (1.5-6.1) and 2.9 (0.5-9.2), respectively. In contrast, the C/P ratio for α-PVP was 1.1 (0.5-1.9). Differences in C/P ratios between methylone and α-PVP were statistically significant (α=0.05).

Although synthetic cathinones may exhibit low to moderate postmortem redistribution, significant variability exists due to site- and time dependent factors. This, in

combination with their overall instability, necessitates careful interpretation of postmortem toxicology results.

Key Words: Synthetic cathinones, Designer drugs, LC-Q/TOF-MS, Postmortem redistribution

POSTMORTEM DISTRIBUTION AND REDISTRIBUTION OF SYNTHETIC CATHINONES

Introduction

Interpreting postmortem toxicology results can be challenging due to the changes that take place in the body after death. Drug movement within the body after death can cause significant variability in blood concentrations (1-3). Postmortem changes in drug concentration have been largely attributed to drug instability or postmortem redistribution (PMR), the latter of which has lived up to its reputation as a "toxicological nightmare" (2). PMR is ideally assessed by comparing postmortem blood concentrations with antemortem specimens, obtained shortly before death. Although this is sometimes possible in hospital deaths, the extent to which a drug is susceptible to PMR is more often limited to the comparison of central (C) and peripheral (P) blood sources. Although other approaches, such as liver/peripheral (L/P) ratios have also been proposed (4), the most widely used approach involves the use of C/P ratios. Notwithstanding this approach, standardized postmortem sampling protocols for toxicology testing have not yet been universally established. Although there is a general consensus that specimen collection should be performed as early as possible during the postmortem interval, the sampling procedure itself, quantity, and selection of specimens varies significantly by jurisdiction (5). While there is no standardized collection protocol, central blood, peripheral blood, and liver are often submitted for toxicology testing, from which C/P and L/P ratios can be determined. Although conclusions should not be drawn from single cases, increases in the C/P ratio (>1) or L/P ratio (>20) in a population of cases may indicate a tendency for the drug to exhibit PMR (4, 6, 7).

The thoracic cavity contains many organs in close proximity, including the lungs, liver, gastrointestinal tract, and myocardium. Depending on physico-chemical properties, including lipophilicity, volume of distribution (Vd), protein binding, and pKa, drugs may have a tendency to accumulate in these organs antemortem. Postmortem, the drug may be released and redistributed, resulting in elevated concentrations in central blood (1, 3, 8, 9). To minimize the influence of PMR, lung, liver and cardiac blood may be sampled from the right side (5, 8). Nonetheless, a peripheral blood specimen is highly preferred because it is anatomically isolated from the thoracic compartment. Although peripheral blood drug concentrations are still subject to variability, they are expected to be more representative of the drug concentration prior to death.

While information regarding PMR potential of synthetic cathinones is still limited, some inferences may be possible from structurally similar drugs that have been more extensively evaluated. Synthetic cathinones are beta-keto amphetamines that are structurally related to both methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). MDMA (pKa 8.7) and methamphetamine (pKa 9.9) are basic drugs with volumes of distribution (Vd) of 3 - 7 L/kg (10). Both have been shown to exhibit some degree of PMR (6, 10). The average C/P ratios for methamphetamine and MDMA were 2.3 (0.9-5.8, n=39) and 2.7 (0.9-4.6, n=7), respectively and average L/P ratios were 5.5 (1.7-9.1 L/kg, n=19) and 6.5 (3.1-8.5 L/kg, n=5), respectively (7, 11-17). Although MDMA may have greater potential for PMR than methamphetamine, the wide range of reported C/P and L/P values highlight the inter-subject and sampling variations (18).

Synthetic cathinones can be structurally differentiated by substituents on the phenyl ring, alkyl chain, and nitrogen terminus (particularly the introduction of a pyrrolidine

group). Many pharmacologic and physico-chemical properties of these relatively new drugs have not yet been established. Like their amphetamine counterparts, they are basic drugs, with pKa values ranging from 7.2 to 8.9 (10, 19, 20). It might be inferred that synthetic cathinones could also exhibit some degree of PMR. However, the influence of the various substituents, including the methylenedioxy and pyrrolidine groups, is not yet well understood.

Synthetic cathinone abuse is well documented and fatalities have been reported (4, 21-29). A compilation of C/P and L/P ratios is shown for published case reports to date (**Table 6.1)**. Although C/P ratios were generally >1, synthetic cathinone L/P ratios were <5 L/kg (with the exception of MDPV). McIntyre proposed the use of L/P ratios, suggesting that the broader range of values might allow for better differentiation of drugs that exhibit PMR (>20 L/kg) from those that do not (<5 L/kg) (4, 30). However, liver is not a homogeneous specimen and drug concentrations within this complex organ are variable (8). Although sampling from the deep right lobe is recommended to avoid contamination or diffusion from the gastrointestinal compartment, sampling protocols vary (5). Due to inter-subject variation and sample collection variation, C/P and L/P ratios from single case studies or very small sample populations may not be representative and should be interpreted with caution.

The purpose of this study was to further investigate the distribution and potential redistribution of synthetic cathinones in fifty cathinone positive fatalities; to compare findings with existing published case reports; and to investigate the influence of structural characteristics or various substituents on the potential for PMR.

Table 6.1. Summary of C/P and L/P ratios for synthetic cathinones from published case reports.

Cathinone	C/P	L/P (L/kg)	Reference
Butylone	N/A	1.7	(21)
Ethylone	1.0	3.6	(4)
MDPV	1.7	23	(22)
	1.6	2.9	(22)
	-	3.0	(22)
	1.3	2.5	(22)
	1.6	19	(22)
	-	9.5	(22)
	1.4	2.2	(31)
	0.7	N/A	(23)
Average	1.3	8.9	
Methylone	-	2.9	(24)
	1.2	-	(25)
	1.0	1.6	(25)
	1.0	3.2	(32)
	1.1	2.7	(26)
	2.1	-	(27)
Average	1.3	2.6	
Pentedrone	-	11	(33)
α-PVP	-	1.1	(28)
	R: 1.4 L: 1.5	1.0	(29)
	-	2.9	(33)
Average	1.45	1.7	
Pyrovalerone	1.4	3.0	(22)

Material and Methods

Chemicals and reagents

Reference standards for 3,4-dimethylmethcathinone (3,4-DMMC), 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (4-FMC), 4-ethylmethcathinone

(4-EMC), 4-methyletehcathinone (4-MEC), α-PVP, buphedrone, butylone, ethcathinone, ethylone, eutylone, 3,4-methylenedioxy-α-pyrrolidinobutiophenone (MDPBP), MDPV, methcathinone, methedrone, methylone, mephedrone, 4-methyl-α-pyrrolidinobutiophenone (MPBP), naphyrone, pentedrone, pentylone, and pyrovalerone were purchased as methanolic standards (1.0 mg/mL) from Cerilliant Corp. (Round Rock, TX, USA). Internal standards methylone-D3, ethylone-D3, butylone-D3, eutylone-D5, MDPV-D8, α-PVP-D8, naphyrone-D5, and mephedrone-D3 were purchased as methanolic 0.1 mg/mL standards from Cerilliant Corp. (Round Rock, TX, USA).

Pooled drug-free urine, preserved with 1% sodium fluoride, was purchased from Utak Laboratories (Valencia, CA, USA) and bovine blood containing 1% sodium fluoride and 0.2% potassium oxalate was purchased from Quad Five (Ryegate, Montana, USA). Drug free human liver was procured from Sam Houston State University's Southeast Texas Applied Forensic Science (STAFS) Facility. Postmortem samples were received from the LA County Department of the Medical Examiner and the North Carolina Office of the Chief Medical Examiner in accordance with an IRB approved study.

Ethyl acetate (HPLC grade), hexane (Optima®), and acetonitrile (LCMS grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (>95%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane (LCMS grade), isopropyl alcohol (LCMS grade), and glacial acetic acid (ACS grade) were purchased from Mallinckrodt Chemicals (St. Louis, MO, USA). Methanol (LCMS grade), concentrated hydrochloric acid (ACS grade), and dibasic sodium phosphate (ACS grade) were obtained from J.T. Baker (Center Valley, MA, USA). Concentrated ammonium hydroxide (Optima®) was obtained from Macron Fine Chemicals (Center Valley, MA, USA).

Monobasic sodium phosphate (ACS grade) was obtained from VWR (Randor, PA, USA). Deionized water was purified in-house using a Millipore Direct-Q® UV Water Purification system (Billerica, MA, USA). PolyChrom Clin II 3 cc (35 mg) solid phase extraction (SPE) columns were obtained from Tecan SP, Inc. (Baldwin Park, CA, USA).

Instrumentation

An Agilent Technologies 6530 LC-Q/TOF-MS equipped with an Agilent 1290 Infinity autosampler was used to analyze samples (Agilent Technologies, Santa Clara, CA, USA). An Agilent Technologies Series 1200 LC system and Agilent Poroshell 120 EC-C18 column (2.1 x 100 mm, 2.7 µm) (equipped with guard column , 2.1 x 5 mm, 2.7 µm) was used for chromatographic separation at 35°C. Nitrogen for the LC-Q/TOF was generated with a Genius 3040 nitrogen generator (Peak Scientific, Billerica, MA, USA). Mobile phase A and B consisted of 0.1% formic acid in deionized water and 0.1% formic acid in acetonitrile, respectively. Using a flow rate of 0.4 mL/min, compounds were separated using gradient elution: 96% A and 4% B (0 – 0.5 mins); 10% B (0.5 – 5 mins); 40% B (5 – 11 mins); 100% B (12 mins). The LC-Q/TOF MS was equipped with an electrospray ionization (ESI) source operated in positive mode under the following conditions: drying gas (N_2), 13 L/min; drying gas temperature, 200ºC; nebulizer, 20 psi; sheath gas temperature, 250ºC; nitrogen sheath gas flow, 12 L/min; capillary voltage, 4000 V; nozzle voltage, 0 V; fragmentor, 150 V; skimmer, 65V. Full analytical details and validation data was previously published (35). Agilent MassHunter software was used for acquisition, qualitative, and quantitative analysis.

Preparation of standards and reagents

Methanolic working standards containing all twenty-two synthetic cathinones were prepared at 0.1 and 1.0 ng/µL for urine calibrators, and 0.2 and 2.0 ng/µL for blood calibrators. A total of 6 non-zero calibrators (5, 10, 250, 100, 250, 350, and 500 ng/mL) were prepared daily in blood and urine. A stock standard of 10 ng/µL was used to prepare high calibrators (>250 ng/mL) for both matrices. Tissue homogenates were fortified using the 0.2, 1.0, or 2.0 ng/µL working solution for the low, medium, and high control, respectively. The working internal standard (IS) solution consisted of all nine deuterated internal standards in methanol at 0.5 ng/µL. Phosphate buffer (pH 6, 0.1M) was prepared from 0.1M solutions of mono and dibasic sodium phosphate solutions. Acidic methanol was prepared using 2% (v/v) concentrated hydrochloric acid in methanol. The elution solvent, consisting of 2% concentrated ammonium hydroxide in 95:5 dichloromethane/isopropyl alcohol (v/v), was prepared daily.

Sample Preparation

Cathinones were isolated from blood and urine using a previously published technique [35]. Urine (1.0 mL) was fortified with IS to achieve a final concentration of 25 ng/mL. Samples were diluted with 2.0 mL of 0.1M phosphate buffer and transferred to SPE columns. A J.T. Baker vacuum manifold (Center Valley, MA, USA) was used for all extractions. Columns were washed with 1.0 mL deionized water and 1.0 mL 1M acetic acid, dried under vacuum, and then washed successively with 1.0 mL hexane, ethyl acetate and methanol. Cathinones were eluted using 1.0 mL of elution solvent, delivered in two 0.5 mL volumes. Following the addition of 30 µL of 2% acidic methanol, extracts were evaporated to dryness under nitrogen at 50°C using a TurboVap LV® (Caliper Life

Sciences, Hopkinton, MA, USA). Samples were reconstituted in 25 µL of 50:50 mobile phase A: B and 1 µL was injected onto the LC-Q/TOF MS for analysis. Vitreous fluid was treated in an analogous fashion.

Blood (2.0 mL) was fortified with IS to achieve final concentration of 25 ng/mL. Cold acetonitrile (4.0 mL) was added with vortex-mixing to precipitate proteins. Following centrifugation at 4,000 RPM (5 mins), the supernatant was transferred to a clean culture tube, diluted with 6.0 mL of 0.1M phosphate buffer, and transferred to an SPE column. Samples were washed with 1.0 mL deionized water and 1.0 mL of 1M acetic acid, dried under vacuum, and then washed with 1.0 mL hexane, 1.0 mL ethyl acetate, 1.0 mL methanol, and 1.0 mL dichloromethane. Elution, acidification, reconstitution and injection of extracts onto the LC-Q/TOF was performed as described above.

Liver homogenates were prepared using one part tissue (0.5 g) with two parts deionized water using a Bead Ruptor 12 (OMNI International, Kennesaw, GA, USA). Full homogenization was achieved using two 30-second pulses (at high speed) in reinforced sample tubes (7 mL) pre-filled with twelve 2.8 mm ceramic beads (OMNI International, Kennesaw, GA, USA). Following the transfer of 0.5 mL homogenate to a clean culture tube, IS (25 ng total) was added. Proteins were precipitated using cold acetonitrile (2.0 mL) and samples were centrifuged at 4,000 RPM (5 mins). The supernatant was transferred to a clean culture tube, diluted with 3.0 mL of 0.1M phosphate buffer, and extracted using the procedure described for blood. Quantitative liver determinations were performed using whole blood calibrators and matrix-matched (liver) controls. Liver homogenates (0.5 mL of a 1:2 homogenate) were fortified with 10, 25, and 50 ng (total) drug, reflecting final liver concentrations of 60, 150, and 300 ng/g. Bias and precision were evaluated using

three replicates over five days. Acceptable criteria for bias and precision was ±20% (34). In addition to whole blood controls, a matrix-matched (liver) control (150 ng/g) was routinely included in each assay. In order to obtain quantitative results within the calibration range of the assay, appropriate dilutions were used if necessary.

Postmortem specimens

A total of fifty cathinone positive cases were identified. A total of 139 specimens were included in the study, comprised of vitreous humor (n=1), urine (n=33), liver (n=22), stomach contents (n=1), central blood (n=51) and peripheral blood (n=31). Central blood was identified as aorta blood (n=33), heart blood (n=5), vena cava blood (n=11), right chest cavity blood (n=1) and central blood (n=1). Peripheral sources were identified as femoral vessel blood (n=17), iliac vein blood (n=10), subclavian vessel blood (n=3), and peripheral blood (n=1). The number of specimens per case (n=50) ranged from one to six. In cases that included central blood or liver, together with a peripheral blood source, the C/P or L/P ratio was estimated (n=21 C/P; n=11 L/P).

Results and discussion

Tissue validation

Assay performance in blood and urine was previously reported, demonstrating limits of quantitation between 0.25 – 5 ng/mL (35). In order to establish validity of the quantitative assay in tissue, precision and bias were assessed using liver fortified with drug at three concentrations in triplicate, over five days. A low control (containing 10 ng total drug in homogenate) was selected to ensure reliable quantitative performance in tissue. Bias for all twenty-two cathinones ranged from -20 to 14.7% (**Table 6.2**). In all but four instances, precision yielded coefficients of variation (%CV) of 20% or less. Intra-assay

CVs for 3-FMC, ethcathinone, MDPBP and MPBP in liver were 0.3-26.4%, 0.7-20.9%, 2.1-20.7% and 0.7-24.7%, respectively. In an abundance of caution, concentrations in liver below the low control (60 ng/g) were reported as <60 ng/g. Extracted ion chromatograms for the twenty-two target drugs are presented in **Figure 6.1** and **Figure 6.2** depicts an authentic liver sample (Case #16) containing methylone at 61 ng/g.

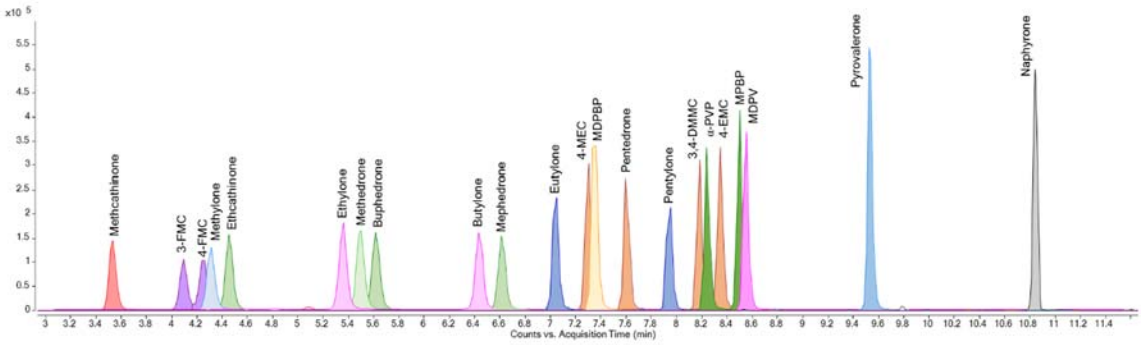

Figure 6.3. Separation of synthetic cathinones in a representative liver extract (60 ng/g). Overlaid extracted ion chromatograms are shown for methcathinone (3.534), 3-FMC (4.096), 4-FMC (4.237), methylone (4.314), ethcathinone (4.452), ethylone (5.361), methedrone (5.503), buphedrone (5.618), butylone (6.432), mephedrone (6.612), eutylone (7.053), 4-MEC (7.307), MDPBP (7.363), pentedrone (7.597), pentylone (7.951), 3,4-DMMC (8.182), α-PVP (8.239), 4-EMC (8.349), MPBP (8.503), MDPV (8.554), pyrovalerone (9.526), naphyrone (10.832).

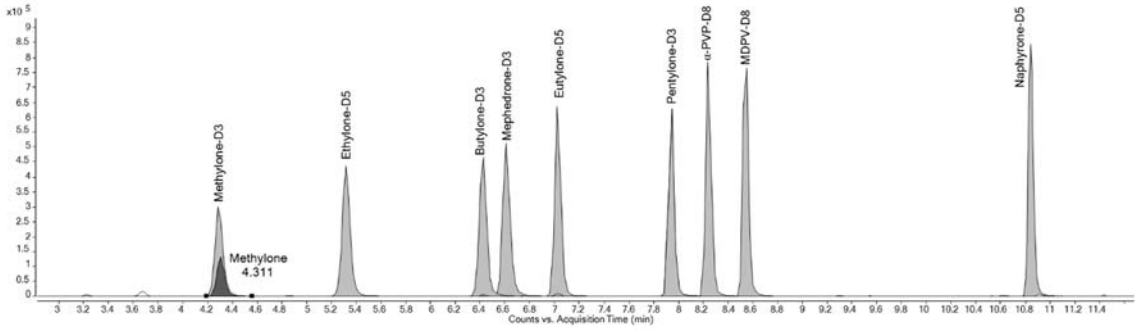

Figure 6.4. Representative postmortem liver extract (Case #16) containing methylone (122 ng/g). Extracted ion chromatograms for the nine internal standards are also shown: methylone-D3 (4.260), ethylone-D5 (5.256), butylone-D3 (6.404), mephedrone-D3 (6.583), eutylone-D5 (7.021), pentylone-d3 (7.918), α-PVP-D8 (8.236), MDPV-D8 (8.525), naphyrone-D5 (10.832).

Table 6.2. Summary of bias, intra- and inter-assay precision in liver. The limit of quantitation (LOQ) in blood is indicated in parentheses for comparison purposes. Drugs identified in the cathinone-positive fatalities are shown in bold.

Cathinone (Blood LOQ, ng/mL)	**Intra-assay Precision (n=3, %CV)**			**Inter-assay Precision (n=15, %CV)**			**Bias (n=15, %)**		
	60 ng/g	150 ng/g	300 ng/g	60 ng/g	150 ng/g	300 ng/g	60 ng/g	150 ng/g	300 ng/g
Methcathinone (2)	3.9-7.6	1.2-11.1	1.2-7.5	7.6	8.9	5.1	-12.5	-3.0	1.3
3-FMC (2)	1.7-8.7	0.3-26.4	2.8-13.5	8.7	16.9	8.5	-20.0	-11.9	-5.9
4-FMC (5)	1.7-13.4	0.4-7.4	3.7-10.7	13.4	9.1	7.6	3.5	6.6	7.8
Methylone* (2)	**1.7-5.9**	**1.9-5.2**	**1.6-7.2**	**3.9**	**3.2**	**4.7**	**-2.8**	**-5.0**	**-1.2**
Ethcathinone (5)	1.6-9.6	0.7-20.9	0.5-9.8	9.6	14.1	8.2	-18.0	-12.0	-6.0
Ethylone* (2)	**4.3-6.0**	**1.1-4.2**	**2.2-4.2**	**6.0**	**4.5**	**4.3**	**-3.7**	**-1.5**	**3.1**
Methedrone* (2)	**3.6-11.4**	**2.4-10.7**	**0.7-5.8**	**11.4**	**5.9**	**3.4**	**-12.6**	**1.3**	**7.9**
Buphedrone (5)	3.3-10.3	1.4-11.1	0.6-11.2	10.3	9.1	5.9	-15.6	-6.9	-1.0
Butylone* (2)	**1.4-6.1**	**2.4-5.9**	**2.1-6.1**	**5.3**	**5.1**	**4.5**	**-2.4**	**-3.2**	**0.2**
Mephedrone (2)	1.9-6.2	0.9-5.2	1.3-4.5	3.8	4.2	3.9	-0.7	-2.3	-0.6
Eutylone (5)	1.3-9.9	1.2-6.3	2.2-6.1	8.1	5.4	4.1	-0.7	-0.8	3.8
4-MEC (5)	1.9-18.5	2.1-5.4	1.5-5.5	18.5	6.3	4.7	-14.2	10.3	12.2
MDPBP (5)	3.1-20.7	2.4-7.2	2.1-4.9	17.3	8.1	6.6	-16.2	5.1	8.9
Pentedrone (5)	1.4-12.6	0.9-9.2	2.4-6.4	12.6	8.6	3.8	-14.5	-2.7	1.0
Pentylone (5)	**2.4-11.7**	**1.9-7.1**	**2.8-7.5**	**8.0**	**5.3**	**6.2**	**-0.7**	**-4.9**	**-1.3**
3,4-DMMC (2)	1.0-8.8	1.4-5.2	1.7-6.6	8.8	12.1	8.5	-8.7	-6.1	0.6
α-PVP* (2)	**0.8-4.6**	**1.5-8.0**	**0.9-5.7**	**4.6**	**6.4**	**8.4**	**3.1**	**-1.5**	**-1.0**
4-EMC (1)	1.6-10.3	0.7-4.8	1.3-4.4	10.3	6.1	3.4	-10.1	0.6	4.4
MPBP (2)	1.3-24.7	1.4-8.7	0.7-18.7	15.8	6.0	10.4	4.3	4.9	14.7
MDPV* (2)	**2.0-6.2**	**2.5-6.1**	**0.8-7.1**	**4.1**	**3.7**	**5.0**	**-4.8**	**-11.0**	**-6.7**
Pyrovalerone (2)	3.0-9.3	0.7-9.5	1.4-7.2	8.4	6.5	5.0	-2.9	2.2	9.5
Naphyrone (1)	1.5-5.0	3.1-5.4	2.2-9.7	4.6	4.4	6.5	-7.4	-4.9	-4.0

Identification of Cathinones in Postmortem Specimens

Of the fifty cases investigated, a total of nine synthetic cathinones were identified, including α-PVP (n=19), methylone (n=18), ethylone (n=15), MDPV (n=6), pentylone

(n=3), butylone (n=1), methedrone (n=2), 4-MEC (n=1), and MDPBP (n=1). Postmortem findings for all specimens are summarized in **Table 6.3.** Concentrations in blood ranged from <2 - 1,090 ng/mL (α-PVP), <2 - 202 ng/mL (methylone), 3 - 2,743 ng/mL (ethylone), 3 - 80 ng/mL (MDPV), and <5 - 322 ng/mL (pentylone). These results highlight the wide range of forensic interest, and the need for sensitive analytical methods to detect low ng/mL concentrations.

More than one synthetic cathinone was identified in seven cases (Case #1, 7, 8, 34, 37, 38 and 44). In two fatalities, α-PVP and pentylone were identified. Ethylone was also detected in combination with methylone, MDPV or α-PVP. As many as four cathinones were detected in one fatality (MDPV, methylone, MDPBP and pentylone), highlighting the problems associated with illicit NPS use. Overall, the most commonly identified cathinones were α-PVP, methylone and ethylone.

Only one vitreous fluid was available for testing (Case #48), but the ethylone concentration (279 ng/mL) was in good agreement with peripheral blood (262 ng/mL). In previously published studies, the distribution of MDPV, methylone, ethylone and mephedrone in vitreous fluid relative to peripheral blood has been highly variable (4, 22, 27, 32, 36). Time dependent variables and cathinone stability may be contributing factors.

Table 6.3. Cathinone-positive specimens (n=139) in a series of fifty fatalities.

Cathinone	Case #	Contents	Concentration (ng/mL or ng/g)	C/P	L/P
4-MEC	6	subclavian vessel blood	57		
Butylone	39	aorta blood	6	0.7	14
		iliac vein blood	8		
		liver	116		
		urine	934		
Ethylone	26	aorta blood	872	1.1	0.2
		iliac vein blood	780		
		liver	170		
		urine	214		
	27	aorta blood	1,271		
		liver	857		
		urine	8,743		
	28	iliac vein blood	10	0.5	
		heart blood	5		
		urine	273		
	29	aorta blood	4		
		vena cava blood	6		
		urine	958		
	30	femoral vessel blood	19	1.0	<3.2
		central blood	19		
		urine	150		
		liver	<60		
	33	femoral vessel blood	298	9.2	
		aorta blood	2,743		
	34	aorta blood	3		
	35	aorta blood	193	2.8	1.7
		femoral vessel blood	69		

(continued)

Cathinone	Case #	Contents	Concentration (ng/mL or ng/g)	C/P	L/P
		urine	>20,000		
		liver	116		
	36	right chest cavity blood	81		
		urine	2,424		
	37	aorta blood	146	2.5	
		iliac vein blood	59		
		urine	32		
	38	urine	310		
	45	femoral blood	267		
	46	heart blood	2,156		
	48	vitreous fluid	279		20
		femoral blood	262		
		stomach contents	6,827		
		liver	5,196		
MDPBP	1	urine	111		
MDPV	1	aorta blood	80	1.0	
		femoral blood	80		
		urine	5,210		
	2	urine	4		
	15	aorta blood	10		
		vena cava blood	35		
		liver	223		
		urine	203		
	37	liver	<60		
	44	heart blood	4		
Methedrone	11	aorta blood	79	1.1	10
		iliac vein blood	70		
		urine	1,213		
		liver	720		

(continued)

Cathinone	Case #	Contents	Concentration (ng/mL or ng/g)	C/P	L/P
Methylone	1	aorta blood	3	1.5	
		femoral blood	2		
		urine	12,100		
	13	aorta blood	23		
		vena cava blood	6		
		urine	856		
		liver	235		
	16	subclavian vessel blood	20	2.4	3.1
		aorta blood	48		
		urine	38,064		
		liver	61		
	17	urine	5		
	18	aorta blood	10		
		vena cava blood	16		
		liver	142		
		urine	>5,000		
	19	aorta blood	14	3.4	
		femoral vessel blood	4		
	20	aorta blood	20	6.1	
		femoral vessel blood	3		
		urine	756		
	21	aorta blood	14		
		vena cava blood	47		
		urine	>5,000		
	22	aorta blood	202		
		vena cava blood	68		
		urine	52		
		liver	216		

(continued)

Cathinone	Case #	Contents	Concentration (ng/mL or ng/g)	C/P	L/P
	23	aorta blood	129		
		vena cava blood	46		
		liver	>300		
		urine	>20,000		
	24	aorta blood	62		
		urine	>5,000		
		liver	594		
		vena cava blood	45		
	34	aorta blood	119	4.2	40
		iliac vein blood	28		
		liver	1,114		
	44	heart blood	2		
	47	liver	77		
		heart blood	<2		
	50	femoral blood	<2		
Pentedrone	4	aorta blood	<5		
Pentylone	1	urine	122		
	7	iliac vein blood	<5		
		aorta blood	<5		
	8	aorta blood	323	2.0	
		iliac vein blood	160		
		urine	>10,000		
α-PVP	3	aorta blood	15	0.5	
		femoral vessel blood	30		
		urine	>5,000		
	5	aorta blood	4		
		vena cava blood	8		
		urine	38		

(continued)

Cathinone	Case #	Contents	Concentration (ng/mL or ng/g)	C/P	L/P
	7	aorta blood	8	1.0	
		iliac vein blood	8		
	8	aorta blood	218	0.9	
		iliac vein blood	234		
		urine	7,580		
	9	subclavian vessel blood	4		
		urine	853		
		femoral vessel blood	<2		
	10	aorta blood	3	1.5	
		peripheral blood	2		
		urine	52		
	12	liver	169		
		vena cava blood	3		
		urine	3,846		
	14	aorta blood	41	1.9	
		iliac vein blood	21		
		urine	3,972		
	25	aorta blood	224	1.1	<0.3
		femoral vessel blood	208		
		liver	<60		
	31	aorta blood	1,090	1.1	<0.1
		iliac vein blood	1,019		
		liver	<60		
	32	aorta blood	75		
		urine	33		
		liver	69		
	38	aorta blood	58	1.3	<1.4
		femoral vessel blood	44		
		urine	1,731		

(continued)

Cathinone	Case #	Contents	Concentration (ng/mL or ng/g)	C/P	L/P
		liver	<60		
	40	femoral vessel blood	84	0.9	
		heart blood	79		
	41	urine	598		
		femoral vessel blood	<2		
	42	femoral vessel blood	7		
		aorta blood	<2		
	43	aorta blood	28		
		vena cava blood	18		
		urine	1,254		
	44	femoral blood	3		
	49	heart blood	<2		

Postmortem redistribution

Twenty-one cases contained both a central and peripheral blood source, which allowed C/P ratios to be determined for seven drugs (**Table 6.4**). The average C/P ratio for α-PVP was 1.1 (0.5 - 1.9, n=9). By comparison, average C/P ratios for methylenedioxy-substituted cathinones ethylone and methylone were 2.9 (0.5 - 9.2, n=6) and 4.0 (1.5 - 6.1, n=5), suggesting greater potential for postmortem redistribution. Although conclusions cannot be drawn form single cases, C/P ratios for butylone, MDPV, methedrone and pentylone were 0.7, 1.0, 1.1, and 2.0, respectively.

In a similar fashion, L/P ratios proposed by McIntyre were also evaluated where possible (n=7) (**Table 6.4**). Consistent with the trends observed with methylenedioxy-substituted cathinones, the highest reported L/P ratios were observed with methylone (40), ethylone (20) and butylone (14). However, L/P ratios were highly variable; ethylone L/P

ratios varied by two orders of magnitude, demonstrating the difficulty associated with individual measurements. Although some measurements exceed the proposed L/P threshold of 20-30, average L/P ratios for all of the cathinones fell below this range, suggesting a lower potential for redistribution.

There were nine instances where both a C/P and L/P ratio were determined. Among the three α-PVP cases, C/P ratios were 1.1 - 1.3 and L/P ratios were <1.4. However, C/P and L/P ratios of 1.1 and 10, respectively were observed for methedrone, and 0.7 and 14 for butylone. These findings further reinforce time dependent factors, postmortem interval and other issues that complicate quantitative comparisons. Results were also compared with previously published studies (**Table 6.4**). C/P ratios were in good agreement for some drugs (e.g. MDPV, α-PVP). Although C/P ranges generally overlapped for the remaining cathinones, significant variability was observed. This is perhaps not unexpected given the relatively small populations and limited number of case reports to date.

Table 6.4. Comparison of C/P and L/P ratios with previously published literature. The mean (range) and number of specimens are summarized for cathinones. Methamphetamine and MDMA are shown for comparison.

Drug	**Current Study**		**Previously Published Literature**		
	C/P	**L/P**	**C/P**	**L/P**	**Reference**
Butylone	0.7 n=1	14 n=1		1.7 n=1	(21)
Ethylone	2.9 (0.5 – 9.2) n=6	7.2 (0.2 – 20) n=3*	0.97 n=1	3.6 n=1	(4)
MDPV	1.0 n=1	-	1.3 (0.7 – 1.7) n=6	8.8 (2.2 – 23) n=7	(22, 23, 31)
Methedrone	1.1	10	-	-	-
Methylone	4.0 (1.5 – 6.1) n=5	12.9 (3.1 – 40) (n=2)	1.3 (1.0 – 2.1) n=5	2.6 (1.6 – 3.2) n=4	(24-27, 32)
Pentylone	2.0 n=1	-			-
α-PVP	1.1 (0.5 – 1.9) n=9	<1.4 n=3	1.4 – 1.5* n=1	1.5 (1.1 – 2.9) n=3	(28, 29, 33)

(continued)

Drug	Current Study		Previously Published Literature		
	C/P	L/P	C/P	L/P	Reference
MDMA	-	-	2.7 (0.9 – 4.6) n=7	6.5 (3.1 – 8.5) n=5	(13-17)
Methamphetamine	-	-	2.3 (0.9 – 5.8) n=39	5.5 (1.7 – 9.1) n=19	(7, 11, 12)

*One case report with left and right cardiac chambers sampled.

The synthetic cathinones with calculated C/P and L/P ratios comprised of secondary amines bearing a ring substituent (methedrone) or methylenedioxy group (methylone, ethylone, butylone, pentylone), and tertiary amines (pyrrolidines) with and without a methylenedioxy group (MDPV and α-PVP, respectively) **(Figure 6.3)**. In general, the secondary amine, methylenedioxy-type cathinones had the highest C/P ratios, indicating that this particular sub population of cathinones may be more susceptible to postmortem redistribution. Butylone was the exception however, but the sample size was limited (n=1). The higher C/P ratios associated with the secondary amine methylenedioxy-substituted cathinones is consistent with MDMA (C/P = 2.7) and methamphetamine (C/P = 2.3) **(Table 6.4)**.

A box plot depicting C/P ratios for α-PVP (n=9), methylone (n=5), and ethylone (n=6) is shown in **(Figure 6.4)**. Statistical significance was investigated using a two-sample *t*-test assuming unequal variances. There was no statistical difference (α=0.05) between methylone and ethylone, the two methylenedioxy-substituted cathinones with larger sample sizes ($t(8)=0.44$, $p=0.67$). The C/P ratios for both pyrrolidine derivatives (tertiary amines), indicated a minimal potential for redistribution. Although they are more lipophilic, the pyrrolidines are less basic than their secondary amine counterparts, due to steric hindrance and solvation of ions. Their reduced tendency to undergo PMR is consistent with the fact that basic drugs are believed to be more susceptible to PMR (37-

39). Statistically, there was a significant difference between α-PVP and methylone ($t(3)=2.99$, $p<0.04$), but not between α-PVP and ethylone ($t(5)=1.29$, $p=0.25$).

Synthetic Cathinone Stability

The distribution of cathinones postmortem is complicated by the instability of these beta-keto amphetamines. We previously investigated the stability of synthetic cathinones in blood and urine (40, 41). Cathinone stability is highly pH, temperature, and analyte dependent. Although all samples included in this study were refrigerated following collection, degradation during the postmortem interval is possible and should be considered. Although pyrrolidine derivatives are relatively stable, ring substituted and unsubstituted secondary amines are significantly less stable. Furthermore, since matrix pH is highly variable, it should be expected that while degradation may be minimal in acidic compartments (e.g. stomach), alkaline conditions will accelerate degradation. This becomes more complicated for biological samples with wide-ranging pH values, such as urine (pH 4-8) (42, 43). It is further complicated by changes in pH post-collection or postmortem. While increases of 2 pH units have been documented in stored urine, the pH of postmortem blood may decrease following death. Quantitative results must be interpreted with caution, and conclusions regarding the distribution of cathinones following death must acknowledge the added complexity that arises due to their limited stability.

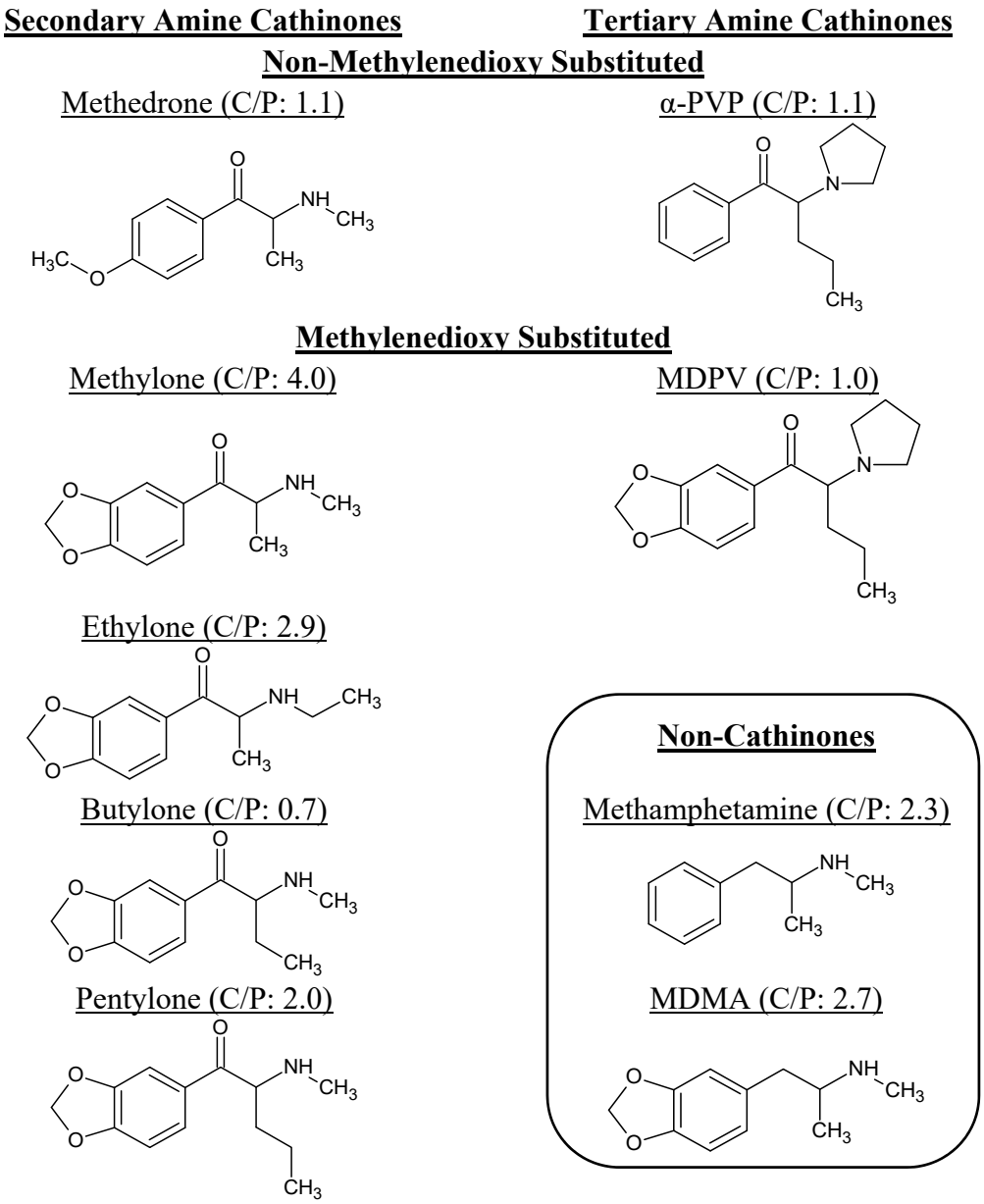

Figure 6.3. Structures and C/P ratios of selected cathinones and non-cathinone species (methamphetamine and MDPV) for comparison.

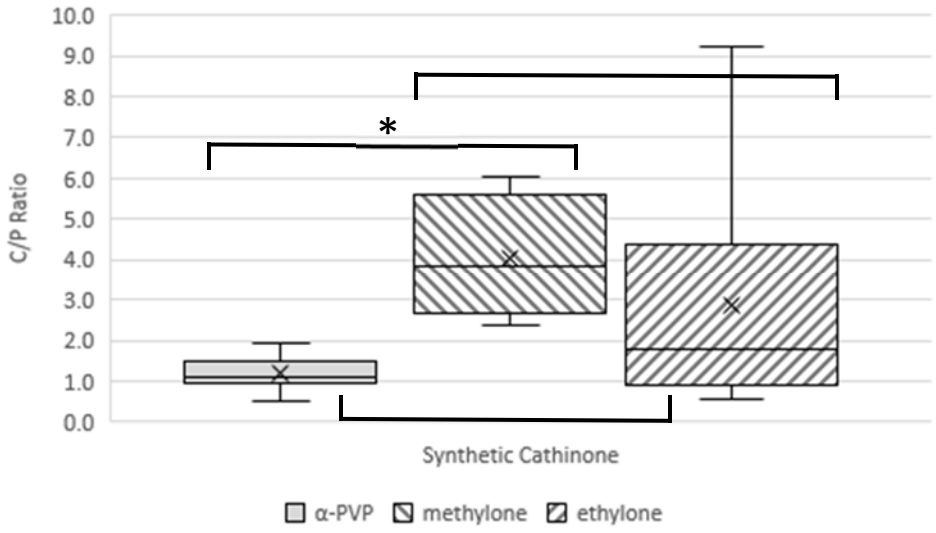

Figure 6.4. Box plots depicting C/P ratios for α-PVP (n=9), methylone (n=5), and ethylone (n=6). Brackets indicate statistical comparisons. Asterisk (*) indicates statistical difference.

Conclusion

The distribution of synthetic cathinones was evaluated in a series of fifty fatalities. C/P and L/P ratios were also evaluated and compared with existing published literature. Although significant variability was observed, cathinones bearing secondary amines and methylenedioxy groups appeared to have the greatest potential for postmortem redistribution. Drugs such as methylone and ethylone may be slightly more susceptible to PMR than either methamphetamine or MDMA. In contrast, pyrrolidines, such as α-PVP and MDPV appear to be less susceptible to PMR.

Pharmacological and physico-chemical properties of the drug can influence postmortem redistribution (8, 44). However, interpretation of postmortem toxicology results is complicated by specimen collection practices, site and time dependent issues, circumstances of death, postmortem interval, decomposition and environmental factors.

While conclusions based on large sample sizes are preferable, published case reports are sometimes limited to single cases or small populations. This is particularly true for new and emerging recreational drugs. Although drug properties can provide insight into a PMR, the tendency of cathinones to degrade in biological evidence further complicates toxicological interpretation.

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References

1. Cook, D. S., Braithwaite, R. A. and Hale, K. A. (2000) Estimating antemortem drug concentrations from postmortem blood samples: The influence of postmortem redistribution. *Journal of Clinical Pathology,* **53**, 282-285.

2. Pounder, D. J. and Jones, G. R. (1990) Post-mortem drug redistribution — a toxicological nightmare. *Forensic Science International,* **45**, 253-263.

3. Jones, G. R. and Pounder, D. J. (1987) Site dependence of drug concentrations in postmortem blood—a case study. *Journal of Analytical Toxicology,* **11**, 186-190.

4. McIntyre, I. M., Hamm, C. E., Sherrard, J. L., Gary, R. D., Burton, C. G. and Mena, O. (2014) Acute 3,4-methylenedioxy-N-ethylcathinone (ethylone) intoxication and related fatality: A case report with postmortem concentrations. *Journal of Analytical Toxicology,* **39**, 225-228.

5. Dinis-Oliveira, R. J., Carvalho, F., Duarte, J. A., Remião, F., Marques, A., Santos, A., *et al.* (2010) Collection of biological samples in forensic toxicology. *Toxicology Mechanisms and Methods,* **20**, 363-414.

6. Drummer, O. H. (2004) Postmortem toxicology of drugs of abuse. *Forensic Science International,* **142**, 101-113.

7. Barnhart, F. E., Fogacci, J. R. and Reed, D. W. (1999) Methamphetamine—a study of postmortem redistribution. *Journal of Analytical Toxicology,* **23**, 69-70.

8. Pélissier-Alicot, A.-L., Gaulier, J.-M., Champsaur, P. and Marquet, P. (2003) Mechanisms underlying postmortem redistribution of drugs: A review. *Journal of Analytical Toxicology,* **27**, 533-544.

9. Skopp, G. (2010) Postmortem toxicology. *Forensic Science, Medicine, and Pathology,* **6**, 314-325.

10. Baselt, R.C. (2017) Disposition of toxic drugs and chemicals in man. 11th ed. Biomedical Publications, Seal Beach, California.

11. Logan, B., Weiss, E. and Harruff, R. (1996) Case report: Distribution of methamphetamine in a massive fatal ingestion. *Journal of Forensic Science,* **41**, 322-323.

12. McIntyre, I., Hamm, C. and Bader, E. (2011) Postmortem methamphetamine distribution. *Journal of Forensic Research,* **2**, 1-3.

13. Elliott, S. P. (2005) Mdma and mda concentrations in antemortem and postmortem specimens in fatalities following hospital admission. *Journal of Analytical Toxicology,* **29**, 296-300.

14. Dams, R., De Letter, E. A., Mortier, K. A., Cordonnier, J. A., Lambert, W. E., Piette, M. H. A., *et al.* (2003) Fatality due to combined use of the designer drugs MDMA and PMA: A distribution study. *Journal of Analytical Toxicology,* **27**, 318-323.

15. Rohrig, T. P. and Prouty, R. W. (1992) Tissue distribution of methylenedioxymethamphetamine. *Journal of Analytical Toxicology,* **16**, 52-53.

16. De Letter, E. A., Bouche, M.-P. L. A., Van Bocxlaer, J. F., Lambert, W. E. and Piette, M. H. A. (2004) Interpretation of a 3,4-methylenedioxymethamphetamine (MDMA) blood level: Discussion by means of a distribution study in two fatalities. *Forensic Science International,* **141**, 85-90.

17. De Letter, E. A., Clauwaert, K. M., Lambert, W. E., Van Bocxlaer, J. F., De Leenheer, A. P. and Piette, M. H. A. (2002) Distribution study of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine in a fatal overdose. *Journal of Analytical Toxicology,* **26**, 113-118.

18. Kennedy, M. C. (2010) Post-mortem drug concentrations. *Internal Medicine Journal,* **40**, 183-187.

19. Zhou, M. J., Bouazzaoui, S., Jones, L. E., Goodrich, P., Bell, S. E. J., Sheldrake, G. N., *et al.* (2015) Isolation and structural determination of non-racemic tertiary cathinone derivatives. *Organic & Biomolecular Chemistry,* **13**, 9629-9636.

20. Gibbons, S. and Zloh, M. (2010) An analysis of the 'legal high' mephedrone. *Bioorganic & Medicinal Chemistry Letters,* **20**, 4135-4139.

21. Rojek, S., Kłys, M., Strona, M., Maciów, M. and Kula, K. (2012) "Legal highs"—toxicity in the clinical and medico-legal aspect as exemplified by suicide with bk-mbdb administration. *Forensic Science International,* **222**, e1-e6.

22. Marinetti, L. J. and Antonides, H. M. (2013) Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: Method development, drug distribution and interpretation of results. *Journal of Analytical Toxicology,* **37**, 135-146.

23. Kesha, K., Boggs, C. L., Ripple, M. G., Allan, C. H., Levine, B., Jufer-Phipps, R., *et al.* (2013) Methylenedioxypyrovalerone ("bath salts"), related death: Case report and review of the literature. *Journal of Forensic Sciences,* **58**, 1654-1659.

24. Shimomura, E. T., Briones, A. J., Warren, W. S., Addison, J. W., Knittel, J. L., Shoemaker, S. A., *et al.* (2016) Case report of methylone, oxymorphone and

ethanol in a fatality case with tissue distribution. *Journal of Analytical Toxicology,* **40**, 543-545.

25. Pearson, J. M., Hargraves, T. L., Hair, L. S., Massucci, C. J., Frazee, C. C., Garg, U., *et al.* (2012) Three fatal intoxications due to methylone. *Journal of Analytical Toxicology,* **36**, 444-451.

26. Cawrse, B. M., Levine, B., Jufer, R. A., Fowler, D. R., Vorce, S. P., Dickson, A. J., *et al.* (2012) Distribution of methylone in four postmortem cases. *Journal of Analytical Toxicology,* **36**, 434-439.

27. Barrios, L., Grison-Hernando, H., Boels, D., Bouquie, R., Monteil-Ganiere, C. and Clement, R. (2016) Death following ingestion of methylone. *International Journal of Legal Medicine,* **130**, 381-385.

28. Potocka-Banaś, B., Janus, T., Majdanik, S., Banaś, T., Dembińska, T. and Borowiak, K. (2017) Fatal intoxication with α-PVP, a synthetic cathinone derivative. *Journal of Forensic Sciences,* **62**, 553-556.

29. Hasegawa, K., Suzuki, O., Wurita, A., Minakata, K., Yamagishi, I., Nozawa, H., *et al.* (2014) Postmortem distribution of α-pyrrolidinovalerophenone and its metabolite in body fluids and solid tissues in a fatal poisoning case measured by LC-MS-MS with the standard addition method. *Forensic Toxicology,* **32**, 225-234.

30. McIntyre, I. M. (2014) Liver and peripheral blood concentration ratio (L/P) as a marker of postmortem drug redistribution: A literature review. *Forensic Science, Medicine, and Pathology,* **10**, 91-96.

31. Wyman, J. F., Lavins, E. S., Engelhart, D., Armstrong, E. J., Snell, K. D., Boggs, P. D., *et al.* (2013) Postmortem tissue distribution of mdpv following lethal intoxication by "bath salts". *Journal of Analytical Toxicology,* **37**, 182-185.

32. McIntyre, I. M., Hamm, C. E., Aldridge, L. and Nelson, C. L. (2013) Acute methylone intoxication in an accidental drowning - a case report. *Forensic Science International,* **231**, e1-3.

33. Sykutera, M., Cychowska, M. and Bloch-Boguslawska, E. (2015) A fatal case of pentedrone and α-pyrrolidinovalerophenone poisoning. *Journal of Analytical Toxicology,* **39**, 324-329.

34. Toxicology, S. W. G. f. F. (2013) Standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology,* **37**, 452-474.

35. Glicksberg, L., Bryand, K. and Kerrigan, S. (2016) Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. *Journal of Chromatography B,* **1035**, 91-103.

36. Adamowicz, P., Tokarczyk, B., Stanaszek, R. and Slopianka, M. (2013) Fatal mephedrone intoxication—a case report. *Journal of Analytical Toxicology,* **37**, 37-42.

37. Dalpe-Scott, M., Degouffe, M., Garbutt, D. and Drost, M. (1995) A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. *Canadian Society of Forensic Science Journal,* **28**, 113-121.

38. Roettger, J. R. (1990) The importance of blood collection site for the determination of basic drugs: A case with fluoxetine and diphenhydramine overdose. *Journal of Analytical Toxicology,* **14**, 191-192.

39. Moriya, F. and Hashimoto, Y. (1999) Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages postmortem. *Journal of Forensic Science,* **44**, 10-16.

40. Glicksberg, L. and Kerrigan, S. (2017) Synthetic cathinone stability in blood. *Journal of Analytical Toxicology,* https://doi.org/10.1093/jat/bkx071.

41. Glicksberg, L. and Kerrigan, S. (in press) Stability of synthetic cathinones in urine. *Journal of Analytical Toxicology.*

42. Fura, A., Harper, T. W., Zhang, H., Fung, L. and Shyu, W. C. (2003) Shift in pH of biological fluids during storage and processing: Effect on bioanalysis. *Journal of Pharmaceutical and Biomedical Analysis,* **32**, 513-522.

43. Cook, J. D., Strauss, K. A., Caplan, Y. H., LoDico, C. P. and Bush, D. M. (2007) Urine pH: The effects of time and temperature after collection. *Journal of Analytical Toxicology,* **31**, 486-496.

44. Ferner, R. E. (2008) Post-mortem clinical pharmacology. *British Journal of Clinical Pharmacology,* **66**, 430-443.

CHAPTER VII

CONCLUSIONS

The synthetic cathinones are powerful psychostimulants that have been associated with impairment, intoxication, and fatal overdose. Forensic toxicology laboratories must be able to identify these new drugs as part of antemortem and postmortem toxicology investigations. Anecdotally, and in a small number of preliminary reports, some of the cathinones have been reported to be unstable. It is important to understand drug stability in biological evidence in order to reliably interpret analytical findings and draw valid conclusions. In this study a systematic and comprehensive approach to evaluate cathinone stability is described in terms of matrix, pH, temperature, concentration, and analyte dependence. This approach will not only aid in the interpretation of toxicological findings, but will also improve our understanding of future designer drugs within the class.

Cathinone stability was evaluated in preserved blood (pH 7) and urine (pH 4 and 8) at two concentrations (100 and 1,000 ng/mL) at four storage temperatures over six months. These were chosen to reflect frozen (-20°C) and refrigerated (4°C) long- and short-term storage temperatures at the laboratory; exposure to ambient (20°C) or room temperature during routine processing and handling; and finally, potential exposure to elevated temperatures during shipping and transport to the laboratory (32°C). A total of nine deuterated internal standards were utilized. All quantitative measurements were performed using liquid chromatography-quadrupole/time of flight mass spectrometry following isolation of the drugs using solid phase extraction. The analytical procedure was fully validated in accordance with the Scientific Working Group for Forensic Toxicology Standard Practices for Method Validation. Extraction efficiencies were 84-104% and 81-

93% in urine and blood, respectively, and limits of quantitation in both matrices were 0.25 – 5 ng/mL. Precision, bias, and matrix effect were all within acceptable thresholds and the assay was free from more than fifty interferences.

Of the twenty-two drugs selected, sixteen were secondary amines. Of these, four were unsubstituted at the benzene ring (methcathinone, ethcathinone, buphedrone, and pentedrone), seven were ring substituted (mephedrone, 4-MEC, 4-EMC, methedrone, 3,4-DMMC, 3-FMC, and 4-FMC) and five were methylenedioxy-substituted (ethylone, methylone, butylone, pentylone, and eutylone). Six tertiary amines (pyrrolidines) were also included, and of these, two were methylenedioxy-substituted.

Drug stability was evaluated to determine analyte, concentration, pH, matrix, and temperature dependence. Although no concentration dependence was observed, cathinone stability was highly analyte dependent. Structural features and substituents within these arylaminoketones exerted significant stabilizing and destabilizing effects. Notably, 3-FMC was the least stable of all of the drugs tested. Significant differences were observed between secondary and tertiary amines. Pyrrolidinyl analogs were inherently more stable, demonstrating far greater resilience than their secondary amine counterparts. With the exception of fluorine substitution, stability within the ring substituted cathinones was not significantly different. Both the unsubstituted and ring substituted cathinones were equally unstable under most conditions. In contrast, however, the methylenedioxy-substituted cathinones were significantly more stable. The stabilizing effect of the methylenedioxy group was observed for both the secondary amines and the pyrrolidines. As a result, cathinone species that contained both a methylenedioxy and a pyrrolidine were the most stable drugs tested. Stability was also highly pH dependent. Cathinones were considerably

more stable under acidic conditions. Degradation of the drug was accelerated dramatically under alkaline conditions, for even the most stable drugs. Significant temperature dependent stability was observed for all cathinones. Exposure to elevated temperature decreased estimated half-lives by several orders of magnitude for some drugs. With the exception of the methylenedioxy-substituted pyrrolidines, significant degradation was observed for all drugs within hours following exposure to elevated temperatures (32ºC). With the exception of 3-FMC, cathinones were stable, or underwent only moderate degradation in blood when frozen. At refrigerated temperatures in blood, all drugs except 3-FMC were stable or underwent moderate losses (<40%) during the first 30 days of storage.

At elevated temperatures in blood, all of the cathinones demonstrated significant (>20%) loss within 5.5 hours (3-FMC) to 7 days (for the most stable methylenedioxy-substituted pyrrolidines). At refrigerated temperature, significant losses were seen within 7 days to more than five months in blood, and 1 day to more than six months in pH 8 urine. These results highlight the critical role of chemical structure among these complex arylaminoketones. Although frozen temperatures provided the greatest protection from loss, this is not necessarily feasible in many laboratories, except for long-term storage. Studies using fortified matrix show that exposure of biological evidence to elevated or ambient temperatures can significantly decrease concentrations over time.

High resolution mass spectrometry was used to investigate possible decomposition products. Although acidic breakdown products have been proposed (particularly for cathinones bearing a secondary amine), identification was precluded due to the nature of

the (alkaline) solid phase extraction. However, *N*-oxide and 2”-oxo degradation products were identified for all tertiary amine cathinones.

Experimental results using fortified, preserved matrix were confirmed using authentic urine specimens (n=180) from cathinone users in accordance with an IRB-approved protocol. Urinary pH ranged from pH 4.5–10 following 5 to 17 months of refrigerated storage. The 180 specimens yielded a total of 164 cathinone positive findings. Of these, quantitative comparisons were made in 156 instances. Results using authentic urine samples from cathinone users were in good agreement with experimentally determined stability data using fortified matrix. This data also underscored the critical importance of specimen pH on overall drug stability. Moreover, the limited degradation of some drugs following extended periods of storage suggest that pH dependent variables were equally as important as conventional time dependent interpretation of drug stability.

Cathinones were also identified in a series of fifty cathinone-positive fatalities. The distribution and postmortem distribution was assessed using central and peripheral blood, urine, vitreous humor, liver, and stomach contents. When cases included either a central blood or liver specimen, together with a peripheral blood sample, central to peripheral (C/P) and liver to peripheral (L/P) ratios were determined (n=21 C/P, n=11 L/P). These were used to evaluate PMR for seven synthetic cathinones, including α-PVP, MDPV, methedrone, butylone, ethylone, methylone, and pentylone. In general, cathinones appeared to exhibit low to moderate PMR (similar to methamphetamine and MDMA). However, cathinones bearing a secondary amine and a methylenedioxy group produced the highest C/P ratios, possibly suggesting greater potential for PMR. In contrast, the lowest C/P ratios were observed for tertiary amines bearing a pyrrolidine group. Differences in

C/P ratios that may arise from these structural characteristics were statistically significant ($\alpha = 0.05$) for α-PVP and methylone.

Forensic toxicology specimens may be subjected to a variety of conditions during sample transport, shipping, storage, and analysis that may cause drug concentrations to change considerably between the time of collection and the time of analysis. Furthermore, information pertaining to synthetic cathinones is still somewhat limited because not all forensic toxicology laboratories routinely screen for these drugs. In order for toxicological results to be reliably interpreted in forensic investigations, factors that influence drug stability must be considered.

With the continued emergence of novel psychoactive substances, it is important to understand pre-analytical factors that may contribute to changes in concentration. The stability of a drug in biological evidence can significantly impact the detection and interpretation of forensic toxicology results in antemortem and postmortem investigations. This research provides the forensic toxicological community with a comprehensive understanding of synthetic cathinone stability which will aid in the interpretation of results. As this class of novel psychoactive substances continues to evolve and grow, the structural influences that impact cathinone stability provides valuable insight into new or yet to be discovered illicit cathinones.

REFERENCES

Adamowicz, P., Gieron, J., Gil, D., Lechowicz, W., Skulska, A. and Tokarczyk, B. (2016) The prevalence of new psychoactive substances in biological material - a three-year review of casework in Poland. *Drug Testing and Analysis,* **8**, 63-70.

Adamowicz, P., Tokarczyk, B., Stanaszek, R. and Slopianka, M. (2013) Fatal mephedrone intoxication—a case report. *Journal of Analytical Toxicology,* **37**, 37-42.

Adamowicz, P. and Tokarczyk, B. (2016) Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. *Drug Testing and Analysis,* **8**, 652-667.

Adamowicz, P., Gieroń, J., Gil, D., Lechowicz, W., Skulska, A., Tokarczyk, B.*, et al.* (2016) Blood concentrations of α-pyrrolidinovalerophenone (α-PVP) determined in 66 forensic samples. *Forensic Toxicology,* **34**, 227-234.

Adamowicz, P., Gil, D., Skulska, A. and Tokarczyk, B. (2013) Analysis of MDPV in blood—determination and interpretation. *Journal of Analytical Toxicology,* **37**, 308-312.

Allegretti, P., de las Mercedes, S., Castro, E. and Furlong, J. (2007) Tautomeric equilibria studies by mass spectrometry. *World Journal of Chemistry,* **2**, 25-62.

Al-Saffar, Y., Stephanson, N. N. and Beck, O. (2013) Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine—experience from the Swedish population. *Journal of Chromatography B,* **930**, 112-120.

Alvarez, J. C., Etting, I., Abe, E., Villa, A. and Fabresse, N. (2017) Identification and quantification of 4-methylethcathinone (4-MEC) and 3,4-methylenedioxypyrovalerone (MDPV) in hair by LC-MS/MS after chronic administration. *Forensic Science International,* **270**, 39-45.

Ammann D., McLaren J.M., Gerostamoulos D., Beyer J. (2012) Detection and quantification of new designer drugs in human blood: part 2 – designer cathinones. *Journal of Analytical Toxicology*, **36**, 381-389.

Anizan, S., Ellefsen, K., Concheiro, M., Suzuki, M., Rice, K. C., Baumann, M. H.*, et al.* (2014) 3, 4-methylenedioxypyrovalerone (MDPV) and metabolites quantification in human and rat plasma by liquid chromatography–high resolution mass spectrometry. *Analytica Chimica Acta,* **827**, 54-63.

Apple, F. S. (2011) A better understanding of the interpretation of postmortem blood drug concentrations. *Journal of Analytical Toxicology,* **35**, 381-383.

Araújo, A. M., Valente, M. J., Carvalho, M., Dias da Silva, D., Gaspar, H., Carvalho, F.*, et al.* (2015) Raising awareness of new psychoactive substances: Chemical analysis and in vitro toxicity screening of 'legal high' packages containing synthetic cathinones. *Archives of Toxicology,* **89**, 757-771.

Archer R.P. (2009) Fluoromethcathinone, a new substance of abuse. *Forensic Science International*, **185**, 10-20.

Aromatario, M., Bottoni, E., Santoni, M. and Ciallella, C. (2012) New "lethal highs": a case of a deadly cocktail of GHB and mephedrone. *Forensic Science International,* **223**, e38-e41.

Babu, N. (2013) DFT studies of molecular structure, equilibium constant for keto-enol tautomerism and geometrical isomerism (E-Z) of 2-amino-1-phenylpropan-1-one (cathinone). *Advances in Applied Science Research: Pelagia Research Library,* **4**, 147-153.

Balikova, M., Zidkova, M., Oktabec, Z., Maresova, V., Linhart, I., Himl, M.*, et al.* (2013) The abuse of 3,4-methylenedioxypyrrolidinobutyrophenone (MDPBP): a case report. *Journal of Forensic Toxicology and Pharmacology,* 2.

Banks, M. L., Worst, T. J., Rusyniak, D. E. and Sprague, J. E. (2014) Synthetic cathinones ("bath salts"). *The Journal of Emergency Medicine,* **46**, 632-642.

Barnhart, F. E., Fogacci, J. R. and Reed, D. W. (1999) Methamphetamine—a study of postmortem redistribution. *Journal of Analytical Toxicology,* **23**, 69-70.

Barrios, L., Grison-Hernando, H., Boels, D., Bouquie, R., Monteil-Ganiere, C. and Clement, R. (2016) Death following ingestion of methylone. *International Journal of Legal Medicine,* **130**, 381-385.

Baselt, R.C. (2017) *Disposition of Toxic Drugs and Chemicals in Man 11th Edition.* Seal Beach, CA: Biomedical Publications, 2017, Print.

Batisse, A., Fortias, M., Bourgogne, E., Gregoire, M., Sec, I. and Djezzar, S. (2014) Case series of 21 synthetic cathinones abuse. *Journal of Clinical Psychopharmacology,* **34**, 411-413.

Baumann, M. H., Partilla, J. S. and Lehner, K. R. (2013) Psychoactive "bath salts": not so soothing. *European Journal of Pharmacology,* **698**, 1-5.

Belhadj-Tahar, H. and Sadeg, N. (2005) Methcathinone: a new postindustrial drug. *Forensic Science International,* **153**, 99-101.

Berrang, B. D., Lewin, A. H. and Carroll, F. I. (1982) Enantiomeric α-aminopropiophenones (cathinone): preparation and investigation. *The Journal of Organic Chemistry,* **47**, 2643-2647.

Bertol, E., Mari, F., Boscolo Berto, R., Mannaioni, G., Vaiano, F. and Favretto, D. (2014) A mixed MDPV and benzodiazepine intoxication in a chronic drug abuser: Determination of MDPV metabolites by LC-HRMS and discussion of the case. *Forensic Science International,* **243**, 149-155.

Bijlsma, L., Sancho, J. V., Hernández, F. and Niessen, W. M. A. (2011) Fragmentation pathways of drugs of abuse and their metabolites based on QTOF MS/MS and MS^E accurate-mass spectra. *Journal of Mass Spectrometry,* **46**, 865-875.

Birkler, R. I. D., Telving, R., Ingemann-Hansen, O., Charles, A. V., Johannsen, M. m. r. a. d. and Andreasen, M. F. m. r. a. d. (2012) Screening analysis for medicinal drugs and drugs of abuse in whole blood using ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC–TOF-MS)—toxicological findings in cases of alleged sexual assault. *Forensic Science International,* **222**, 154-161.

Borek, H. A. and Holstege, C. P. (2012) Hyperthermia and multiorgan failure after abuse of “bath salts” containing 3,4-methylenedioxypyrovalerone. *Annals of Emergency Medicine,* **60**, 103-105.

Braun, U., Shulgin, A. T. and Braun, G. (1980) Centrally active N-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylenedioxyamphetamine). *Journal of Pharmaceutical Sciences,* **69**, 192-195.

Busardò, F. P., Kyriakou, C., Tittarelli, R., Mannocchi, G., Pantano, F., Santurro, A., *et al.* Assessment of the stability of mephedrone in ante-mortem and post-mortem blood specimens. *Forensic Science International,* **256**, 28-37.

Butzbach, D. M. (2010) The influence of putrefaction and sample storage on post-mortem toxicology results. *Forensic Science, Medicine, and Pathology,* **6**, 35-45.

Cameron, K. N., Kolanos, R., Solis, E., Glennon, R. A. and De Felice, L. J. (2013) Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter. *British Journal of Pharmacology,* **168**, 1750-1757.

Capriola M. (2013) Synthetic cathinone abuse. Clinical Pharmacology, **5**, 109-115.

Carbone, P., Carbone, D. L., Carstairs, S. and Luzi, S. A. (2012) Sudden cardiac death associated with methylone use. *American Journal of Clinical Pathology,* **138**, A320-A320.

Cawrse, B. M., Levine, B., Jufer, R. A., Fowler, D. R., Vorce, S. P., Dickson, A. J., *et al.* (2012) Distribution of methylone in four postmortem cases. *Journal of Analytical Toxicology,* **36**, 434-439.

Chappell, J. S. and Lee, M. M. (2010) Cathinone preservation in khat evidence via drying. *Forensic Science International,* **195**, 108-120.

Chen, X. (2015) Simultaneous determination of four designer drugs and their major metabolites by liquid chromatography–mass spectrometry. *Journal of Chromatography B,* **992**, 1-7.

Chung, H., Lee, J. and Kim, E. (2016) Trends of novel psychoactive substances (NPSs) and their fatal cases. *Forensic Toxicology,* **34**, 1-11.

Concheiro, M., Anizan, S., Ellefsen, K. and Huestis, M. A. (2013) Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. *Analytical and Bioanalytical Chemistry,* **405**, 9437-9448.

Concheiro, M., Castaneto, M., Kronstrand, R. and Huestis, M. A. (2015) Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography–high resolution mass spectrometry and library matching. *Journal of Chromatography A,* **1397**, 32-42.

Cook, D. S., Braithwaite, R. A. and Hale, K. A. (2000) Estimating antemortem drug concentrations from postmortem blood samples: the influence of postmortem redistribution. *Journal of Clinical Pathology,* **53**, 282.

Cook, J. D., Strauss, K. A., Caplan, Y. H., LoDico, C. P. and Bush, D. M. (2007) Urine pH: the effects of time and temperature after collection. *Journal of Analytical Toxicology,* **31**, 486-496.

Coppola M., Mondola R. (2012) Synthetic cathinones: Chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as “bath salts” or “plant food”. *Toxicology Letters*, **211**, 144-149.

Cosbey, S. H., Peters, K. L., Quinn, A. and Bentley, A. (2013) Mephedrone (methylmethcathinone) in toxicology casework: a Northern Ireland perspective. *Journal of Analytical Toxicology,* **37**, 74-82.

Cozzi, N. V., Sievert, M. K., Shulgin, A. T., Jacob, P. and Ruoho, A. E. (1999) Inhibition of plasma membrane monoamine transporters by β-ketoamphetamines. *European Journal of Pharmacology,* **381**, 63-69.

Dal Cason, T. A. (1997) The characterization of some 3,4-methylenedioxycathinone (MDCATH) homologs. *Forensic Science International,* **87**, 9-53.

Dalpe-Scott, M., Degouffe, M., Garbutt, D. and Drost, M. (1995) A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. *Canadian Society of Forensic Science Journal,* **28**, 113-121.

Dams, R., De Letter, E. A., Mortier, K. A., Cordonnier, J. A., Lambert, W. E., Piette, M. H. A.*, et al.* (2003) Fatality due to combined use of the designer drugs MDMA and PMA: a distribution study. *Journal of Analytical Toxicology,* **27**, 318-323.

de Castro A., Lendoiro E., Fernández-Vega H., Steinmeyer S., López-Rivadulla M., Cruz A. (2014) Liquid chromatography tandem mass spectrometry determination of selected synthetic cathinones and two piperazines in oral fluid. Cross reactivity study with an on-site immunoassay device. *Journal of Chromatography A*, **1374**, 93-101.

De Felice, L. J., Glennon, R. A. and Negus, S. S. (2014) Synthetic cathinones: Chemical phylogeny, physiology, and neuropharmacology. *Life Sciences,* **97**, 20-26.

De Letter, E. A., Bouche, M.-P. L. A., Van Bocxlaer, J. F., Lambert, W. E. and Piette, M. H. A. (2004) Interpretation of a 3,4-methylenedioxymethamphetamine (MDMA) blood level: discussion by means of a distribution study in two fatalities. *Forensic Science International,* **141**, 85-90.

De Letter, E. A., Clauwaert, K. M., Lambert, W. E., Van Bocxlaer, J. F., De Leenheer, A. P. and Piette, M. H. A. (2002) Distribution study of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine in a fatal overdose. *Journal of Analytical Toxicology,* **26**, 113-118.

den Hollander, B., Rozov, S., Linden, A.-M., Uusi-Oukari, M., Ojanperä, I. and Korpi, E. R. (2013) Long-term cognitive and neurochemical effects of "bath salt" designer drugs methylone and mephedrone. *Pharmacology Biochemistry and Behavior,* **103**, 501-509.

DeRuiter J., Hayes L., Valaer A., Clark C.R., Noggle F.T. (1994) Methcathinone and Designer Analogues: Synthesis, Stereochemical Analysis, and Analytical Properties. *Journal of Chromatographic Science*, **32**, 552-564.

Derungs, A., Schietzel, S., Meyer, M. R., Maurer, H. H., Krähenbühl, S. and Liechti, M. E. (2011) Sympathomimetic toxicity in a case of analytically confirmed recreational use of naphyrone (naphthylpyrovalerone). *Clinical Toxicology,* **49**, 691-693.

Dickson A.J., Vorce S.P., Levine B., Past M.R. (2010) Multiple-Drug Toxicity Caused by the Coadministration of 4-Methylmethcathinone (Mephedrone) and Heroin. *Journal of Analytical Toxicology*, **34**, 162-168.

Dinis-Oliveira, R. J., Carvalho, F., Duarte, J. A., Remião, F., Marques, A., Santos, A., *et al.* (2010) Collection of biological samples in forensic toxicology. *Toxicology Mechanisms and Methods,* **20**, 363-414.

Drummer, O. H. (2004) Postmortem toxicology of drugs of abuse. *Forensic Science International,* **142**, 101-113.

Dumestre-Toulet, V., Brault, S., Labadie, M. and Penouil-Pucheu, F. (2017) Madness with five dollars: two new cases of non-lethal poisoning flakka (α-PVP). *Toxicologie Analytique et Clinique,* **29**, 111-116.

Ellefsen, K. N., Concheiro, M., Suzuki, M., Rice, K. C., Elmore, J. S., Baumann, M. H., *et al.* (2015) Quantification of methylone and metabolites in rat and human plasma by liquid chromatography-tandem mass spectrometry. *Forensic Toxicology,* **33**, 202-212.

Elliott, S. and Evans, J. (2014) A 3-year review of new psychoactive substances in casework. *Forensic Science International,* **243**, 55-60.

Elliott, S. P. (2005) MDMA and MDA concentrations in antemortem and postmortem specimens in fatalities following hospital admission. *Journal of Analytical Toxicology,* **29**, 296-300.

Elliott, S., Sedefov, R. and Evans-Brown, M. (2017) Assessing the toxicological significance of new psychoactive substances in fatalities. *Drug Testing and Analysis,* 1-7.

Eshleman, A. J., Wolfrum, K. M., Hatfield, M. G., Johnson, R. A., Murphy, K. V. and Janowsky, A. (2013) Substituted methcathinones differ in transporter and receptor interactions. *Biochemical Pharmacology,* **85**, 1803-1815.

European Monitoring Centre for Drugs and Drug Addiction. (2017) European Drug Report 2017: Trends and Developments. Available at http://www.emcdda.europa.eu/.

Ezaki, J., Ro, A., Hasegawa, M. and Kibayashi, K. (2016) Fatal overdose from synthetic cannabinoids and cathinones in Japan: demographics and autopsy findings. *The American Journal of Drug and Alcohol Abuse,* **42**, 520-529.

Ferner, R. E. (2008) Post-mortem clinical pharmacology. *British Journal of Clinical Pharmacology,* **66**, 430-443.

Fornal, E. (2013) Formation of odd-electron product ions in collision-induced fragmentation of electrospray-generated protonated cathinone derivatives: aryl - primary amino ketones. *Rapid Communications in Mass Spectrometry,* **27**, 1858-1866.

Fornal, E. (2013) Identification of substituted cathinones: 3,4-methylenedioxy derivatives by high performance liquid chromatography–quadrupole time of flight mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis,* **81–82**, 13-19.

Fornal, E. (2014) Study of collision-induced dissociation of electrospray-generated protonated cathinones. *Drug Testing and Analysis,* **6**, 705-715.

Forrester, M. B. (2012) Synthetic cathinone exposures reported to Texas poison centers. *The American Journal of Drug and Alcohol Abuse,* **38**, 609-615.

Fura, A., Harper, T. W., Zhang, H., Fung, L. and Shyu, W. C. (2003) Shift in ph of biological fluids during storage and processing: effect on bioanalysis. *Journal of Pharmaceutical and Biomedical Analysis,* **32**, 513-522.

Garrett, E. R., Seyda, K. and Marroum, P. (1991) High performance liquid chromatographic assays of the illicit designer drug "ecstasy", a modified amphetamine, with applications to stability, partitioning and plasma protein binding. *Acta Pharm Nord,* **3**, 9-14.

Gatch, M. B., Dolan, S. B. and Forster, M. J. (2015) Comparative behavioral pharmacology of three pyrrolidine-containing synthetic cathinone derivatives. *Journal of Pharmacology and Experimental Therapuetics,* **354**, 103.

Gerace, E.; Petrarulo, M.; Bison, F.; Salomone, A.; Vincenti, M. (2014) Toxicological findings in a fatal multidrug intoxication involving mephedrone. *Forensic Science International*, **243**, 68-73.

German, C. L., Fleckenstein, A. E. and Hanson, G. R. (2014) Bath salts and synthetic cathinones: an emerging designer drug phenomenon. *Life Sciences,* **97**, 2-8.

Gerostamoulos, D., Beyer, J., Staikos, V., Tayler, P., Woodford, N. and Drummer, O. H. (2012) The effect of the postmortem interval on the redistribution of drugs: A comparison of mortuary admission and autopsy blood specimens. *Forensic Science, Medicine, and Pathology,* **8**, 373-379.

Gibbons, S. and Zloh, M. (2010) An analysis of the 'legal high' mephedrone. *Bioorganic & Medicinal Chemistry Letters,* **20**, 4135-4139.

Gil, D., Adamowicz, P., Skulska, A., Tokarczyk, B. and Stanaszek, R. (2013) Analysis of 4-MEC in biological and non-biological material—three case reports. *Forensic Science International,* **228**, e11-e15.

Glicksberg L., Bryand K., Kerrigan S. (2016) Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. *Journal of Chromatography B*, **1035**, 91-103.

Glicksberg, L.; Kerrigan, S. (2017) Stability of synthetic cathinones in blood. *Journal of Analytical Toxicology*, https://doi.org/10.1093/jat/bkx071.

Glicksberg, L.; Kerrigan, S. (2017) Stability of synthetic cathinones in urine. *Journal of Analytical Toxicology*, in press.

Grapp, M., Kaufmann, C. and Ebbecke, M. (2017) Toxicological investigation of forensic cases related to the designer drug 3,4-methylenedioxypyrovalerone (MDPV): Detection, quantification and studies on human metabolism by GC-MS. *Forensic Science International,* **273**, 1-9.

Gregg, R. A. and Rawls, S. M. (2014) Behavioral pharmacology of designer cathinones: a review of the preclinical literature. *Life Sciences,* **97**, 27-30.

Grueninger, D., Englert, R. (2011) Determination of the amphetamine-like designer drugs methcathinone and 4-methylmethcathinone in urine by LC-MS/MS. *Annales de Toxicologie Analytique,* **23**, 7-14.

Guale, F., Shahreza, S., Walterscheid, J. P., Chen, H.-H., Arndt, C., Kelly, A. T., *et al.* (2013) Validation of LC–TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens. *Journal of Analytical Toxicology,* **37**, 17-24.

Gwak, S., Arroyo-Mora, L. E. and Almirall, J. R. (2015) Qualitative analysis of seized synthetic cannabinoids and synthetic cathinones by gas chromatography triple quadrupole tandem mass spectrometry. *Drug Testing and Analysis,* **7**, 121-130.

Gygi, M. P., Gibb, J. W. and Hanson, G. R. (1996) Methcathinone: an initial study of its effects on monoaminergic systems. *Journal of Pharmacology and Experimental Therapuetics,* **276**, 1066.

Gygi, M. P., Fleckenstein, A. E., Gibb, J. W. and Hanson, G. R. (1997) Role of endogenous dopamine in the neurochemical deficits induced by methcathinone. *Journal of Pharmacology and Experimental Therapuetics,* **283**, 1350-1355.

Hadlock, G. C., Webb, K. M., McFadden, L. M., Chu, P. W., Ellis, J. D., Allen, S. C., *et al.* (2011) 4-methylmethcathinone (mephedrone): Neuropharmacological effects of a designer stimulant of abuse. *Journal of Pharmacology and Experimental Therapuetics S,* **339**, 530-536.

Hagan, K. S. and Reidy, L. (2015) Detection of synthetic cathinones in victims of sexual assault. *Forensic Science International,* **257**, 71-75.

Han, E., Kim, E., Hong, H., Jeong, S., Kim, J., In, S., *et al.* (2012) Evaluation of postmortem redistribution phenomena for commonly encountered drugs. *Forensic Science International,* **219**, 265-271.

Hasegawa, K., Suzuki, O., Wurita, A., Minakata, K., Yamagishi, I., Nozawa, H., *et al.* (2014) Postmortem distribution of α-pyrrolidinovalerophenone and its metabolite in body fluids and solid tissues in a fatal poisoning case measured by LC-MS-MS with the standard addition method. *Forensic Toxicology,* **32**, 225-234.

Helfer, A. G., Turcant, A., Boels, D., Ferec, S., Lelièvre, B., Welter, J., *et al.* (2015) Elucidation of the metabolites of the novel psychoactive substance 4-methyl-n-ethyl-cathinone (4-MEC) in human urine and pooled liver microsomes by GC-MS and LC-HR-MS/MS techniques and of its detectability by GC-MS or LC-MS^n standard screening approaches. *Drug Testing and Analysis,* **7**, 368-375.

Hilberg, T., *et al.* (1999) The extent of postmortem drug redistribution in a rat model. *Journal of Forensic Sciences,* **44**, 956-962.

Huestis, M. A. and Cone, E. J. (2007) Methamphetamine disposition in oral fluid, plasma, and urine. *Annals of the New York Academy of Sciences,* **1098**, 104-121.

Ibáñez, M., Pozo, Ó. J., Sancho, J. V., Orengo, T., Haro, G. and Hernández, F. (2016) Analytical strategy to investigate 3,4-methylenedioxypyrovalerone (MDPV) metabolites in consumers' urine by high-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry,* **408**, 151-164.

Johnson, R. D. and Botch-Jones, S. R. (2013) The stability of four designer drugs: MDPV, mephedrone, BZP and TFMPP in three biological matrices under various storage conditions. *Journal of Analytical Toxicology,* **37**, 51-55.

Jones, G. R. and Pounder, D. J. (1987) Site dependence of drug concentrations in postmortem blood—a case study. *Journal of Analytical Toxicology,* **11**, 186-190.

Joshi, M., Cetroni, B., Camacho, A., Krueger, C. and Midey, A. J. (2014) Analysis of synthetic cathinones and associated psychoactive substances by ion mobility spectrometry. *Forensic Science International,* **244**, 196-206.

Kalix, P. (1981) Cathinone, an alkaloid from khat leaves with an amphetamine-like releasing effect. *Psychopharmacology,* **74**, 269-270.

Kamata, H. T., Shima, N., Zaitsu, K., Kamata, T., Miki, A., Nishikawa, M., *et al.* (2006) Metabolism of the recently encountered designer drug, methylone, in humans and rats. *Xenobiotica,* **36**, 709-723.

Karch, S. B. (2015) Cathinone neurotoxicity ("the 3Ms"). *Current Neuropharmacology,* **13**, 21-25.

Katselou, M., Papoutsis, I., Nikolaou, P., Spiliopoulou, C. and Athanaselis, S. (2016) A-PVP ("flakka"): A new synthetic cathinone invades the drug arena. *Forensic Toxicology,* **34**, 41-50.

Katz, D.P.; Bhattacharya, D.; Bhattacharya, S.; Deruiter, J.; Clark, C.R.; Suppiramanian, V.; et al. (2014) Synthetic cathinones: “A khat and mouse game”. *Toxicology Letters*, **229**, 349-356.

Kelly, J. P. (2011) Cathinone derivatives: a review of their chemistry, pharmacology and toxicology. *Drug Testing and Analysis,* **3**, 439-453.

Kennedy, M. C. (2010) Post-mortem drug concentrations. *Internal Medicine Journal,* **40**, 183-187.

Kerrigan, S. (2013) Sampling, Storage and Stability, in: A. Negrusz, G. Cooper (Eds.), Clarke's Analytical Forensic Toxicology 2 Ed, Pharmaceutical Press, London, England, pp 335-347.

Kerrigan S., Savage M., Cavazos C., Bella P. (2015) Thermal Degradation of Synthetic Cathinones: Implications for Forensic Toxicology. *Journal of Analytical Toxicology*, **40**, 1-11 .

Kesha, K., Boggs, C. L., Ripple, M. G., Allan, C. H., Levine, B., Jufer-Phipps, R.*, et al.* (2013) Methylenedioxypyrovalerone ("bath salts"), related death: case report and review of the literature. *Journal of Forensic Sciences,* **58**, 1654-1659.

Khreit, O. I. G., Grant, M. H., Zhang, T., Henderson, C., Watson, D. G. and Sutcliffe, O. B. (2013) Elucidation of the phase I and phase II metabolic pathways of (±)-4′-methylmethcathinone (4-MMC) and (±)-4′-(trifluoromethyl)methcathinone (4-TFMMC) in rat liver hepatocytes using LC-MS and LC-MS^2. *Journal of Pharmaceutical and Biomedical Analysis,* **72**, 177-185.

Klavž, J., Gorenjak, M. and Marinšek, M. (2016) Suicide attempt with a mix of synthetic cannabinoids and synthetic cathinones: Case report of non-fatal intoxication with

AB-CHMINACA, AB-FUBINACA, alpha-PHP, alpha-PVP and 4-CMC. *Forensic Science International,* **265**, 121-124.

Knoy, J. L., Peterson, B. L. and Couper, F. J. (2014) Suspected impaired driving case involving α-pyrrolidinovalerophenone, methylone and ethylone. *Journal of Analytical Toxicology,* **38**, 615-617.

Kovács, K., Tóth, A. R. and Kereszty, É. M. (2012) A new designer drug: methylone related death. *Orvosi Hetilap,* **153**, 271-276.

Kriikku, P., Wilhelm, L., Schwarz, O. and Rintatalo, J. (2011) New designer drug of abuse: 3, 4-methylenedioxypyrovalerone (MDPV). Findings from apprehended drivers in Finland. *Forensic Science International,* **210**, 195-200.

Lee, D., Chronister, C. W., Hoyer, J. and Goldberger, B. A. (2015) Ethylone-related deaths: toxicological findings. *Journal of Analytical Toxicology,* **39**, 567-571.

Leffler, A. M., Smith, P. B., de Armas, A. and Dorman, F. L. (2014) The analytical investigation of synthetic street drugs containing cathinone analogs. *Forensic Science International,* **234**, 50-56.

Leikin, J. B. and Watson, W. A. (2003) Post-mortem toxicology: what the dead can and cannot tell us. *Journal of Toxicology: Clinical Toxicology,* **41**, 47-56.

Li X., Uboh C.E., Soma L.R., Liu Y., Guan F., Aurand C.R. (2014) Sensitive hydrophilic interaction liquid chromatography/tandem mass spectrometry method for rapid detection, quantification and confirmation of cathinone-derived designer drugs for doping control in equine plasma. *Rapid Communications in Mass Spectrometry*, **28**, 217-229.

Liveri, K., Constantinou, M. A., Afxentiou, M. and Kanari, P. (2016) A fatal intoxication related to MDPV and pentedrone combined with antipsychotic and antidepressant substances in Cyprus. *Forensic Science International,* **265**, 160-165.

Logan, B., Weiss, E. and Harruff, R. (1996) Case report: distribution of methamphetamine in a massive fatal ingestion. *Journal of Forensic Sciences*, **41**, 322-323.

Loi, B., Corkery, J. M., Claridge, H., Goodair, C., Chiappini, S., Gimeno Clemente, C.*, et al.* (2015) Deaths of individuals aged 16–24 years in the UK after using mephedrone. *Human Psychopharmacology: Clinical and Experimental,* **30**, 225-232.

López-Arnau, R., Martínez-Clemente, J., Carbó, M. l., Pubill, D., Escubedo, E. and Camarasa, J. (2013) An integrated pharmacokinetic and pharmacodynamic study of a new drug of abuse, methylone, a synthetic cathinone sold as "bath salts". *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* **45**, 64-72.

Lusthof, K. J., Oosting, R., Maes, A., Verschraagen, M., Dijkhuizen, A. and Sprong, A. G. A. (2011) A case of extreme agitation and death after the use of mephedrone in the Netherlands. *Forensic Science International,* **206**, E93-E95.

Maas, A., Wippich, C., Madea, B. and Hess, C. (2015) Driving under the influence of synthetic phenethylamines: a case series. *International Journal of Legal Medicine,* **129**, 997-1003.

Marinetti, L. J. and Antonides, H. M. (2013) Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method

development, drug distribution and interpretation of results. *Journal of Analytical Toxicology,* **37**, 135-146.

Martínez-Clemente, J., López-Arnau, R., Carbó, M., Pubill, D., Camarasa, J. and Escubedo, E. (2013) Mephedrone pharmacokinetics after intravenous and oral administration in rats: relation to pharmacodynamics. *Psychopharmacology,* **229**, 295-306.

Maskell, P. D., De Paoli, G., Seneviratne, C. and Pounder, D. J. (2011) Mephedrone (4-methylmethcathinone)-related deaths. *Journal of Analytical Toxicology,* **35**, 188-191.

Maskell, P. D., Seetohul, L. N., Livingstone, A. C., Cockburn, A. K., Preece, J. and Pounder, D. J. (2013) Stability of 3,4-methylenedioxymethampetamine (MDMA), 4-methylmethcathinone (mephedrone) and 3-trifluromethylphenylpiperazine (3-TFMPP) in formalin solution. *Journal of Analytical Toxicology,* **37**, 440-446.

Matsuta S., Katagi M., Nishioka H., Kamata H., Sasaki K., Shima N. (2014) Structural characterization of cathinone-type designer drugs by EI mass spectrometry. *Japanese Journal of Forensic Science and Technology,* **19**, 77-89.

McDermott, S. D., Power, J. D., Kavanagh, P. and O'Brien, J. (2011) The analysis of substituted cathinones. part 2: An investigation into the phenylacetone based isomers of 4-methylmethcathinone and n-ethylcathinone. *Forensic Science International,* **212**, 13-21.

McGraw, M. and McGraw, L. Bath salts: not as harmless as they sound. *Journal of Emergency Nursing,* **38**, 582-588.

McIntyre, I. M. (2014) Liver and peripheral blood concentration ratio (L/P) as a marker of postmortem drug redistribution: A literature review. *Forensic Science, Medicine, and Pathology,* **10**, 91-96.

McIntyre, I. M., Hamm, C. E., Aldridge, L. and Nelson, C. L. (2013) Acute methylone intoxication in an accidental drowning - a case report. *Forensic Science International,* **231**, e1-3.

McIntyre, I.M.; Hamm, C.E.; Sherrard, J.L.; Gary, R.D.; Burton, C.G.; Mena, O. (2014) Acute 3,4-methylenedioxy-N-ethylcathinone (Ethylone) intoxication and related fatality: a case report with postmortem concentrations. *Journal of Analytical Toxicology*, **39**, 225-228.

McIntyre, I., Hamm, C. and Bader, E. (2011) Postmortem methamphetamine distribution. *Journal of Forensic Research,* **2**, 1-3.

Meyer, M. R., Wilhelm, J., Peters, F. T. and Maurer, H. H. (2010) Beta-keto amphetamines: Studies on the metabolism of the designer drug mephedrone and toxicological detection of mephedrone, butylone, and methylone in urine using gas chromatography–mass spectrometry. *Analytical & Bioanalytical Chemistry,* **397**, 1225-1233.

Meyer, M. R., Vollmar, C., Schwaninger, A. E., Wolf, E. and Maurer, H. H. (2012) New cathinone-derived designer drugs 3-bromomethcathinone and 3-fluoromethcathinone: Studies on their metabolism in rat urine and human liver microsomes using GC-MS and LC–high-resolution MS and their detectability in urine. *Journal of Mass Spectrometry,* **47**, 253-262.

Meyer, M. R., Du, P., Schuster, F. and 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.

Michaelis, W., Russel, J. H. and Schindler, O. (1970) Metabolism of pyrovalerone hydrochloride. *Journal of Medicinal Chemistry,* **13**, 497-503.

Miller, B., Kim, J. and Concheiro, M. (2017) Stability of synthetic cathinones in oral fluid samples. *Forensic Science International,* **274**, 13-21.

Minakata, K., Yamagishi, I., Nozawa, H., Hasegawa, K., Wurita, A., Gonmori, K., *et al.* (2014) MALDI-TOF mass spectrometric determination of four pyrrolidino cathinones in human blood. *Forensic Toxicology,* **32**, 169-175.

Miotto, K., Striebel, J., Cho, A. K. and Wang, C. (2013) Clinical and pharmacological aspects of bath salt use: a review of the literature and case reports. *Drug and Alcohol Dependence,* **132**, 1-12.

Morad, A., Al-Meshal, I., Nasir, M. and El-Feraly, F. (1989) High-performance liquid chromatographic determination of (−)-cathinone in plasma. *Chromatographia,* **27**, 201-204.

Moriya, F. and Hashimoto, Y. (1999) Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages postmortem. *Journal of Forensic Science,* **44**, 10-16.

Mueller, D. M. and Rentsch, K. M. (2012) Generation of metabolites by an automated online metabolism method using human liver microsomes with subsequent

identification by LC-MS^n, and metabolism of 11 cathinones. *Analytical and Bioanalytical Chemistry,* **402**, 2141-2151.

Murray, B. L., Murphy, C. M. and Beuhler, M. C. (2012) Death following recreational use of designer drug "bath salts" containing 3, 4-methylenedioxypyrovalerone (MDPV). *Journal of Medical Toxicology,* **8**, 69-75.

Namera A., Urabe S., Saito T., Torikoshi-Hatano A., Shiraishi H., Arima Y., et al. (2013) A fatal case of 3,4-methylenedioxypyrovalerone poisoning: coexistence of alpha-pyrrolidinobutiophenone and alpha-pyrrolidinovalerophenone in blood and/or hair. *Forensic Toxicology*, **31**, 338-43.

National Conference of State Legislatures (NCSL), 2015. Synthetic drug threats. Updated 13 Jan 2015: http://www.ncsl.org/research/civil-and-criminal-justice/synthetic-drug-threats.aspx. Last accessed April 2017.

Neifeld, J. R., Regester, L. E., Holler, J. M., Vorce, S. P., Magluilo, J. J., Ramos, G.*, et al.* (2016) Ultrafast screening of synthetic cannabinoids and synthetic cathinones in urine by rapidfire-tandem mass spectrometry. *Journal of Analytical Toxicology,* **40**, 379-387.

Nieddu, M., Burrai, L., Trignano, C. and Boatto, G. (2014) Cross-reactivities of 39 new amphetamine designer drugs on three abuse drugs urinary screening tests. *Forensic Toxicology,* **32**, 132-138.

Nieddu, M., Burrai, L., Baralla, E., Pasciu, V., Varoni, M. V., Briguglio, I.*, et al.* (2016) ELISA detection of 30 new amphetamine designer drugs in whole blood, urine and oral fluid using neogen® "amphetamine" and "methamphetamine/mdma" kits. *Journal of Analytical Toxicology,* **40**, 492-497.

Nielsen, M. K. K., Johansen, S. S., Dalsgaard, P. W. and Linnet, K. (2010) Simultaneous screening and quantification of 52 common pharmaceuticals and drugs of abuse in hair using UPLC–TOF-MS. *Forensic Science International,* **196**, 85-92.

O'Byrne P.M., Kavanagh P.V., McNamara S.M., Stokes S.M. (2013) Screening of stimulants including designer drugs in urine using a liquid chromatography tandem mass spectrometry system. *Journal of Analytical Toxicology*, **37**, 64-73.

Odoardi, S., Romolo, F. S. and Strano-Rossi, S. (2016) A snapshot on NPS in Italy: distribution of drugs in seized materials analysed in an Italian forensic laboratory in the period 2013–2015. *Forensic Science International,* **265**, 116-120.

Ojanperä I.A., Heikman P.K., Rasanen I.J. (2011) Urine analysis of 3, 4-methylenedioxypyrovalerone in opioid-dependent patients by gas chromatography–mass spectrometry. *Therapeutic Drug Monitoring*, **33**, 257-263.

Olesti, E., Pujadas, M., Papaseit, E., Pérez-Mañá, C., Pozo, Ó. J., Farré, M.*, et al.* (2017) GC-MS quantification method for mephedrone in plasma and urine: Application to human pharmacokinetics. *Journal of Analytical Toxicology,* **41**, 100-106.

Paillet-Loilier, M., Cesbron, A., Le Boisselier, R., Bourgine, J. and Debruyne, D. (2014) Emerging drugs of abuse: current perspectives on substituted cathinones. *Substance Abuse and Rehabilitation,* **5**, 37-52.

Palamar, J. J., Acosta, P., Sherman, S., Ompad, D. C. and Cleland, C. M. (2016) Self-reported use of novel psychoactive substances among attendees of electronic dance music venues. *The American Journal of Drug and Alcohol Abuse,* **42**, 624-632.

Pasin D., Bidny S., Fu S. (2015) Analysis of new designer drugs in post-mortem blood using high-resolution mass spectrometry. *Journal of Analytical Toxicology*, **39**, 163-171.

Paul B.D., Cole K.A. (2001) Cathinone (khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. *Journal of Analytical Toxicology*, **25**, 525-530.

Paul M., Ippisch J., Herrmann C., Guber S., Schultis W. (2014) Analysis of new designer drugs and common drugs of abuse in urine by a combined targeted and untargeted LC-HR-QTOFMS approach. *Analytical and Bioanalytical Chemistry*, **406**, 4425-4441.

Pawlik, E., Plässer, G., Mahler, H. and Daldrup, T. (2012) Studies on the phase I metabolism of the new designer drug 3-fluoromethcathinone using rabbit liver slices. *International Journal of Legal Medicine,* **126**, 231-240.

Pearson, J.M.; Hargraves, T.L.; Hair, L.S.; Massucci, C.J.; Frazee, C.C.; Garg, U.; et al. (2012) Three fatal intoxications due to methylone. *Journal of Analytical Toxicology*, **36**, 444-451.

Pedersen, A. J., Dalsgaard, P. W., Rode, A. J., Rasmussen, B. S., Müller, I. B., Johansen, S. S., *et al.* (2013) Screening for illicit and medicinal drugs in whole blood using fully automated SPE and ultra-high-performance liquid chromatography with TOF-MS with data-independent acquisition. *Journal of Separation Science,* **36**, 2081-2089.

Pedersen, A. J., Reitzel, L. A., Johansen, S. S. and Linnet, K. (2013) In vitro metabolism studies on mephedrone and analysis of forensic cases. *Drug Testing and Analysis,* **5**, 430-438.

Pélissier-Alicot, A.-L., Gaulier, J.-M., Champsaur, P. and Marquet, P. (2003) Mechanisms underlying postmortem redistribution of drugs: a review. *Journal of Analytical Toxicology,* **27**, 533-544.

Penders, T. M., Gestring, R. E. and Vilensky, D. A. (2012) Intoxication delirium following use of synthetic cathinone derivatives. *The American Journal of Drug and Alcohol Abuse,* **38**, 616-617.

Peters, F. T., Meyer, M. R., Fritschi, G. and Maurer, H. H. (2005) Studies on the metabolism and toxicological detection of the new designer drug 4′-methyl-α-pyrrolidinobutyrophenone (MPBP) in rat urine using gas chromatography–mass spectrometry. *Journal of Chromatography B,* **824**, 81-91.

Peters, J. R., Keasling, R., Brown, S. D. and Pond, B. B. (2016) Quantification of synthetic cathinones in rat brain using HILIC–ESI-MS/MS. *Journal of Analytical Toxicology,* **40**, 718-725.

Petrie, M., Lynch, K. L., Ekins, S., Chang, J. S., Goetz, R. J., Wu, A. H. B., *et al.* (2013) Cross-reactivity studies and predictive modeling of "bath salts" and other amphetamine-type stimulants with amphetamine screening immunoassays. *Clinical Toxicology,* **51**, 83-91.

Pichini, S. s. p. i. i., Rotolo, M. C., García, J., Girona, N., Leal, L., García-Algar, O., *et al.* (2014) Neonatal withdrawal syndrome after chronic maternal consumption of 4-methylethcathinone. *Forensic Science International,* **245**, e33-e35.

Potocka-Banaś, B.; Janus, T.; Majdanik, S.; Banaś, T.; Dembińska, T.; Borowiak, K. (2017) Fatal intoxication with α-PVP, a synthetic cathinone derivative. *Journal of Forensic Science*, **62**, 553-556.

Pounder, D. J. and Jones, G. R. (1990) Post-mortem drug redistribution — a toxicological nightmare. *Forensic Science International,* **45**, 253-263.

Power J.D., McDermott S.D., Talbot B., O'Brien J.E., Kavanagh P. (2012) The analysis of amphetamine-like cathinone derivatives using positive electrospray ionization with in-source collision-induced dissociation. *Rapid Communications in Mass Spectrometry*, **26**, 2601-2611.

Prosser, J. and Nelson, L. (2012) The toxicology of bath salts: a review of synthetic cathinones. *Journal of Medical Toxicology,* **8**, 33-42.

Rech, M. A., Donahey, E., Cappiello Dziedzic, J. M., Oh, L. and Greenhalgh, E. (2015) New drugs of abuse. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy,* **35**, 189-197.

Regester L.E., Chmiel J.D., Holler J.M., Vorce S.P., Levine B., Bosy T.Z. (2015) Determination of designer drug cross-reactivity on five commercial immunoassay screening kits. *Journal of Analytical Toxicology,* **39**, 144-151.

Roettger, J. R. (1990) The importance of blood collection site for the determination of basic drugs: A case with fluoxetine and diphenhydramine overdose. *Journal of Analytical Toxicology,* **14**, 191-192.

Rohrig, T. P. and Prouty, R. W. (1992) Tissue distribution of methylenedioxymethamphetamine. *Journal of Analytical Toxicology,* **16**, 52-53.

Rojek, S., Kłys, M., Maciów-Głąb, M., Kula, K. and Strona, M. (2014) Cathinones derivatives-related deaths as exemplified by two fatal cases involving methcathinone with 4-methylmethcathinone and 4-methylethcathinone. *Drug Testing and Analysis,* **6**, 770-777.

Rojek, S., Kłys, M., Strona, M., Maciów, M. and Kula, K. (2012) "Legal highs"—toxicity in the clinical and medico-legal aspect as exemplified by suicide with bk-MBDB administration. *Forensic Science International,* **222**, e1-e6.

Rojek, S., Kula, K., Maciów-Głąb, M. and Kłys, M. (2016) New psychoactive substance α-PVP in a traffic accident case. *Forensic Toxicology,* **34**, 403-410.

Rosenbaum, C. D., Carreiro, S. P. and Babu, K. M. (2012) Here today, gone tomorrow…and back again? A review of herbal marijuana alternatives (K2, spice), synthetic cathinones (bath salts), kratom, salvia divinorum, methoxetamine, and piperazines. *Journal of Medical Toxicology,* **8**, 15-32.

Saito, T., Namera, A., Osawa, M., Aoki, H. and Inokuchi, S. (2013) SPME–GC–MS analysis of α-pyrrolidinovaleorophenone in blood in a fatal poisoning case. *Forensic Toxicology,* **31**, 328-332.

Sauer, C., Peters, F. T., Haas, C., Meyer, M. R., Fritschi, G. and Maurer, H. H. (2009) New designer drug α-pyrrolidinovalerophenone (PVP): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. *Journal of Mass Spectrometry,* **44**, 952-964.

Schneir, A., Ly, B. T., Casagrande, K., Darracq, M., Offerman, S. R., Thornton, S., *et al.* (2014) Comprehensive analysis of "bath salts" purchased from California stores and the internet. *Clinical Toxicology,* **52**, 651-658.

Scientific Working Group for Forensic Toxicology (SWGTOX). (2013) Standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*, **37**, 452-474.

Shima, N., Katagi, M., Kamata, H., Matsuta, S., Nakanishi, K., Zaitsu, K., *et al.* (2013) Urinary excretion and metabolism of the newly encountered designer drug 3, 4-dimethylmethcathinone in humans. *Forensic Toxicology,* **31**, 101-112.

Shima, N., Katagi, M., Kamata, H., Matsuta, S., Sasaki, K., Kamata, T., *et al.* (2014) Metabolism of the newly encountered designer drug α-pyrrolidinovalerophenone in humans: Identification and quantitation of urinary metabolites. *Forensic Toxicology,* **32**, 59-67.

Shimomura, E. T., Briones, A. J., Warren, W. S., Addison, J. W., Knittel, J. L., Shoemaker, S. A., *et al.* (2016) Case report of methylone, oxymorphone and ethanol in a fatality case with tissue distribution. *Journal of Analytical Toxicology,* **40**,1-3.

Shin, H.-S., Shin, Y.-S. O., Lee, S. and Park, B.-B. (1996) Detection and identification of pyrovalerone and its hydroxylated metabolite in the rat. *Journal of Analytical Toxicology,* **20**, 568-572.

Simmler, L. D., Buser, T. A., Donzelli, M., Schramm, Y., Dieu, L. H., Huwyler, J., *et al.* (2013) Pharmacological characterization of designer cathinones in vitro. *British Journal of Pharmacology,* **168**, 458-470.

Skopp, G. (2010) Postmortem toxicology. *Forensic Science, Medicine, and Pathology,* **6**, 314-325.

Smith, J. P., Sutcliffe, O. B. and Banks, C. E. (2015) An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). *Analyst,* **140**, 4932-4948.

Smith, P., Cole, R., Hamilton, S., West, K., Morley, S. and Maskell, P. (2016) Reporting two fatalities associated with the use of 4-methylethcathinone (4-MEC) and a review of the literature. *Journal of Analytical Toxicology,* **40**, 553-560.

Soh, Y. N. A. and Elliott, S. (2014) An investigation of the stability of emerging new psychoactive substances. *Drug Testing and Analysis,* **6**, 696-704.

Sorensen 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**, 727-736.

Spiller, H. A., Ryan, M. L., Weston, R. G. and Jansen, J. (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. *Clinical Toxicology,* **49**, 499-505.

Strano-Rossi, S., Cadwallader, A. B., de la Torre, X. and Botre, F. (2010) Toxicological determination and in vitro metabolism of the designer drug methylenedioxypyrovalerone (MDPV) by gas chromatography/mass spectrometry and liquid chromatography/quadrupole time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry,* **24**, 2706-2714.

Sundström, M., Pelander, A., Angerer, V., Hutter, M., Kneisel, S. and Ojanperä, I. (2013) A high-sensitivity ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening

synthetic cannabinoids and other drugs of abuse in urine. *Analytical and Bioanalytical Chemistry,* **405**, 8463-8474.

Swortwood, M. J., Boland, D. M. and DeCaprio, A. P. (2013) Determination of 32 cathinone derivatives and other designer drugs in serum by comprehensive LC-QQQ-MS/MS analysis. *Anal Bioanal Chem,* **405**, 1383-1397.

Swortwood, M. J., Hearn, W. L. and DeCaprio, A. P. (2014) Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays. *Drug Testing and Analysis,* **6**, 716-727.

Sykutera, M. and Bloch-Bogusławska, E. (2015) A fatal case of 3, 4-dimethylmethcathinone poisoning. *Problems of forensic sciences,* **102**, 138-148.

Sykutera, M., Cychowska, M. and Bloch-Boguslawska, E. (2015) A fatal case of pentedrone and α-pyrrolidinovalerophenone poisoning. *Journal of Analytical Toxicology,* **39**, 324-329.

Szendrei, K. (1980) The Chemistry of Khat. *Bulletin on Narcotics,* **32,** 5-36.

Thornton, S. L., Gerona, R. R. and Tomaszewski, C. A. (2012) Psychosis from a bath salt product containing flephedrone and MDPV with serum, urine, and product quantification. *Journal of Medical Toxicology,* **8**, 310-313.

Toennes, S. W. and Kauert, G. F. (2002) Excretion and detection of cathinone, cathine, and phenylpropanolamine in urine after kath chewing. *Clin Chem,* **48**, 1715-1719.

Torrance, H. and Cooper, G. (2010) The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland. *Forensic Science International,* **202**, e62-e63.

Truscott, S. M., Crittenden, N. E., Shaw, M. A., Middleberg, R. A. and Jortani, S. A. (2013) Violent behavior and hallucination in a 32-year-old patient. *Clinical Chemistry,* **59**, 612-615.

Tsujikawa, K., Kuwayama, K., Kanamori, T., Iwata, Y. T. and Inoue, H. (2013) Thermal degradation of alpha-pyrrolidinopentiophenone during injection in gas chromatography/mass spectrometry. *Forensic Science International,* **231**, 296-299.

Tsujikawa, K., Yamamuro, T., Kuwayama, K., Kanamori, T., Iwata, Y. T. and Inoue, H. (2015) Instability of the hydrochloride salts of cathinone derivatives in air. *Forensic Science International,* **248**, 48-54.

Tsujikawa, K., Mikuma, T., Kuwayama, K., Miyaguchi, H., Kanamori, T., Iwata, Y. T., *et al.* (2012) Degradation pathways of 4-methylmethcathinone in alkaline solution and stability of methcathinone analogs in various pH solutions. *Forensic Science International,* **220**, 103-110.

Tyrkkö, E., Pelander, A., Ketola, R. A. and Ojanperä, I. (2013) In silico and in vitro metabolism studies support identification of designer drugs in human urine by liquid chromatography/quadrupole-time-of-flight mass spectrometry. *Analytical and Bioanalytical Chemistry,* **405**, 6697-6709.

U.S. Drug Enforcement Administration. (2011) Schedules of controlled substances: temporary placement of three synthetic cathinones in Schedule I: Final Order. Federal Register, 76, 65371-65375.

U.S. Drug Enforcement Administration. (2014) Schedules of controlled substances: temporary placement of 10 synthetic cathinones into Schedule I: Final order. Federal Register, 79, 12938-12943.

U.S. Drug Enforcement Administration: Diversion Control Division (2011) National forensic laboratory information system special report: Synthetic cannabinoids and synthetic cathinones reported in NFLIS: 2009-2010. Springfield, VA: U.S. Drug

U.S. Drug Enforcement Administration: Diversion Control Division (2011) National forensic laboratory information system special report: Synthetic cannabinoids and synthetic cathinones reported in NFLIS: 2010-2013. Springfield, VA: U.S. Drug Enforcement Administration.

U.S. Drug Enforcement Administration: Diversion Control Division (2016) Synthetic Cannabinoids and Synthetic Cathinones Reported in NFLIS, 2013-2015. Springfield, VA: U..S. Drug Enforcement Administration.

Umebachi, R., Aoki, H., Sugita, M., Taira, T., Wakai, S., Saito, T., *et al.* (2016) Clinical characteristics of α-pyrrolidinovalerophenone (α-PVP) poisoning. *Clinical Toxicology,* **54**, 563-567.

Uralets, V., Rana, S., Morgan, S. and Ross, W. (2014) Testing for designer stimulants: metabolic profiles of 16 synthetic cathinones excreted free in human urine. *Journal of Analytical Toxicology,* **38**, 233-241.

Usui, K., Aramaki, T., Hashiyada, M., Hayashizaki, Y. and Funayama, M. (2014) Quantitative analysis of 3,4-dimethylmethcathinone in blood and urine by liquid chromatography–tandem mass spectrometry in a fatal case. *Legal Medicine,* **16**, 222-226.

Vaiano, F., Busardò, F. P., Palumbo, D., Kyriakou, C., Fioravanti, A., Catalani, V., *et al.* (2016) A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. *Journal of Pharmaceutical and Biomedical Analysis,* **129**, 441-449.

Verstraete, A. G. (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. *Therapeutic Drug Monitoring,* **26**, 200-205.

Warrick, B. J., Hill, M., Hekman, K., Christensen, R., Goetz, R., Casavant, M. J., *et al.* (2013) A 9-state analysis of designer stimulant,"bath salt," hospital visits reported to poison control centers. *Annals of Emergency Medicine,* **62**, 244-251.

Watterson, L. R., Kufahl, P. R., Nemirovsky, N. E., Sewalia, K., Grabenauer, M., Thomas, B. F., *et al.* (2014) Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone (mdpv). *Addiction Biology,* **19**, 165-174.

Westphal, F., Junge, T., Girreser, U., Greibl, W. and Doering, C. (2012) Mass, NMR and IR spectroscopic characterization of pentedrone and pentylone and identification of their isocathinone by-products. *Forensic Science International,* **217**, 157-167.

Wiergowski, M., Woźniak, M. K., Kata, M. and Biziuk, M. (2016) Determination of MDPBP in postmortem blood samples by gas chromatography coupled with mass spectrometry. *Monatshefte für Chemie - Chemical Monthly,* **147**, 1415-1421.

Wikström, M., Thelander, G., Nyström, I. and Kronstrand, R. (2010) Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone). *Journal of Analytical Toxicology,* **34**, 594-598.

Winstock, A. R., Mitcheson, L. R., Deluca, P., Davey, Z., Corazza, O. and Schifano, F. (2011) Mephedrone, new kid for the chop? *Addiction,* **106**, 154-16.

Winstock, A., Mitcheson, L., Ramsey, J., Davies, S., Puchnarewicz, M. and Marsden, J. (2011) Mephedrone: use, subjective effects and health risks. *Addiction,* **106**, 1991-1996.

Wood, D. M. and Dargan, P. I. (2012) Mephedrone (4-methylmethcathinone): what is new in our understanding of its use and toxicity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* **39**, 227-233.

Wood, D., Davies, S., Puchnarewicz, M., Button, J., Archer, R., Ovaska, H.*, et al.* (2010) Recreational use of mephedrone (4-methylmethcathinone, 4-MMC) with associated sympathomimetic toxicity. *Journal of Medical Toxicology,* **6**, 327-330.

Wright T.H., Harris C. (2016) Twenty-One Cases Involving Alpha-Pyrrolidinovalerophenone (α-PVP). *Journal of Analytical Toxicology*, **40**, 396-402.

Wright, T.H.; Cline-Parhamovich, K.; Lajoie, D.; Parsons, L.; Dunn, M.; Ferslew, K.E. (2013) Deaths involving methylenedioxypyrovalerone (MDPV) in upper east Tennessee. *Journal of Forensic Science*, **58**, 1558-1562.

Wyman, J. F., Lavins, E. S., Engelhart, D., Armstrong, E. J., Snell, K. D., Boggs, P. D.*, et al.* (2013) Postmortem tissue distribution of mdpv following lethal intoxication by "bath salts". *Journal of Analytical Toxicology,* **37**, 182-185.

Yarema, M. C. and Becker, C. E. (2005) Key concepts in postmortem drug redistribution. *Clinical Toxicology,* **43**, 235-241.

Zaitsu, K., Katagi, M., Kamata, H. T., Kamata, T., Shima, N., Miki, A., *et al.* (2009) Determination of the metabolites of the new designer drugs bk-MBDB and bk-MDEA in human urine. *Forensic Science International,* **188**, 131-139.

Zawilska J.B., Wojcieszak J. (2013) Designer cathinones—An emerging class of novel recreational drugs. Forensic Science International, **231**, 42-53.

Zawilska, J. B. and Andrzejczak, D. (2015) Next generation of novel psychoactive substances on the horizon – a complex problem to face. *Drug and Alcohol Dependence,* **157**, 1-17.

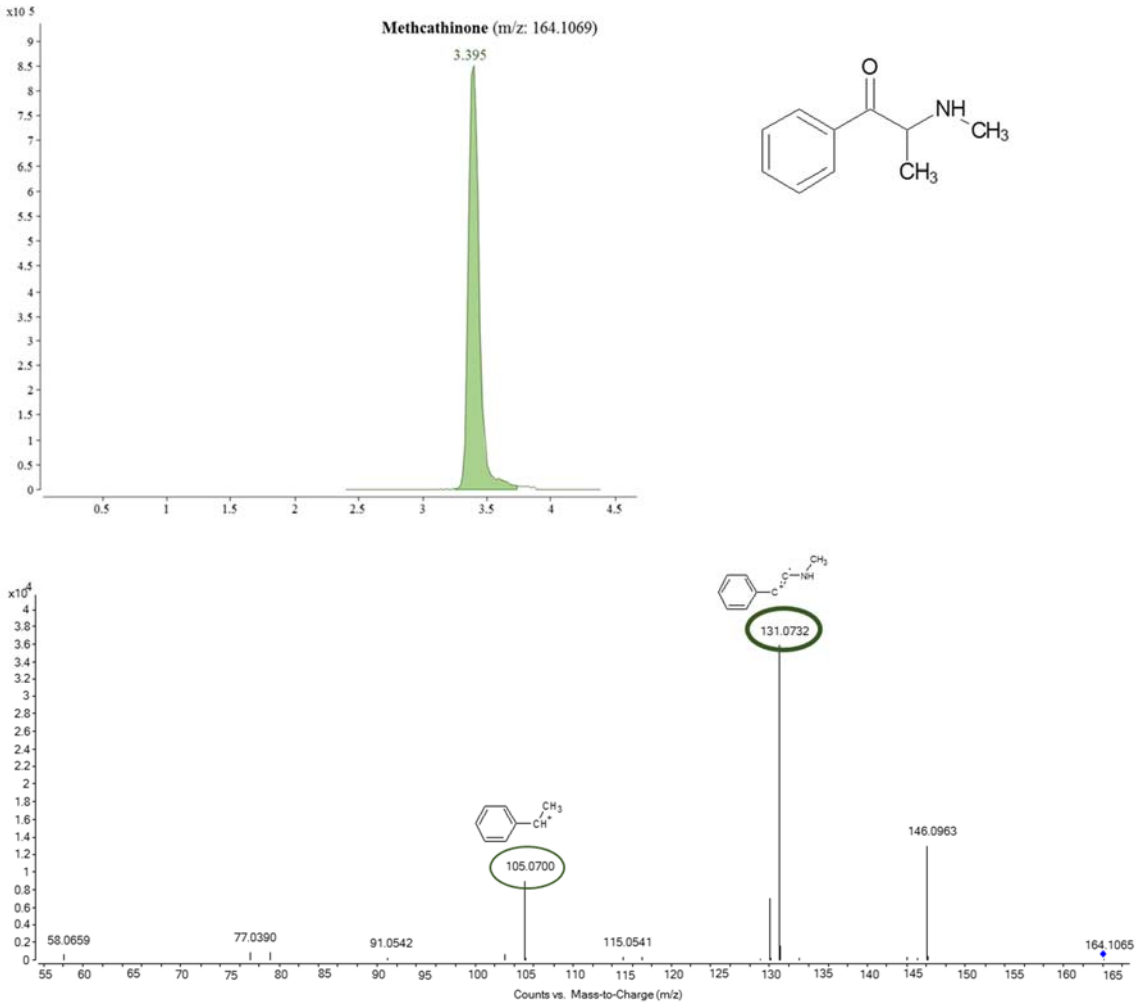
Zhou, M. J., Bouazzaoui, S., Jones, L. E., Goodrich, P., Bell, S. E. J., Sheldrake, G. N., *et al.* (2015) Isolation and structural determination of non-racemic tertiary cathinone derivatives. *Organic & Biomolecular Chemistry,* **13**, 9629-9636.

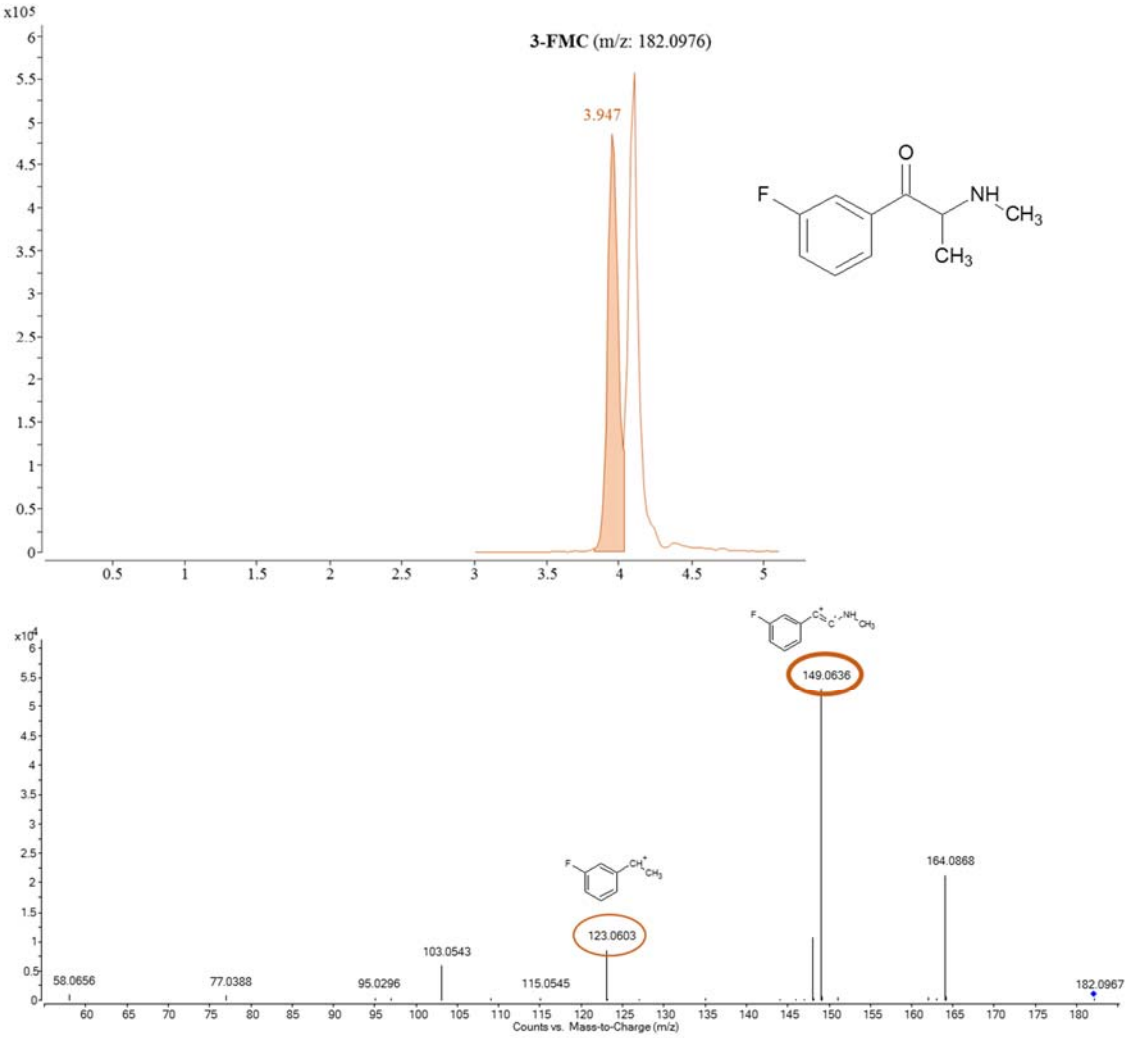
Zuba, D. (2012) Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods. *Trac-Trends in Analytical Chemistry,* **32**, 15-30.

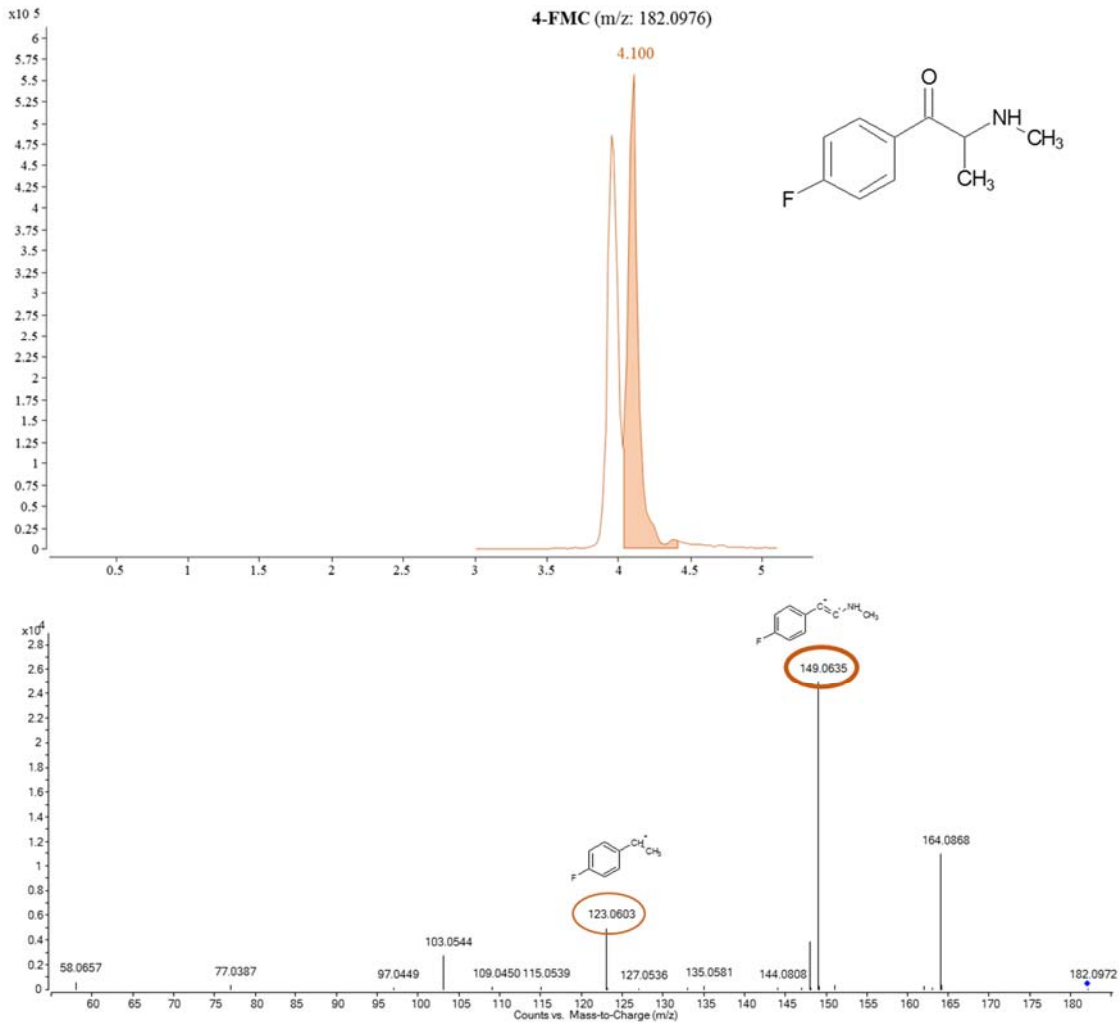
Zuba, D., Adamowicz, P. and Byrska, B. (2013) Detection of buphedrone in biological and non-biological material – two case reports. *Forensic Science International,* **227**, 15-20.

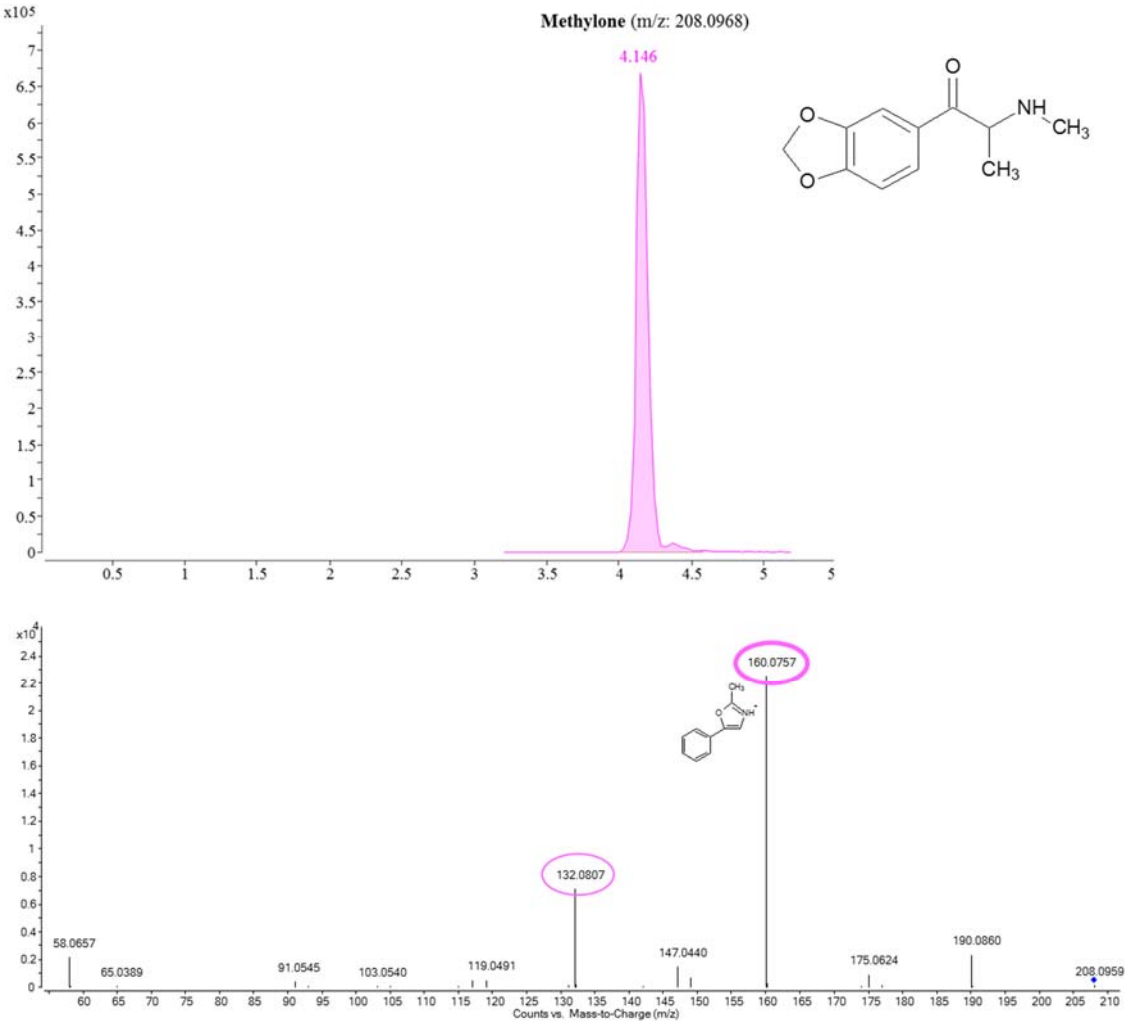
APPENDIX A: INDIVIDUAL EXTRACTED ION CHROMATOGRAMS AND MS/MS SPECTRA FOR TWENTY-TWO SYNTHETIC CATHINONES

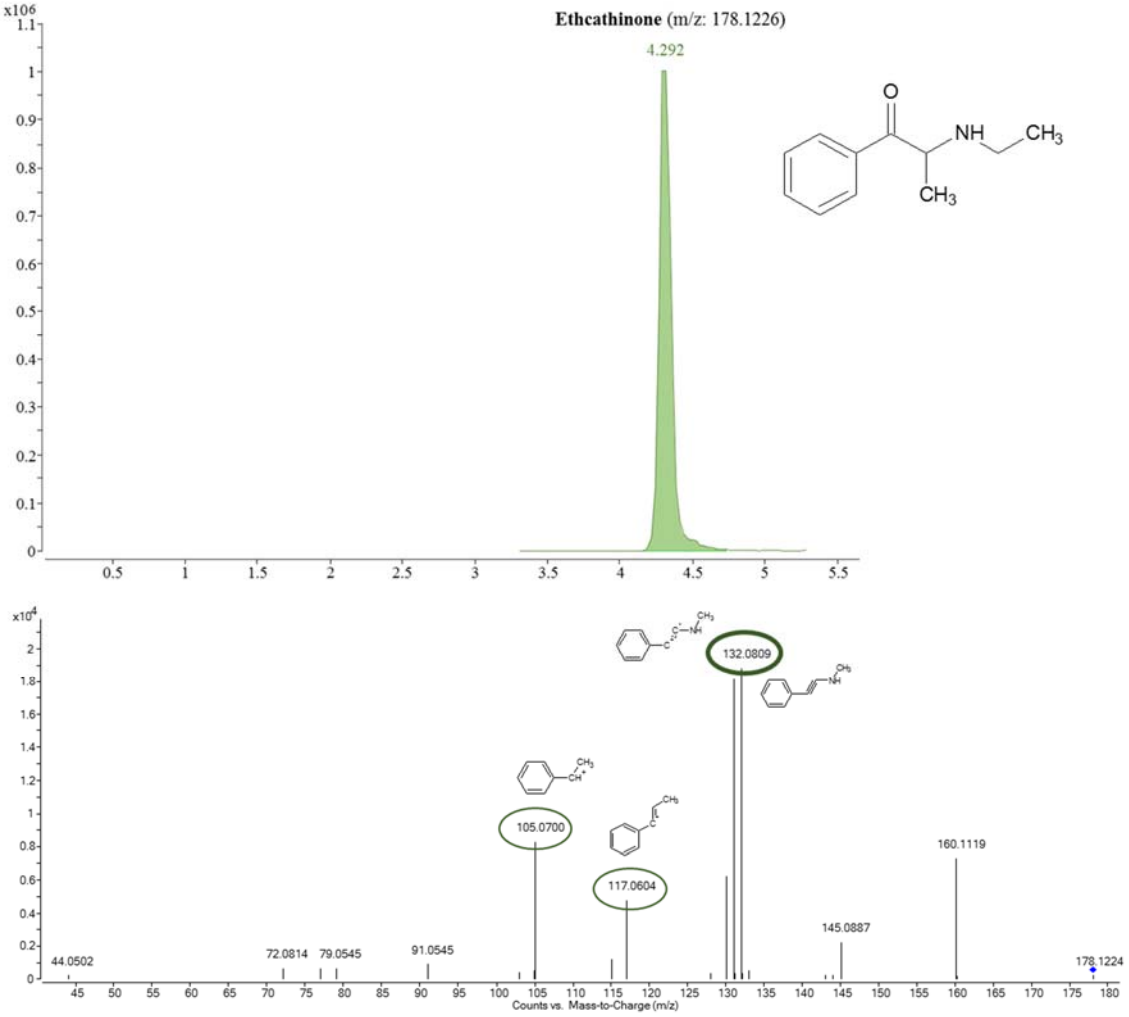
Extracted ion chromatograms (EICs) for twenty-two synthetic cathinones with chemical structure and MS/MS spectra. Quantifier (bold) and qualifier ions identified with predicted fragment structure.

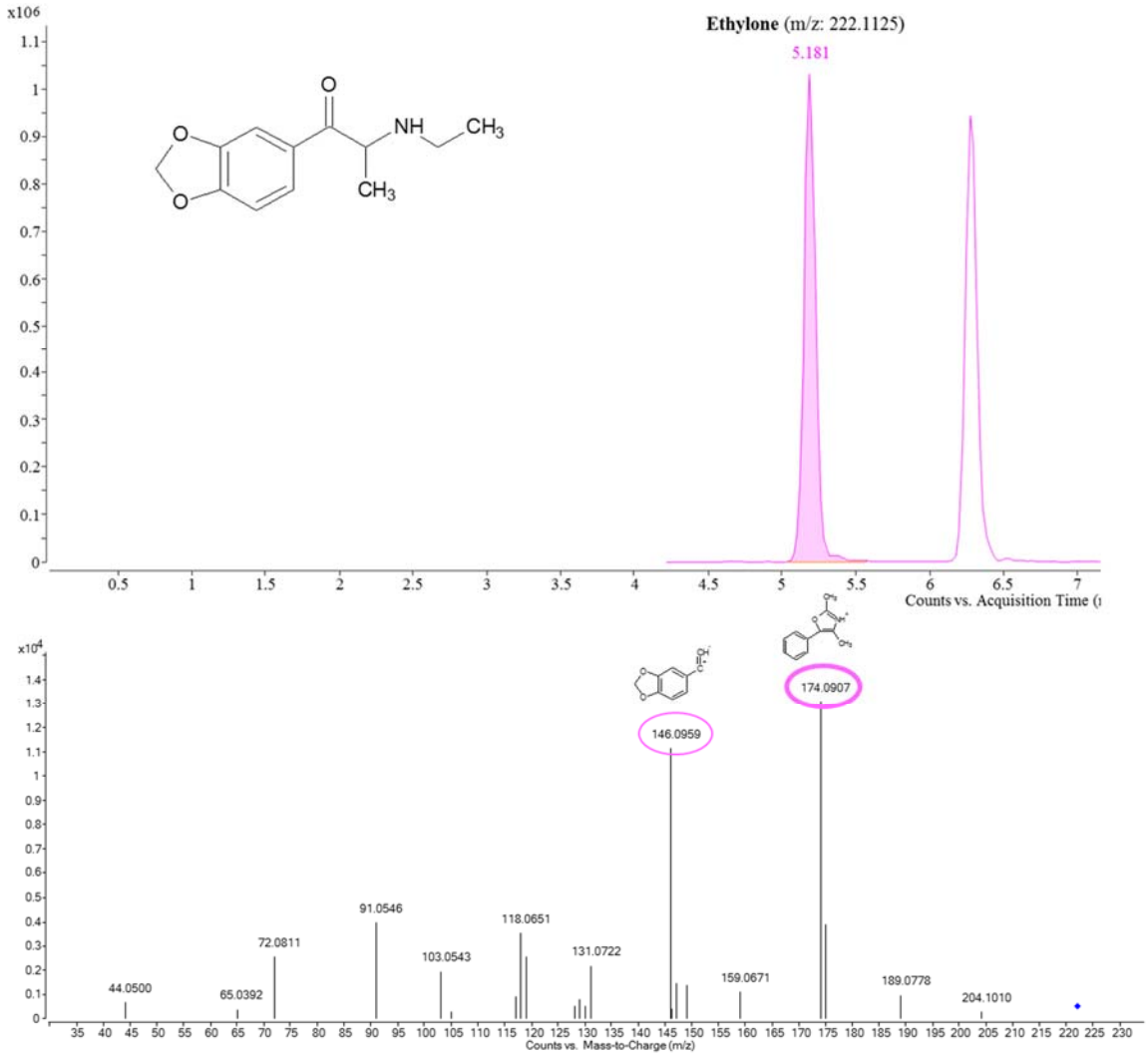


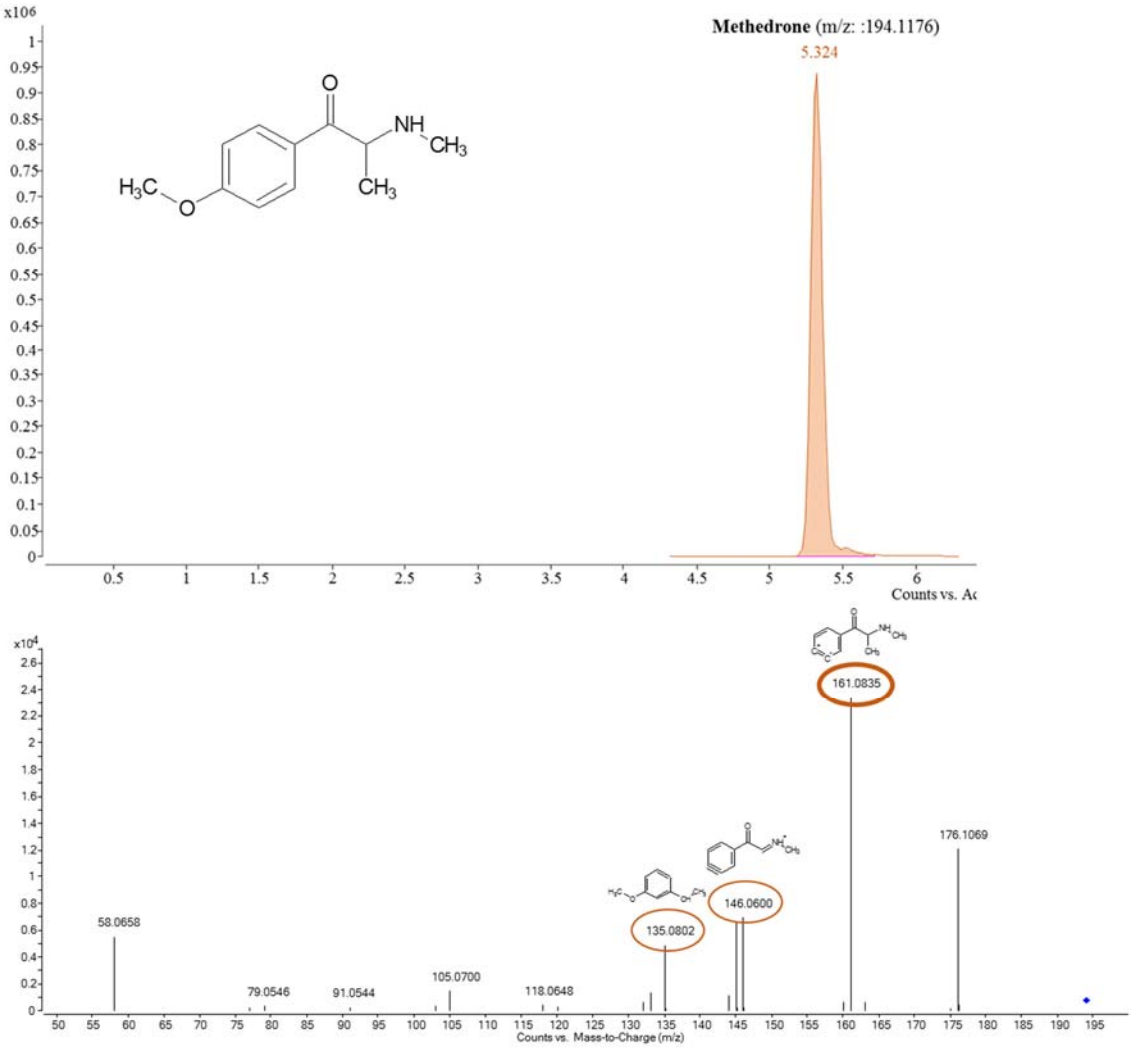

3-FMC (m/z: 182.0976)
3.947
x105
F
O
NH
CH3
CH3
x10^4
149.0636
164.0868
123.0603
103.0543
58.0656
77.0388
95.0296
115.0545
182.0967
Counts vs. Mass-to-Charge (m/z)

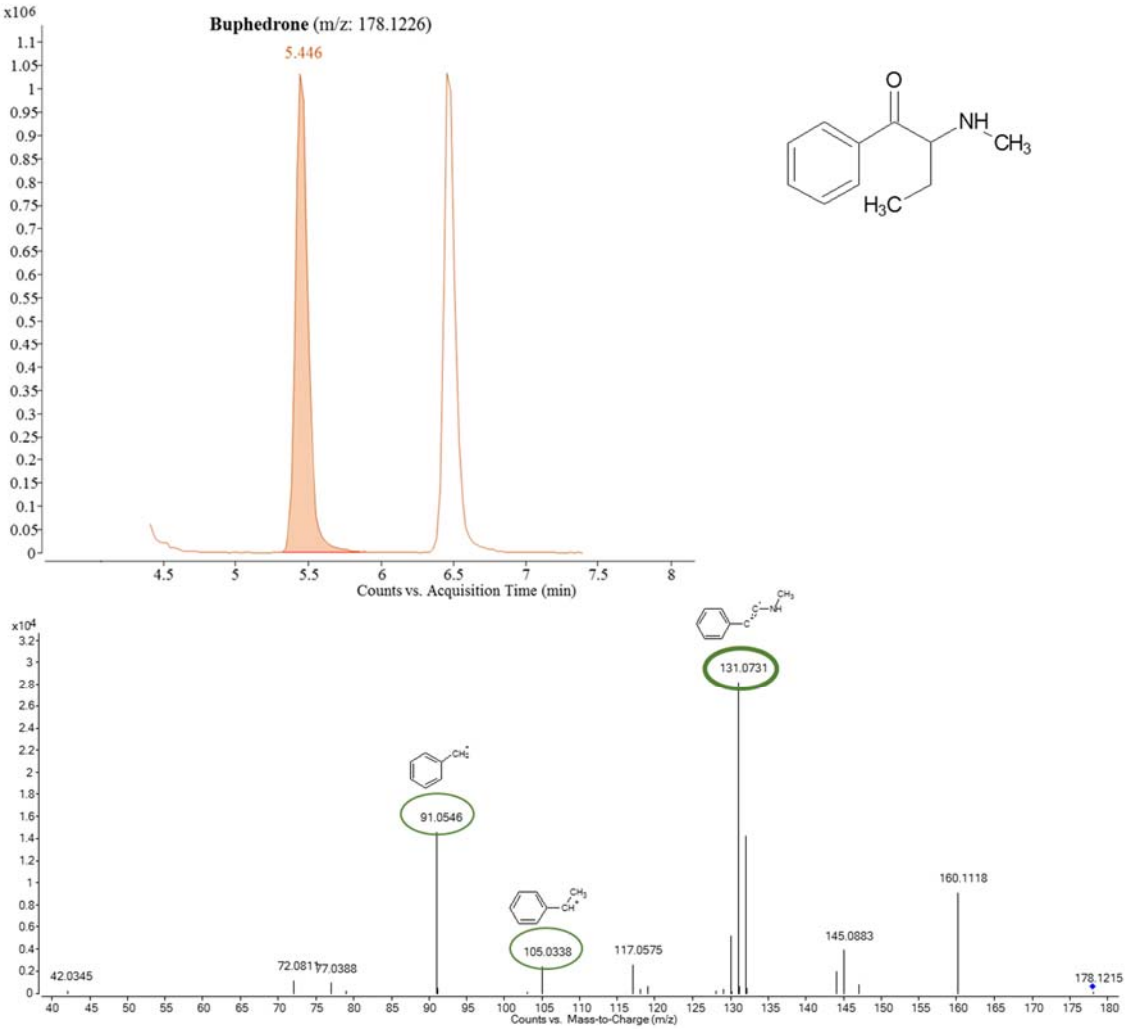

4-FMC (m/z: 182.0976)
4.100
x10 5
O
NH
CH3
CH3
F
x10 4
149.0635
164.0868
123.0603
103.0544
58.0657
77.0387
97.0449
109.0450
115.0539
127.0536
135.0581
144.0808
182.0972
Counts vs. Mass-to-Charge (m/z)

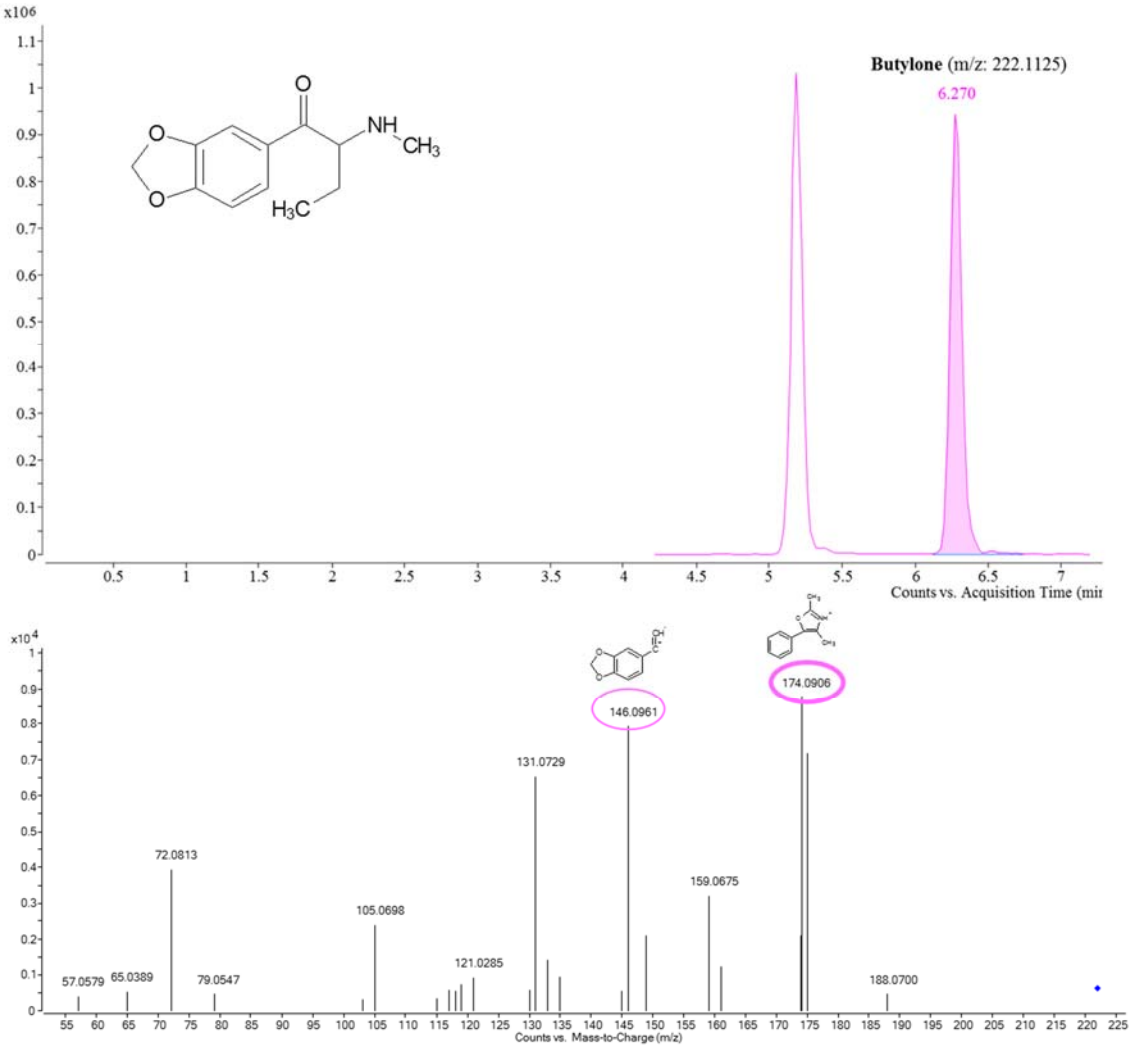

Methylone (m/z: 208.0968)
4.146
x105
O
NH
CH3
CH3
O
O
x10^4
160.0757
132.0807
58.0657
65.0389
91.0545
103.0540
119.0491
147.0440
175.0624
190.0860
208.0959
Counts vs. Mass-to-Charge (m/z)

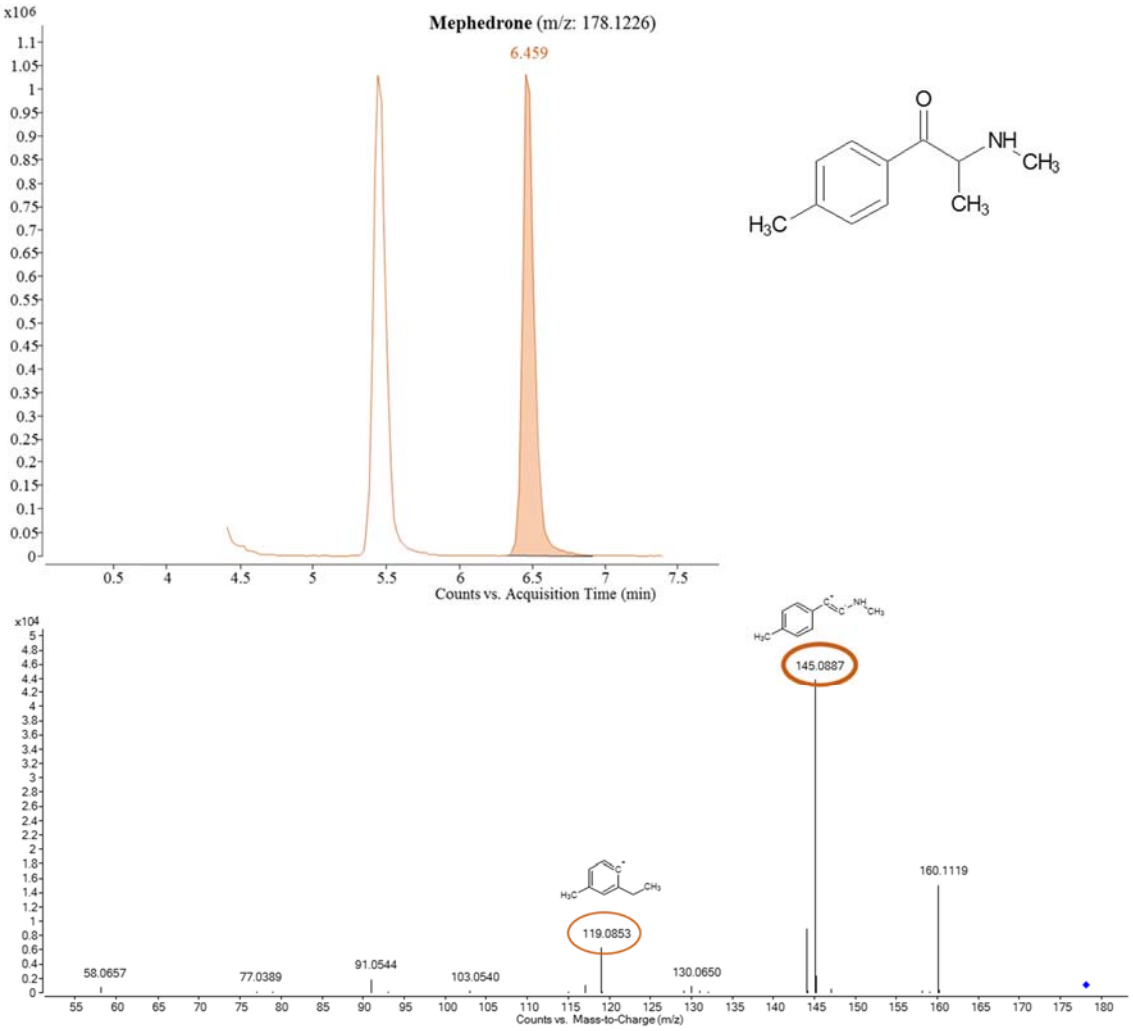

Ethcathinone (m/z: 178.1226)
4.292
x106
O
NH
CH3
CH3
x10^4
132.0809
105.0700
117.0604
160.1119
145.0887
44.0502
72.0814
79.0545
91.0545
178.1224
Counts vs. Mass-to-Charge (m/z)

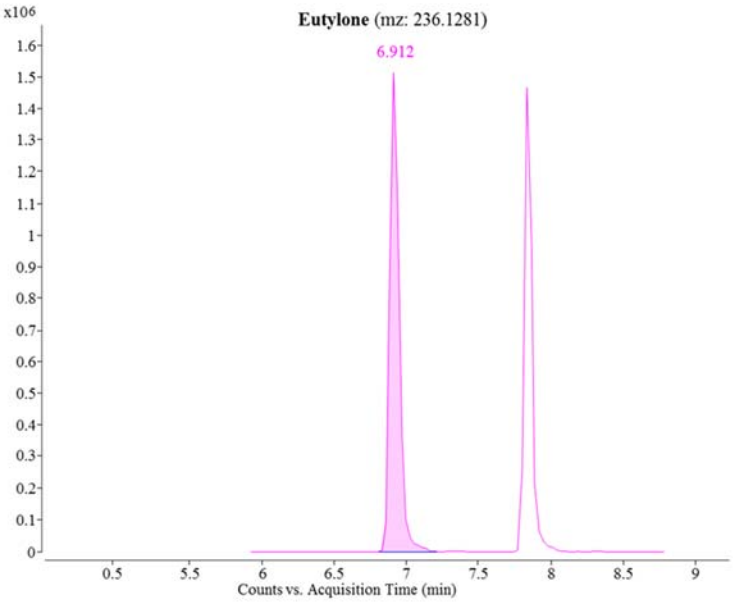

Ethylone (m/z: 222.1125)
5.181
Counts vs. Acquisition Time (
146.0959
174.0907
91.0546
118.0651
72.0811
103.0543
131.0722
44.0500
65.0392
159.0671
189.0778
204.1010
Counts vs. Mass-to-Charge (m/z)

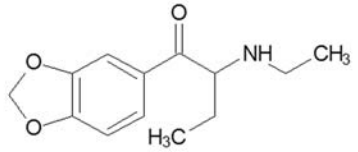

Methedrone (m/z: :194.1176)
5.324
Counts vs. Ac
161.0835
176.1069
146.0600
135.0802
58.0658
79.0546
91.0544
105.0700
118.0648
Counts vs. Mass-to-Charge (m/z)

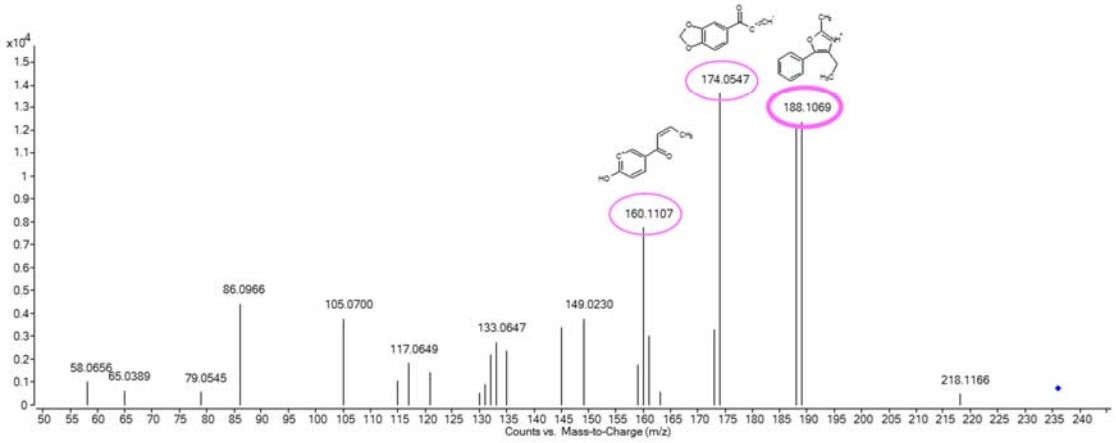

Buphedrone (m/z: 178.1226)
5.446
Counts vs. Acquisition Time (min)
131.0731
91.0546
105.0338
117.0575
145.0883
160.1118
178.1215
42.0345
72.0811
77.0388
Counts vs. Mass-to-Charge (m/z)

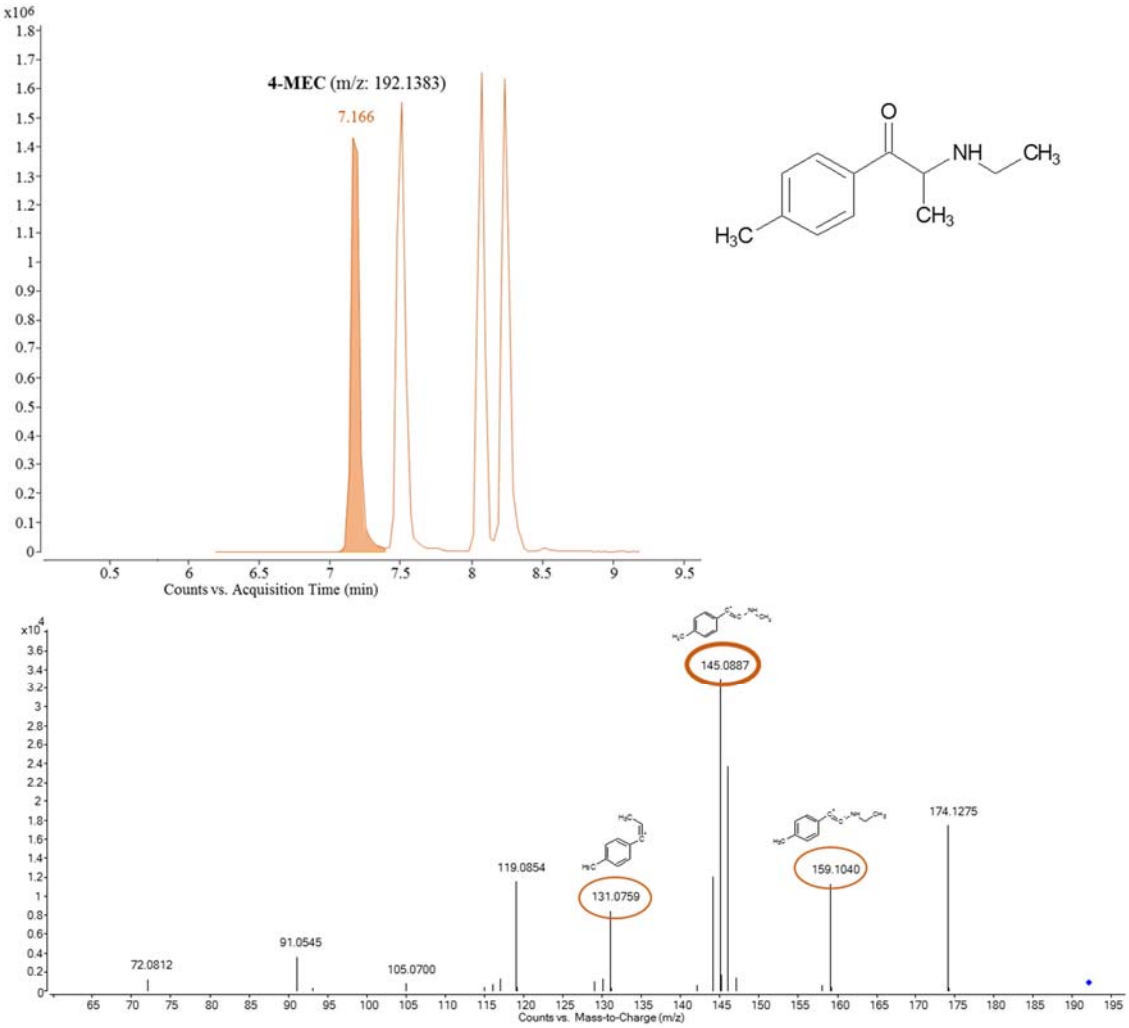

x106
Butylone (m/z: 222.1125)
6.270
Counts vs. Acquisition Time (mi
x10 4
146.0961
174.0906
131.0729
72.0813
159.0675
105.0698
121.0285
57.0579
65.0389
79.0547
188.0700
Counts vs. Mass-to-Charge (m/z)

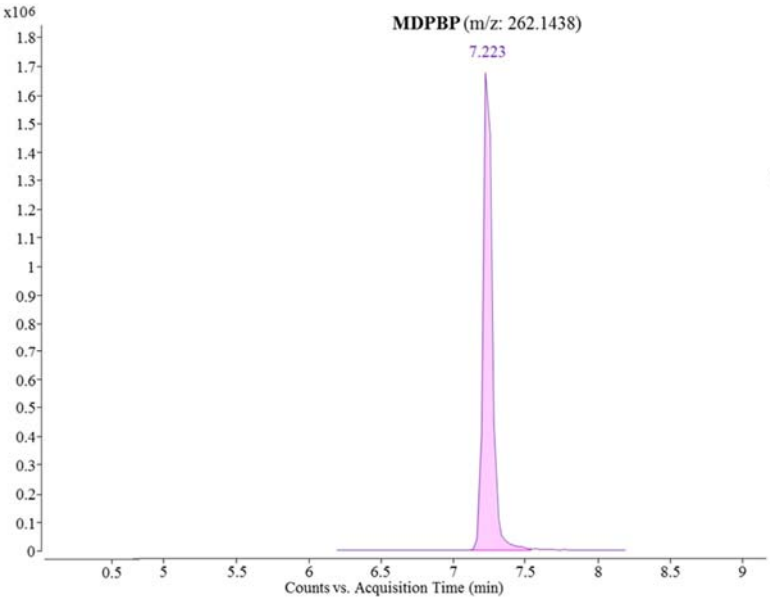

Mephedrone (m/z: 178.1226)
6.459
Counts vs. Acquisition Time (min)
145.0887
160.1119
119.0853
58.0657
77.0389
91.0544
103.0540
130.0650
Counts vs. Mass-to-Charge (m/z)

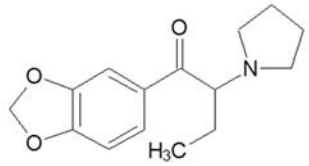

x106
Eutylone (mz: 236.1281)
6.912
Counts vs. Acquisition Time (min)

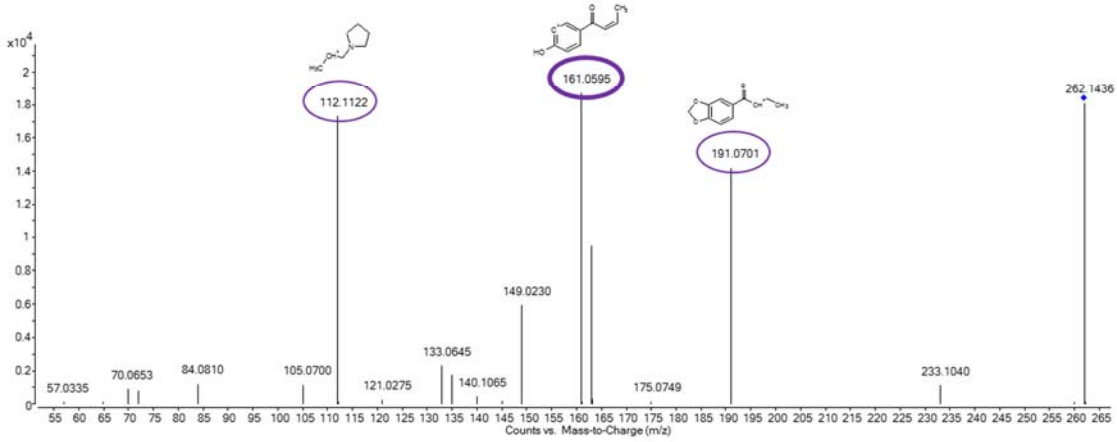

O
NH
CH3
O
O
H3C

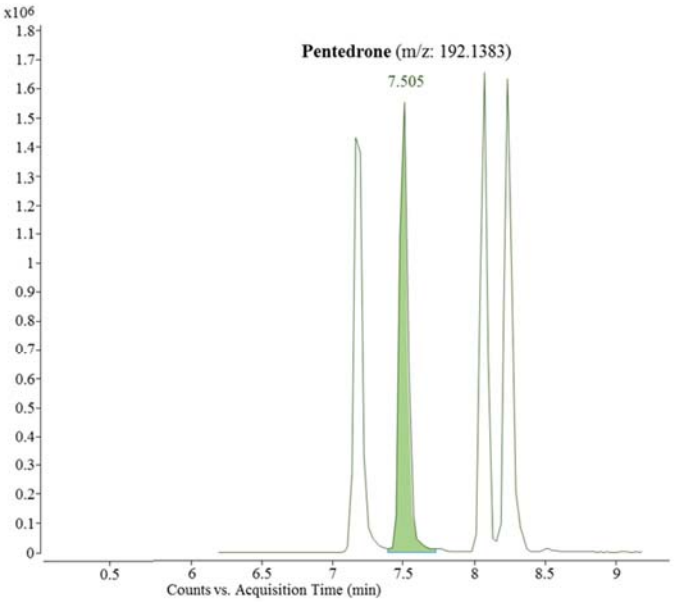

x10^4
174.0547
188.1069
160.1107
86.0966
105.0700
149.0230
133.0647
117.0649
58.0656
65.0389
79.0545
218.1166
Counts vs. Mass-to-Charge (m/z)

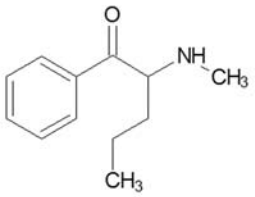

4-MEC (m/z: 192.1383)
7.166
Counts vs. Acquisition Time (min)
145.0887
174.1275
119.0854
131.0759
159.1040
91.0545
72.0812
105.0700
Counts vs. Mass-to-Charge (m/z)

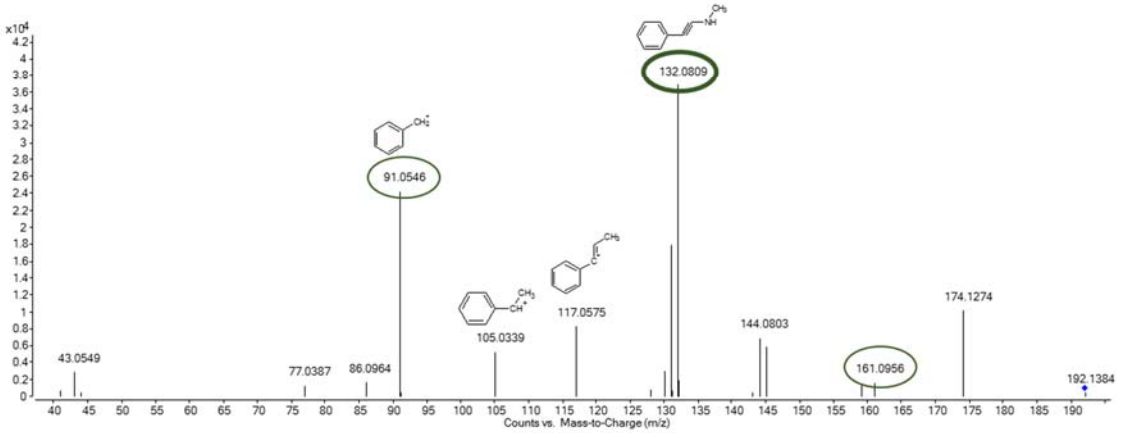

x106
MDPBP (m/z: 262.1438)
7.223
Counts vs. Acquisition Time (min)

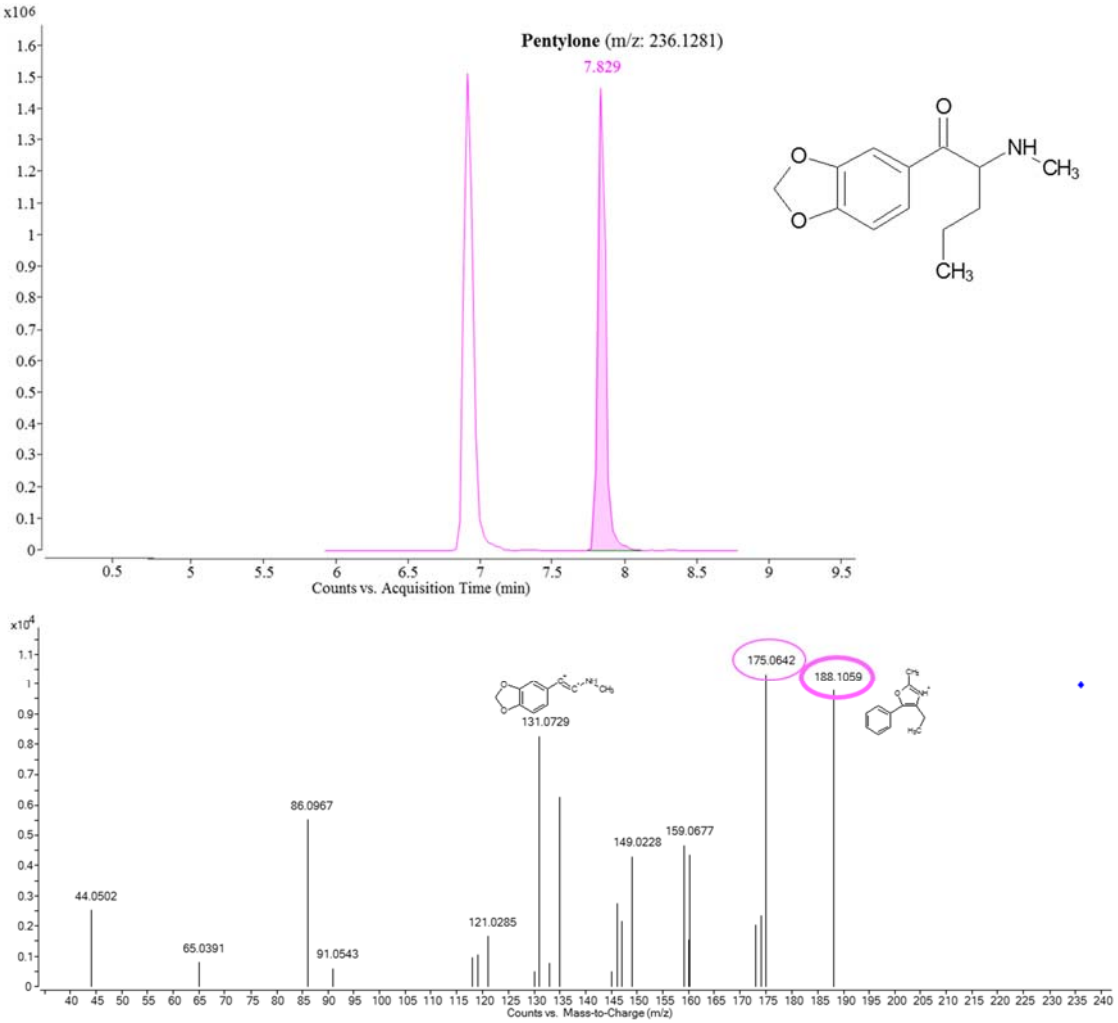

O
N
O
O
H3C

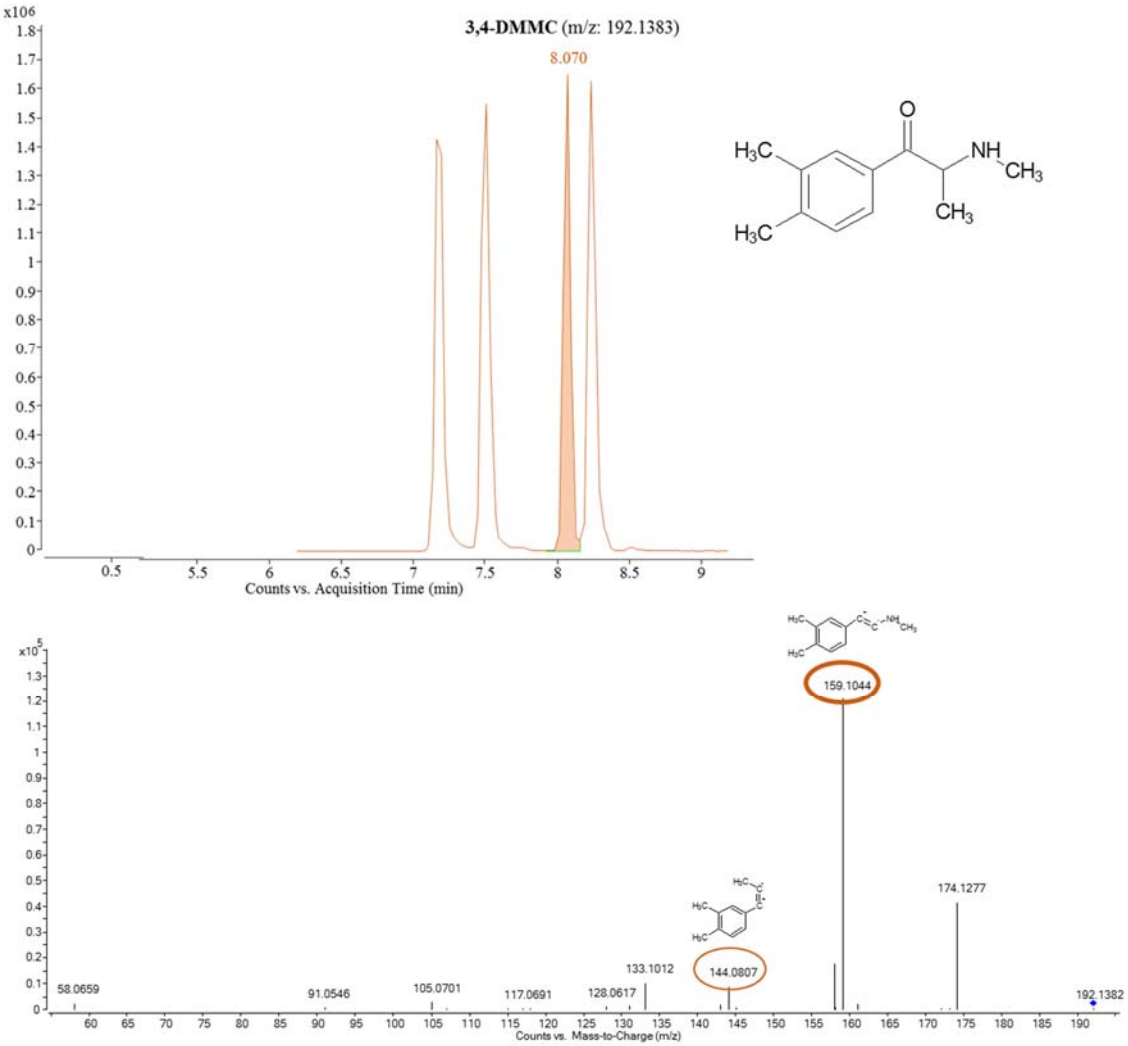

x10^4
112.1122
161.0595
191.0701
262.1436
149.0230
133.0645
57.0335
70.0653
84.0810
105.0700
121.0275
140.1065
175.0749
233.1040
Counts vs. Mass-to-Charge (m/z)

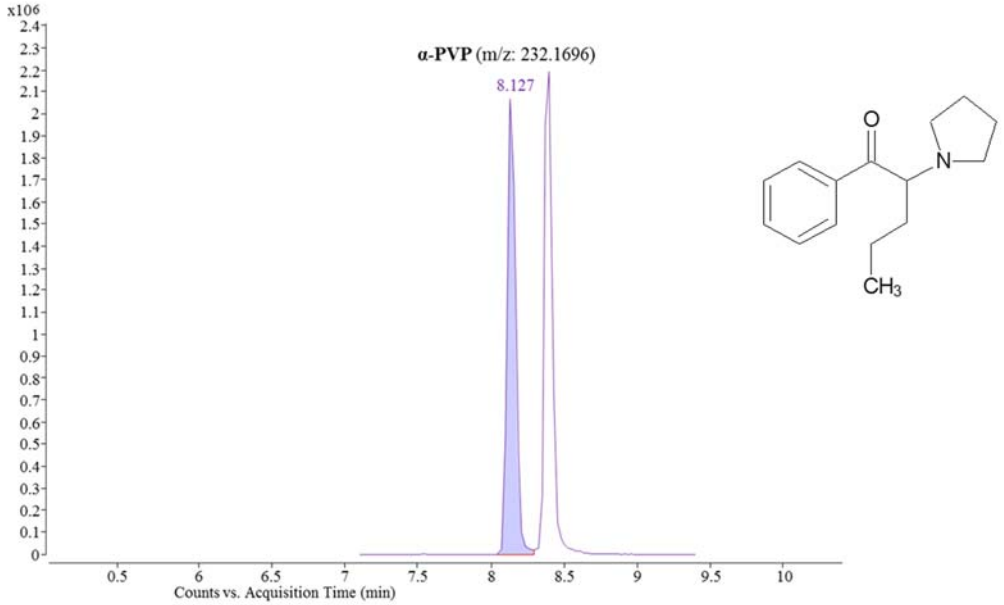

x10^6
Pentedrone (m/z: 192.1383)
7.505
Counts vs. Acquisition Time (min)

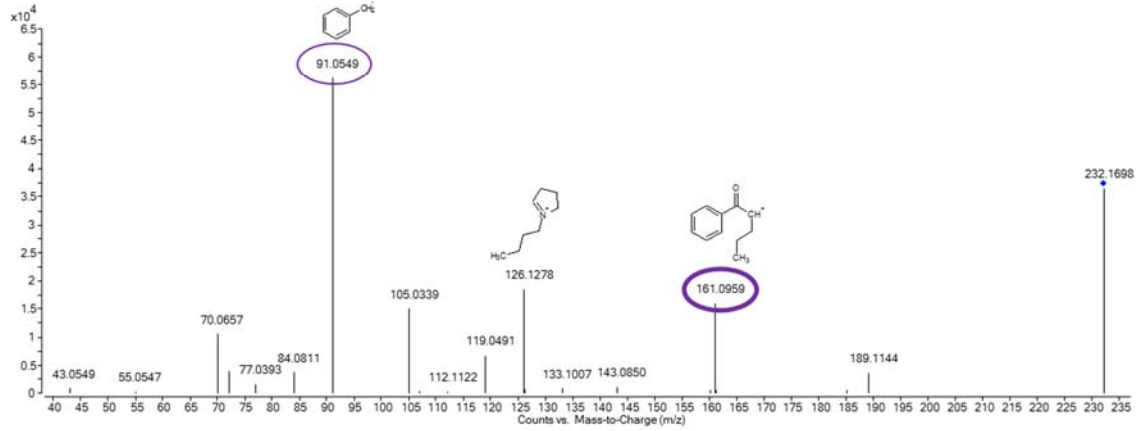

O
NH
CH3
CH3

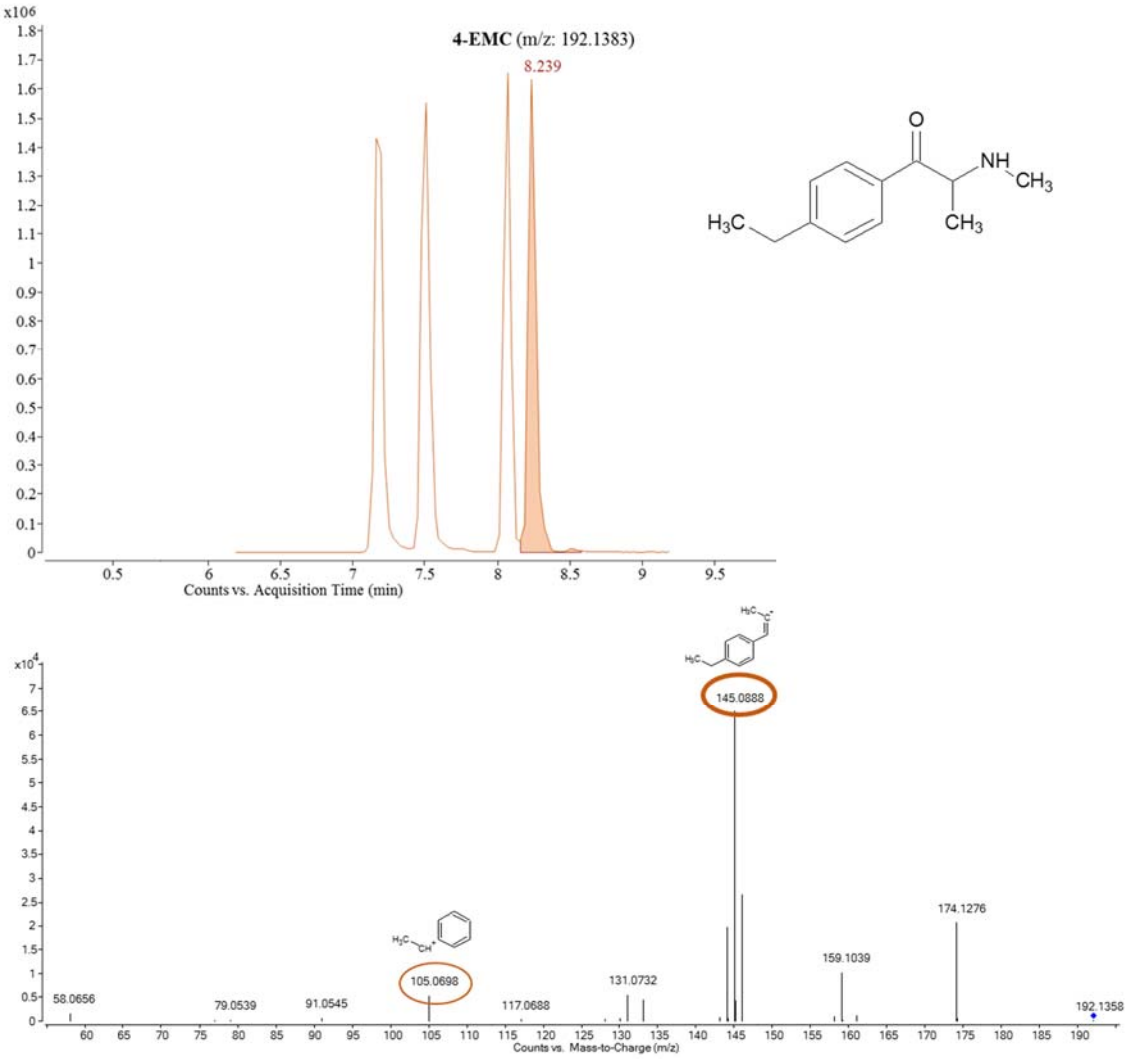

x10^4
132.0809
91.0546
105.0339
117.0575
144.0803
174.1274
43.0549
77.0387
86.0964
161.0956
192.1384
Counts vs. Mass-to-Charge (m/z)

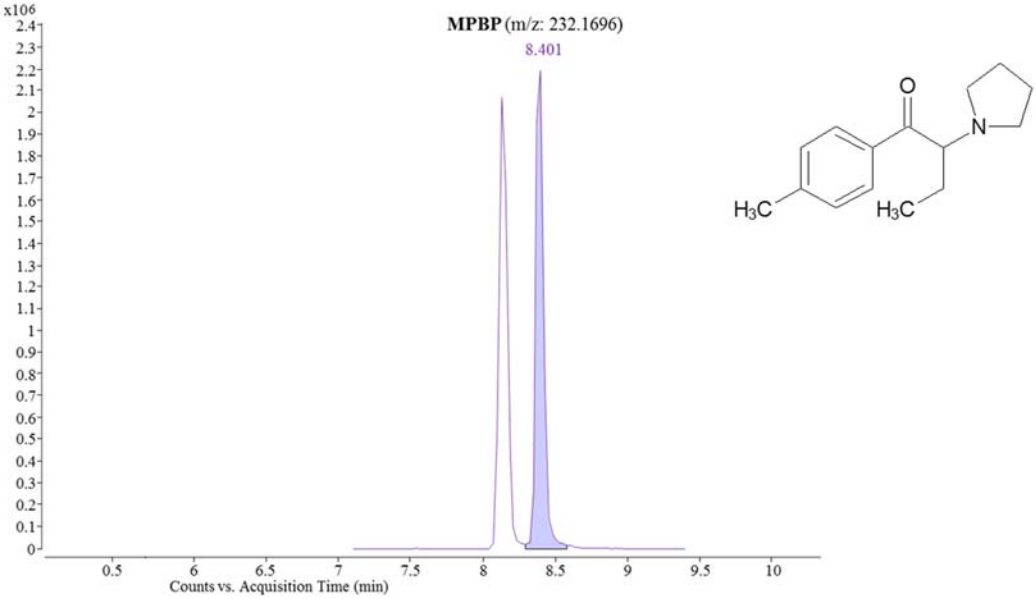

Pentylone (m/z: 236.1281)
7.829
Counts vs. Acquisition Time (min)
175.0642
188.1059
131.0729
86.0967
149.0228
159.0677
44.0502
121.0285
65.0391
91.0543
Counts vs. Mass-to-Charge (m/z)

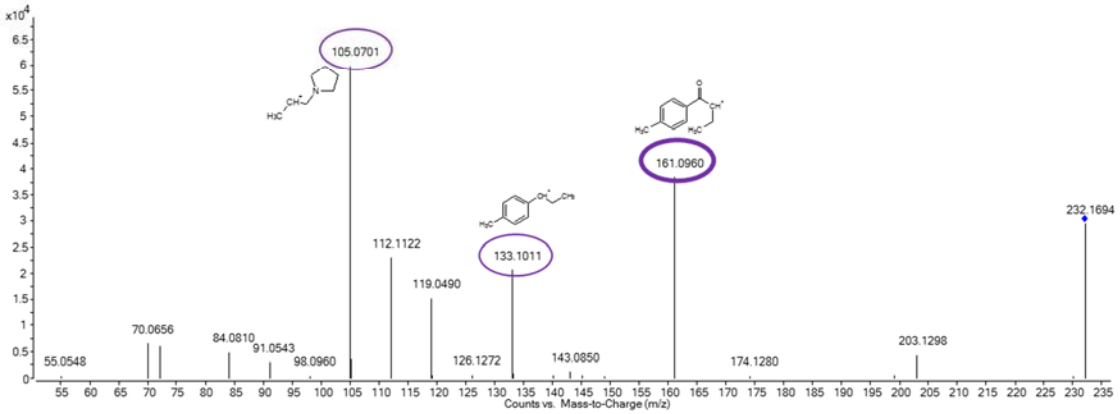

3,4-DMMC (m/z: 192.1383)
8.070
x106
Counts vs. Acquisition Time (min)
159.1044
174.1277
144.0807
133.1012
58.0659
91.0546
105.0701
117.0691
128.0617
192.1382
Counts vs. Mass-to-Charge (m/z)


α-PVP (m/z: 232.1696)
8.127
Counts vs. Acquisition Time (min)
O
N
CH3

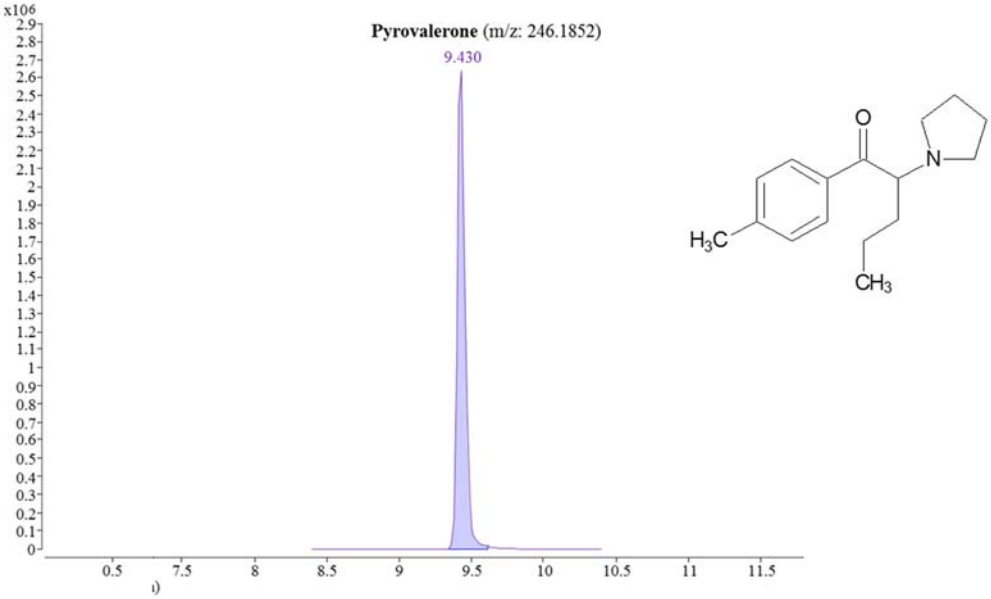

91.0549
232.1698
126.1278
161.0959
105.0339
70.0657
119.0491
84.0811
77.0393
43.0549
55.0547
112.1122
133.1007
143.0850
189.1144
Counts vs. Mass-to-Charge (m/z)

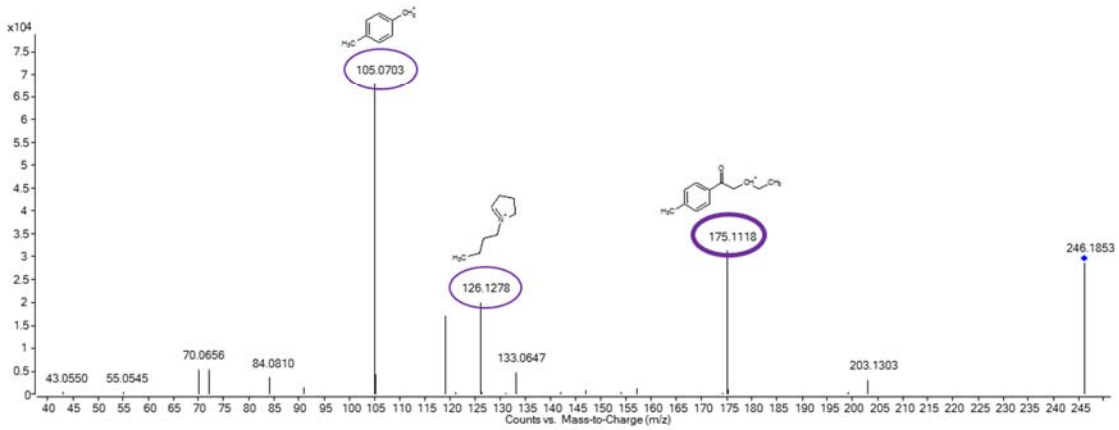

4-EMC (m/z: 192.1383)
8.239
Counts vs. Acquisition Time (min)
145.0888
105.0698
58.0656
79.0539
91.0545
117.0688
131.0732
159.1039
174.1276
192.1358
Counts vs. Mass-to-Charge (m/z)

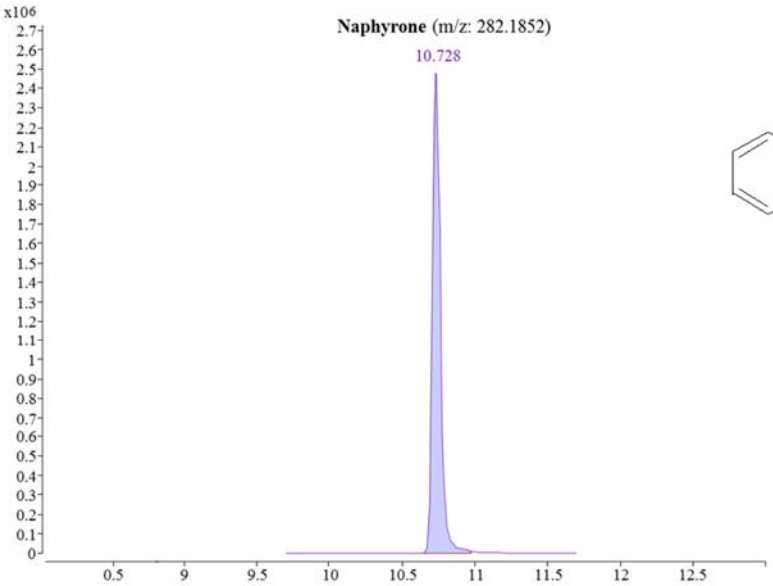

MPBP (m/z: 232.1696)
8.401
x106
Counts vs. Acquisition Time (min)
O
N
H3C
H3C

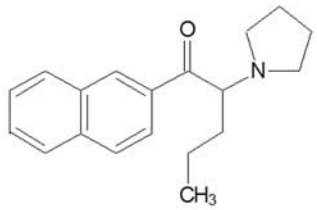

x10^4
105.0701
161.0960
133.1011
232.1694
112.1122
119.0490
70.0656
84.0810
91.0543
98.0960
55.0548
126.1272
143.0850
174.1280
203.1298
Counts vs. Mass-to-Charge (m/z)

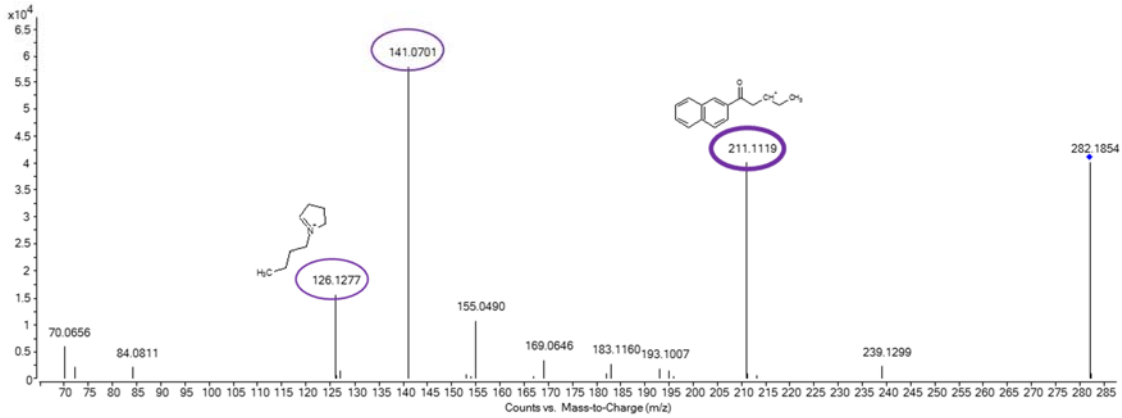

MDPV (m/z: 276.1594)
8.452
Counts vs. Acquisition Time (min)
126.1276
135.0439
149.0230
175.0751
205.0856
276.1594
233.1035
154.1224
121.0281
84.0811
72.0812
55.0183
Counts vs. Mass-to-Charge (m/z)


x106
Pyrovalerone (m/z: 246.1852)
9.430
O
N
H3C
CH3
i)


x104
105.0703
126.1278
175.1118
246.1853
43.0550
55.0545
70.0656
84.0810
133.0647
203.1303
Counts vs. Mass-to-Charge (m/z)

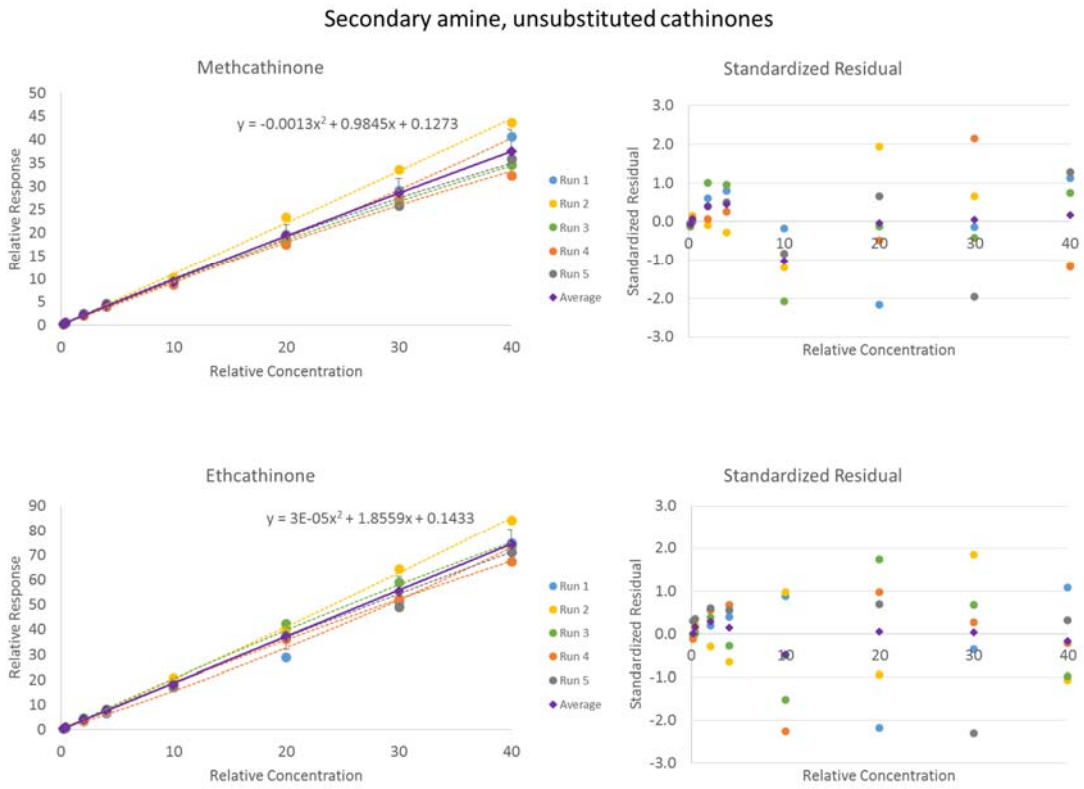

x106
Naphyrone (m/z: 282.1852)
10.728
0.5
9
9.5
10
10.5
11
11.5
12
12.5

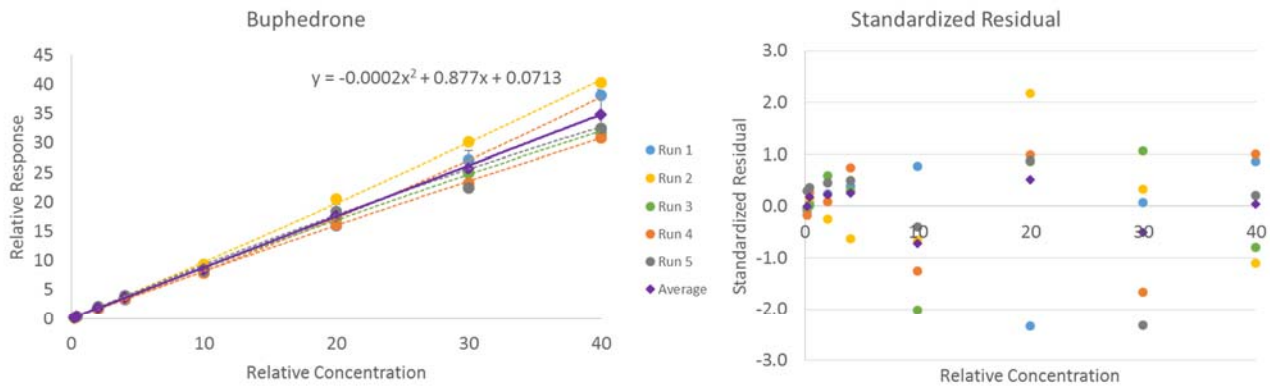

O
N
CH3

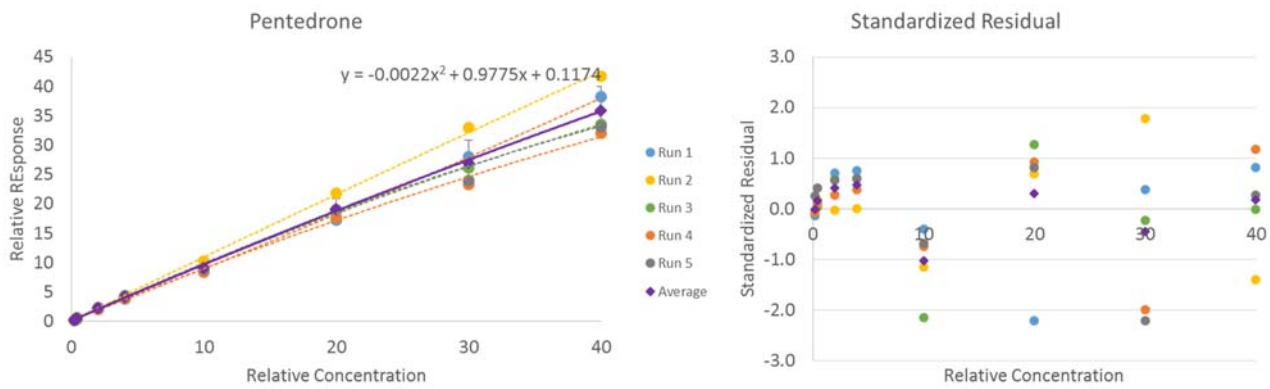

x10^4
141.0701
211.1119
282.1854
126.1277
155.0490
70.0656
84.0811
169.0646
183.1160
193.1007
239.1299
Counts vs. Mass-to-Charge (m/z)

APPENDIX B: CALIBRATION CURVES AND RESIDUAL PLOTS FOR SYNTHETIC CATHINONES IN BLOOD

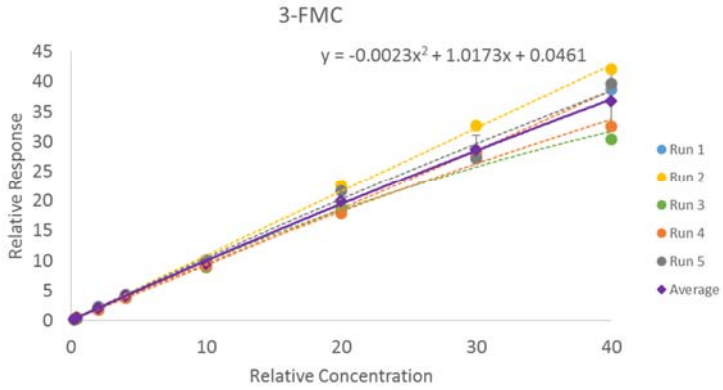
Calibration curves, quadratic, weighted (1/x), for synthetic cathinones in blood organized by sub-population. Axes expressed as relative concentration and relative response. Calibrator concentrations were 10, 25, 100, 250, 500, 750, and 1,000 ng/mL.

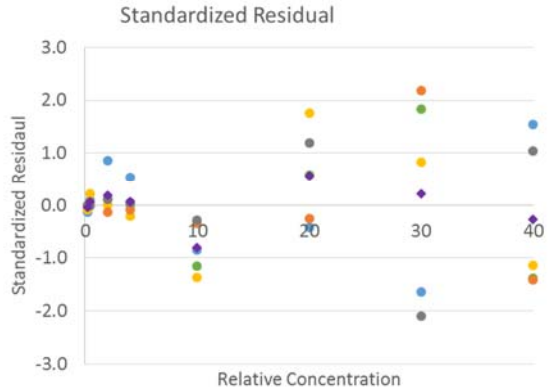


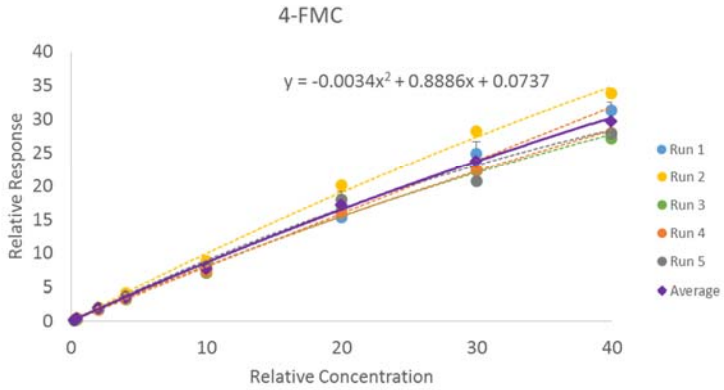

Buphedrone
y = -0.0002x² + 0.877x + 0.0713
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

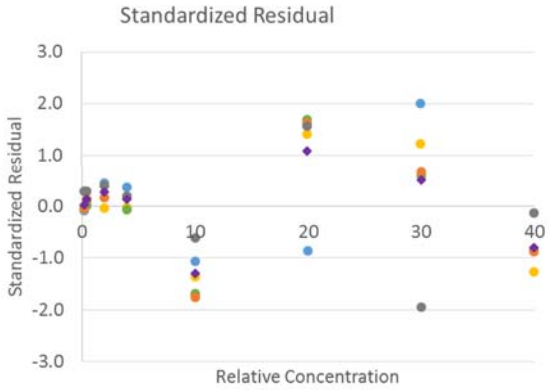

Pentedrone
y = -0.0022x² + 0.9775x + 0.1174
Relative REsponse
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

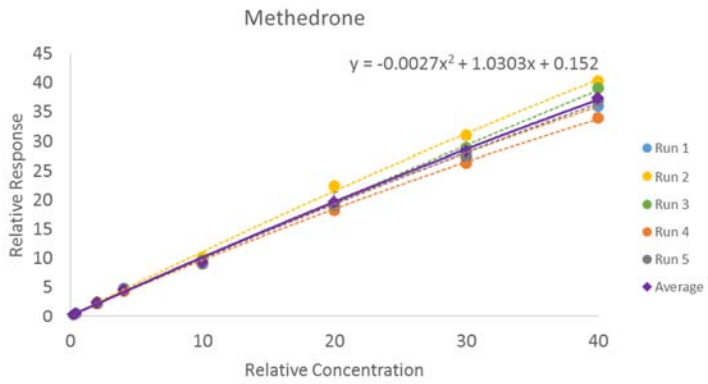
Secondary amine, substituted cathinones

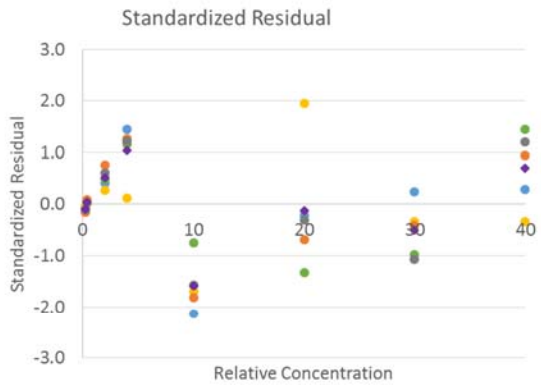


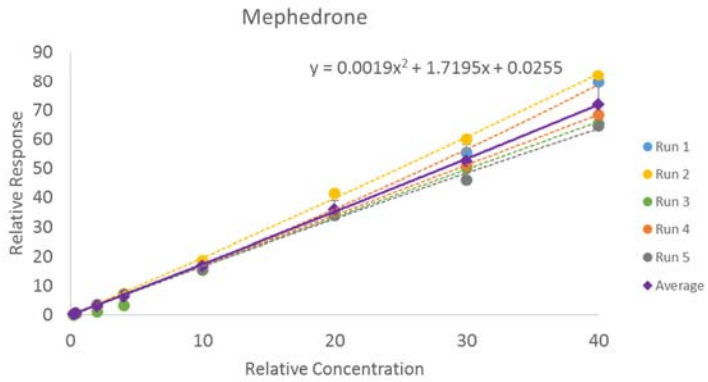


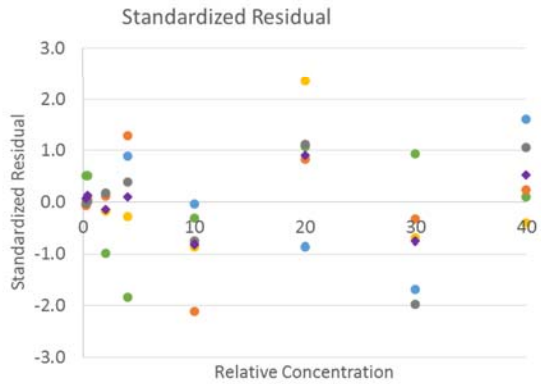


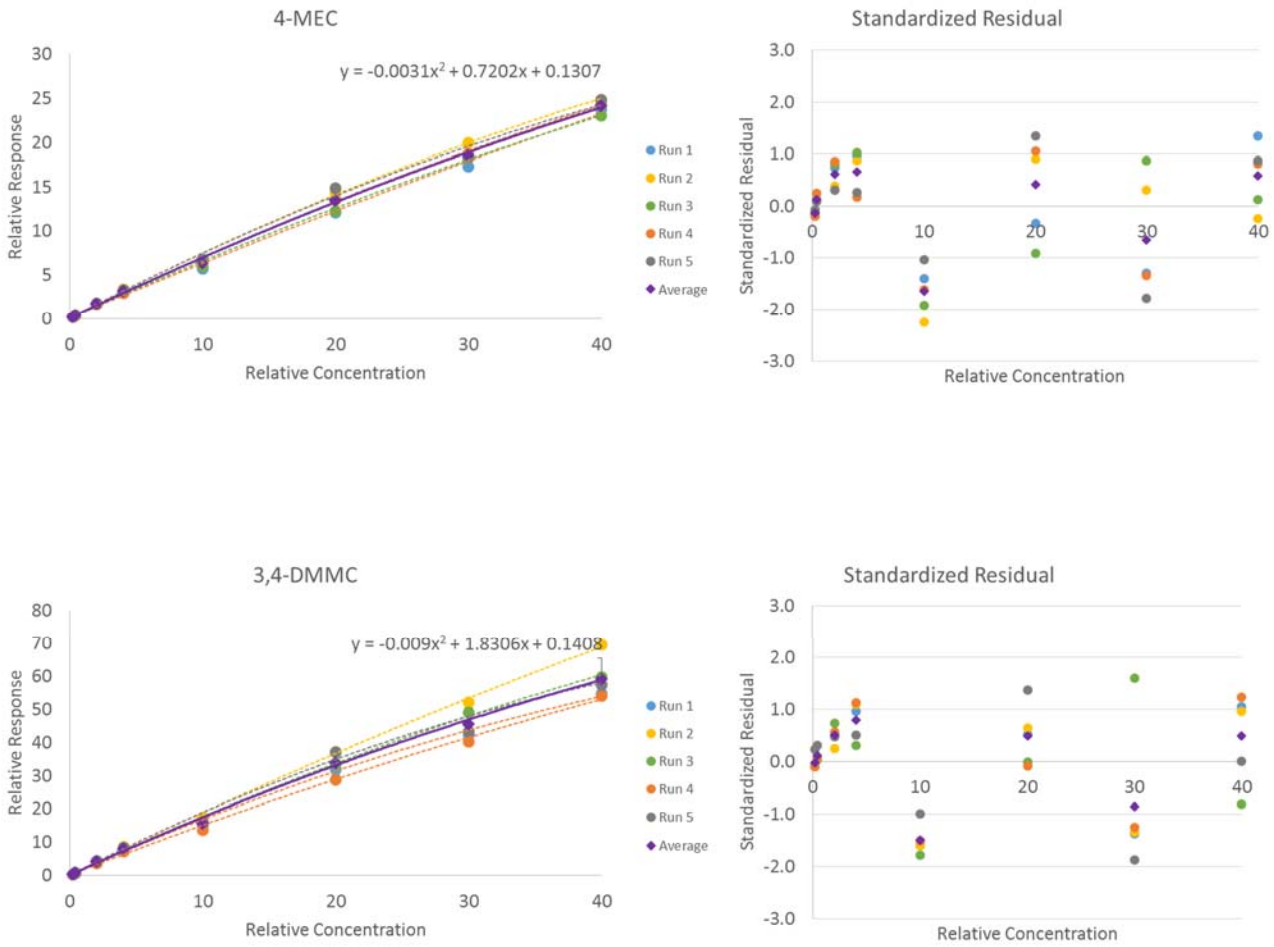


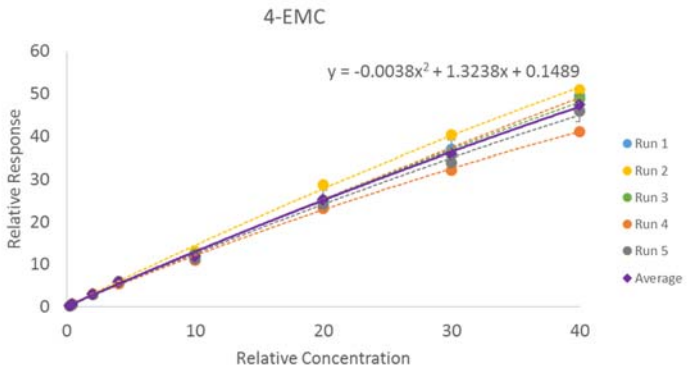

Methedrone
y = -0.0027x² + 1.0303x + 0.152
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

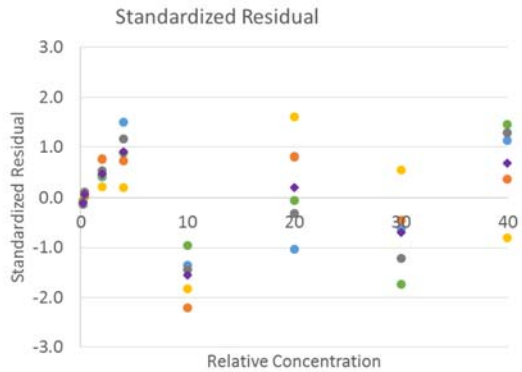

Standardized Residual
Standardized Residual
Relative Concentration


Mephedrone
y = 0.0019x² + 1.7195x + 0.0255
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

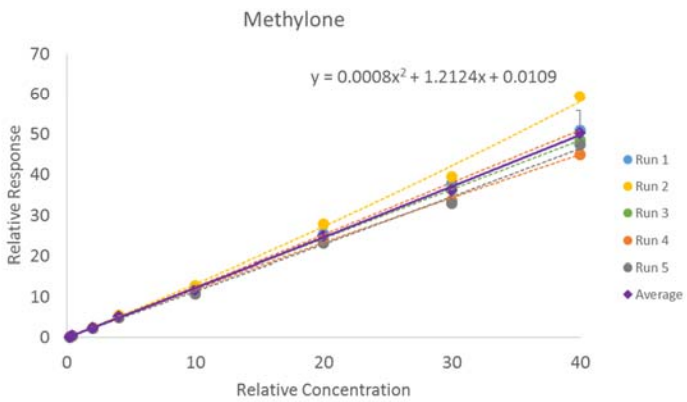

Standardized Residual
Standardized Residual
Relative Concentration

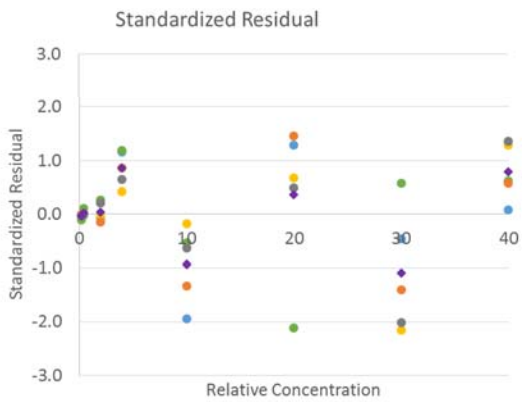

4-MEC
y = -0.0031x² + 0.7202x + 0.1307
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration
3,4-DMMC
y = -0.009x² + 1.8306x + 0.1408
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

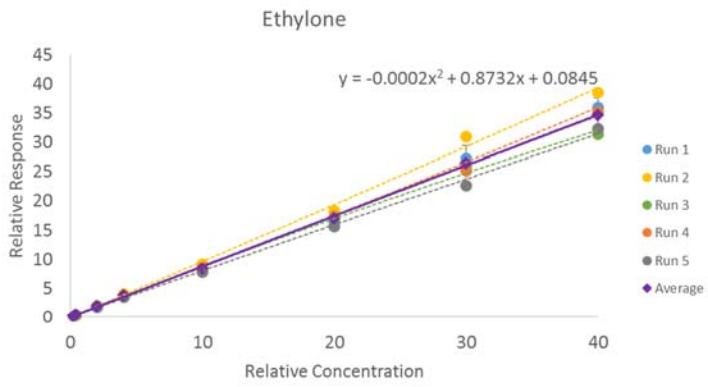


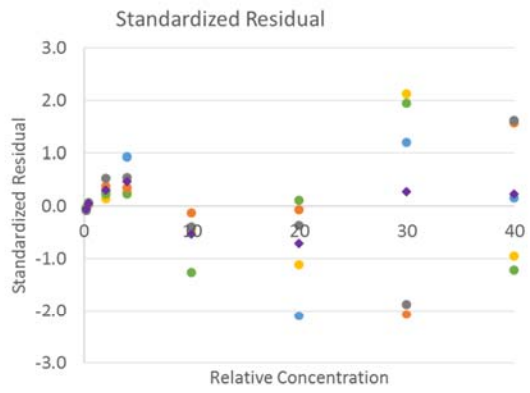


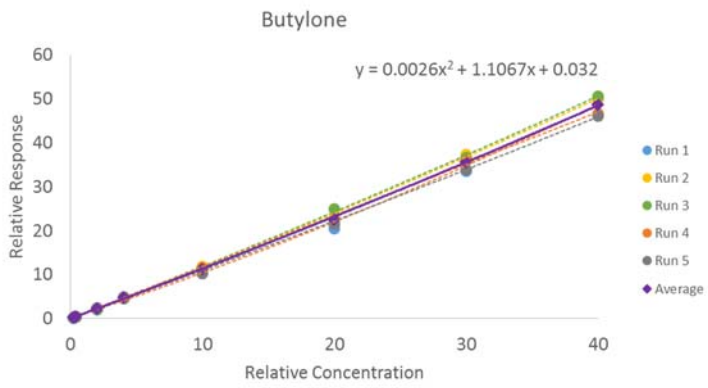
Secondary amine, methylenedioxy-type cathinones

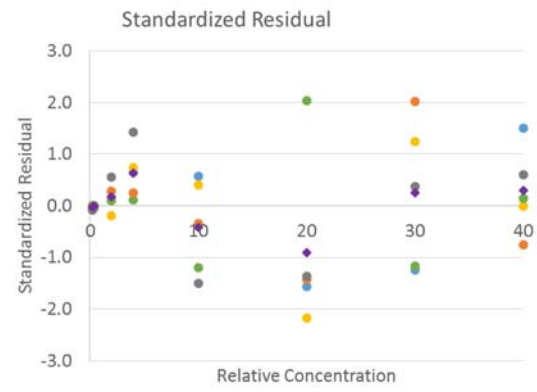


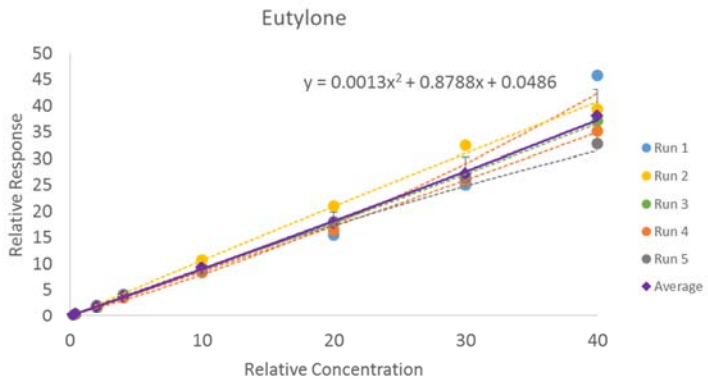


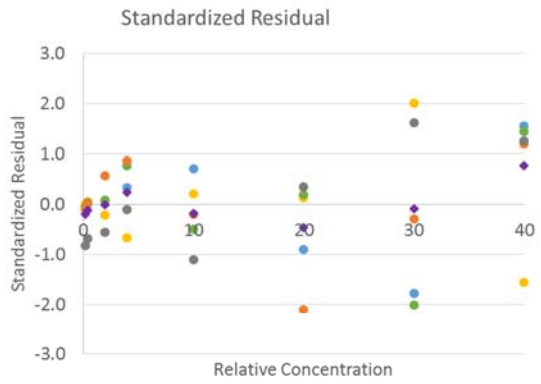

Ethylone
y = -0.0002x² + 0.8732x + 0.0845
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

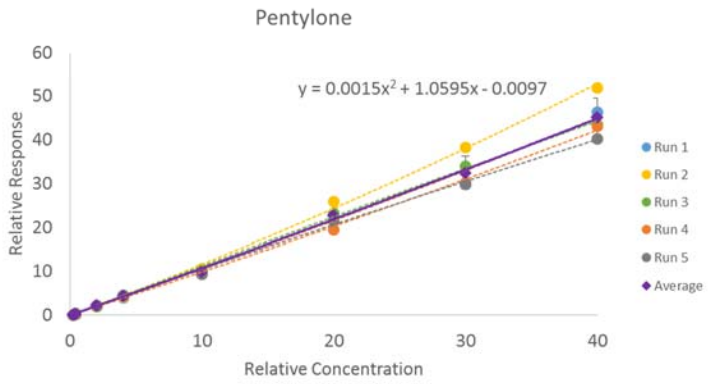

Standardized Residual
Standardized Residual
Relative Concentration

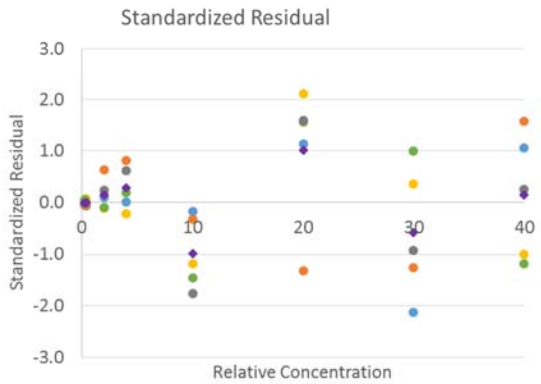

Butylone
y = 0.0026x² + 1.1067x + 0.032
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average


Standardized Residual
Standardized Residual
Relative Concentration

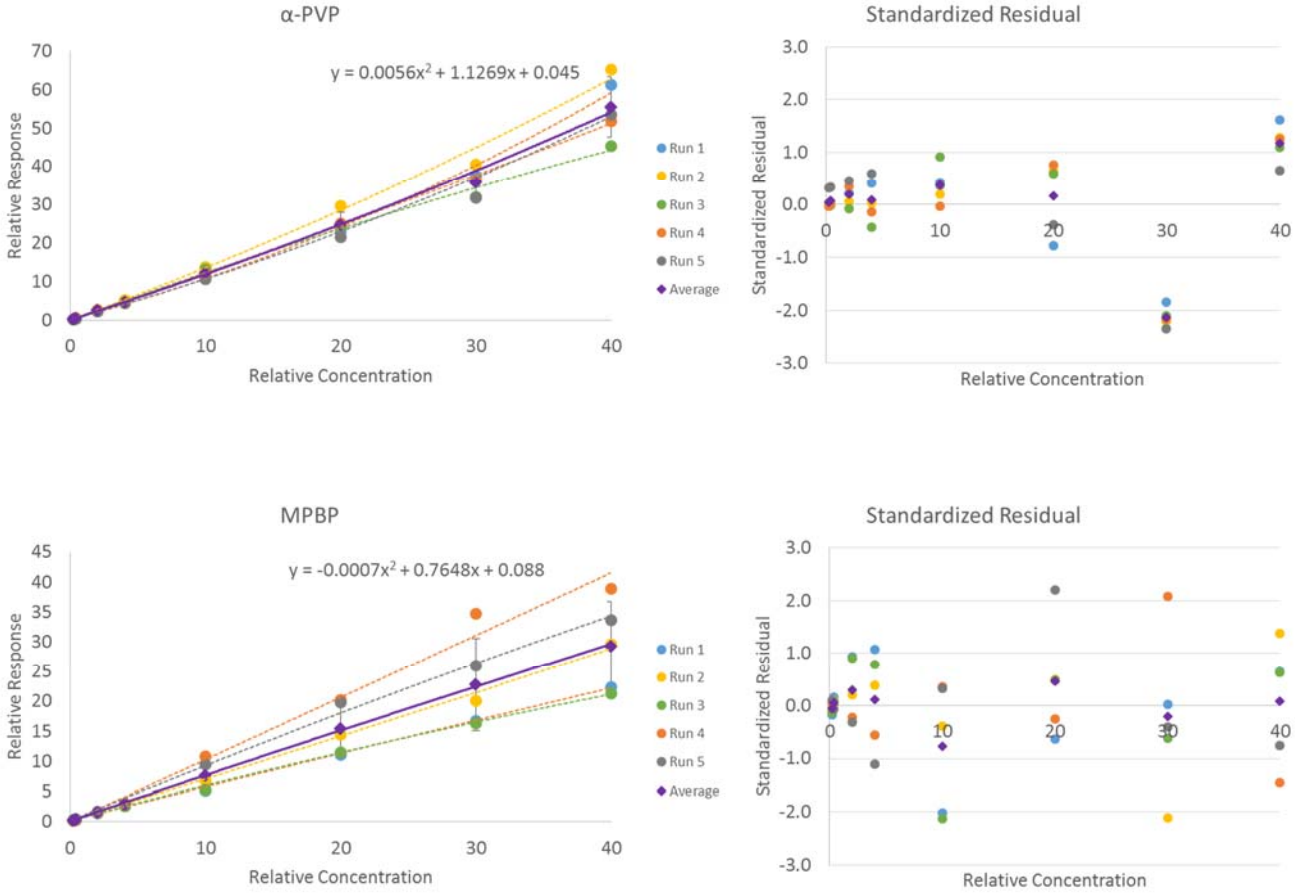

Eutylone
y = 0.0013x² + 0.8788x + 0.0486
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

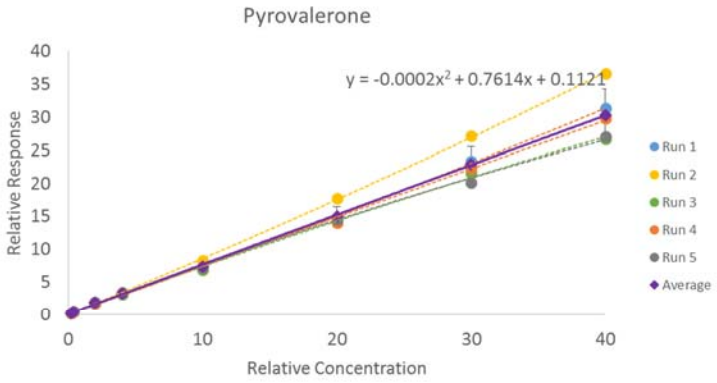

Standardized Residual
Standardized Residual
Relative Concentration

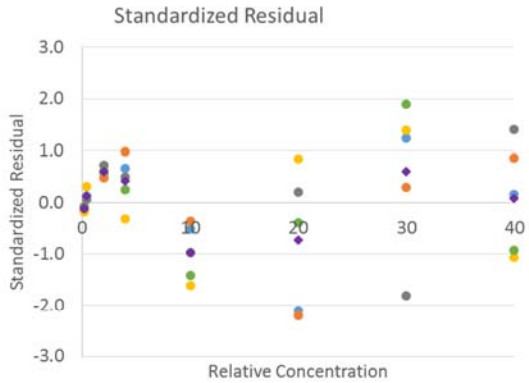

Pentylone
y = 0.0015x² + 1.0595x - 0.0097
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

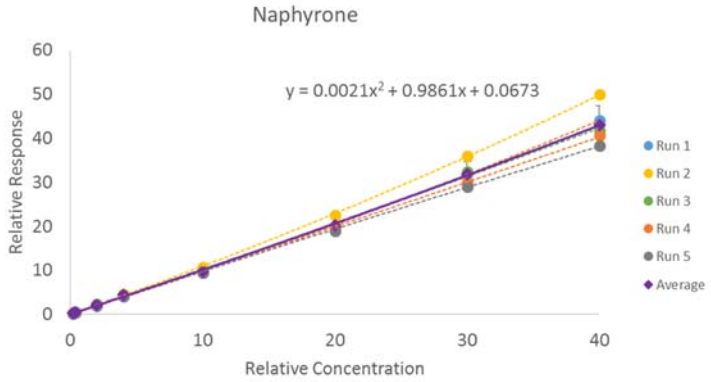

Standardized Residual
Standardized Residual
Relative Concentration

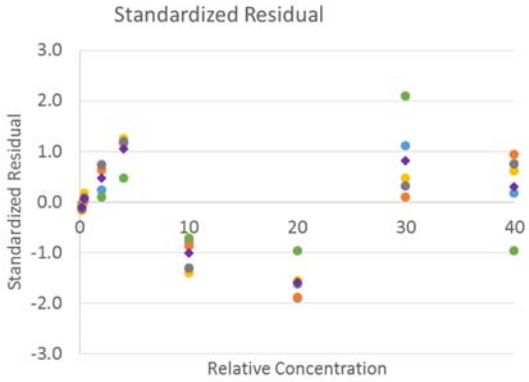
Tertiary amine (pyrrolidine) cathinones



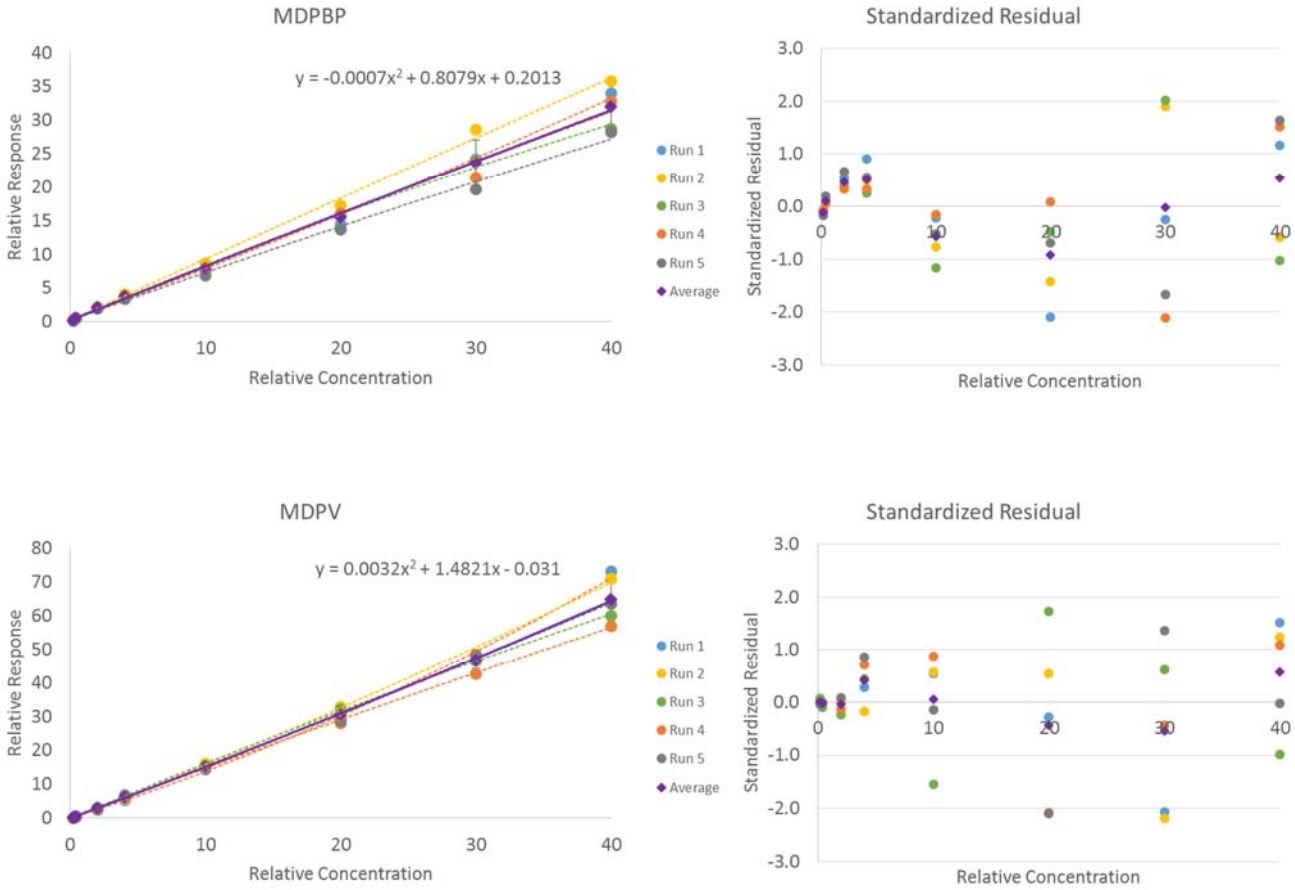

Pyrovalerone
y = -0.0002x² + 0.7614x + 0.1121
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average


Standardized Residual
Standardized Residual
Relative Concentration


Naphyrone
y = 0.0021x² + 0.9861x + 0.0673
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

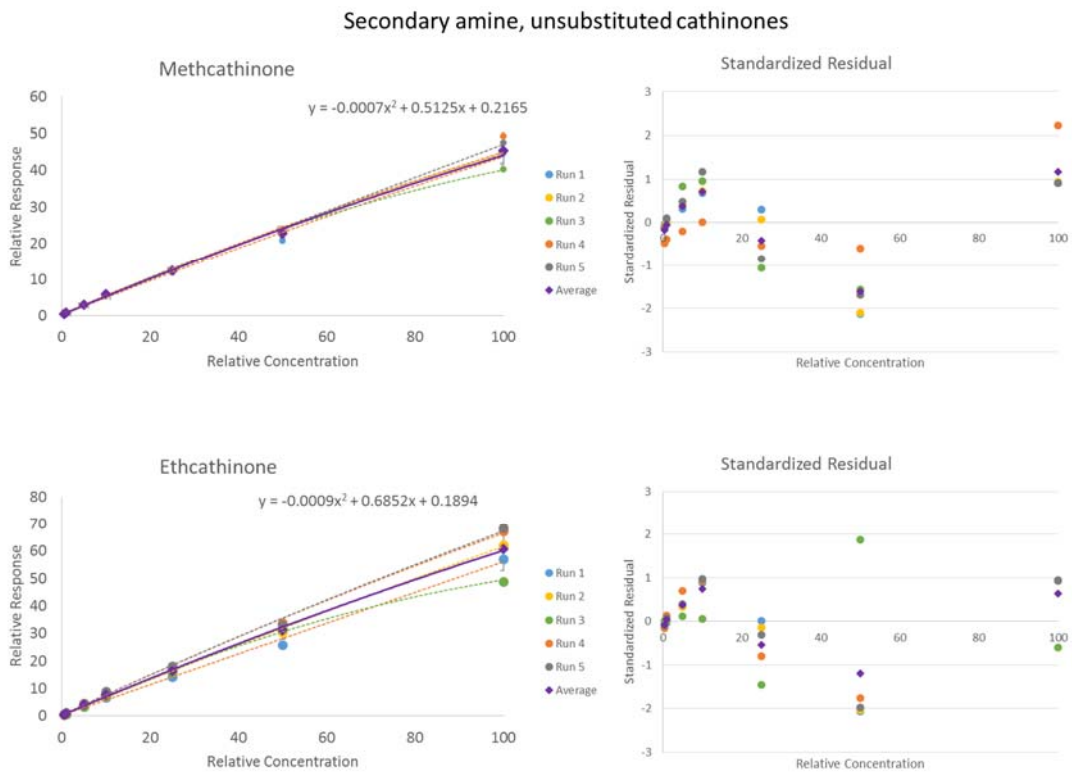

Standardized Residual
Standardized Residual
Relative Concentration

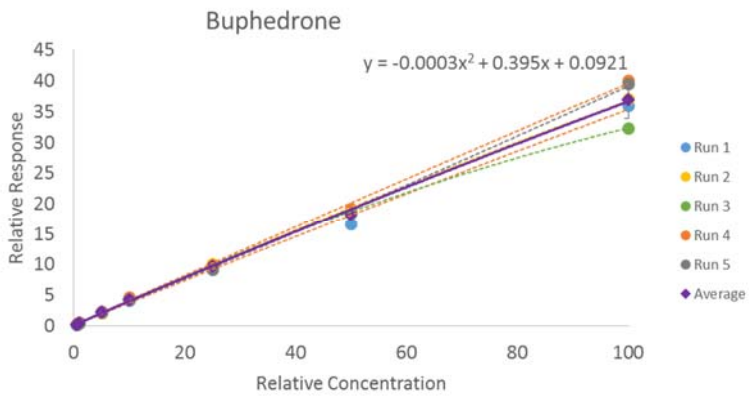
Tertiary amine (pyrrolidine), methylenedioxy-type cathinones

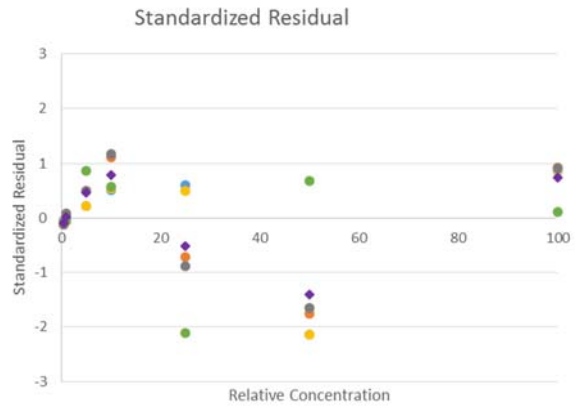


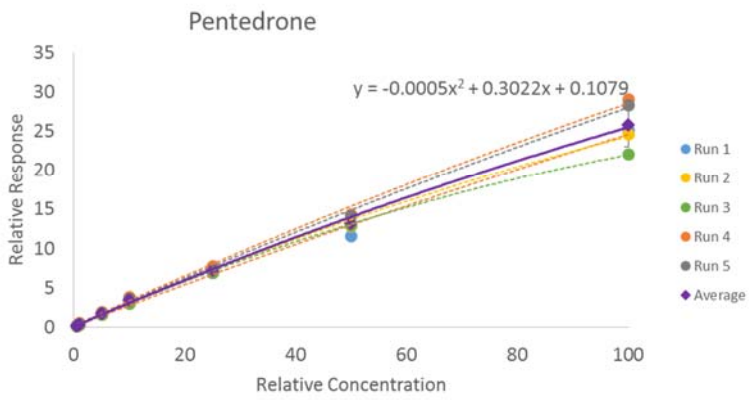
APPENDIX C: CALIBRATION CURVES AND RESIDUAL PLOTS FOR SYNTHETIC CATHINONES IN URINE

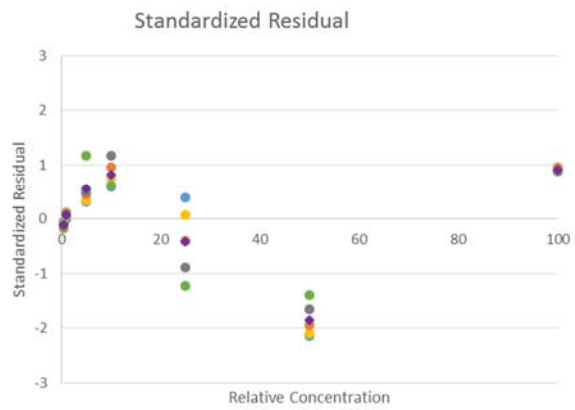
Calibration curves, quadratic, weighted (1/x), for synthetic cathinones in urine organized by sub-population. Axes expressed as relative concentration and relative response. Calibrators included 10, 25, 100, 250, 500, and 1,000 ng/mL.



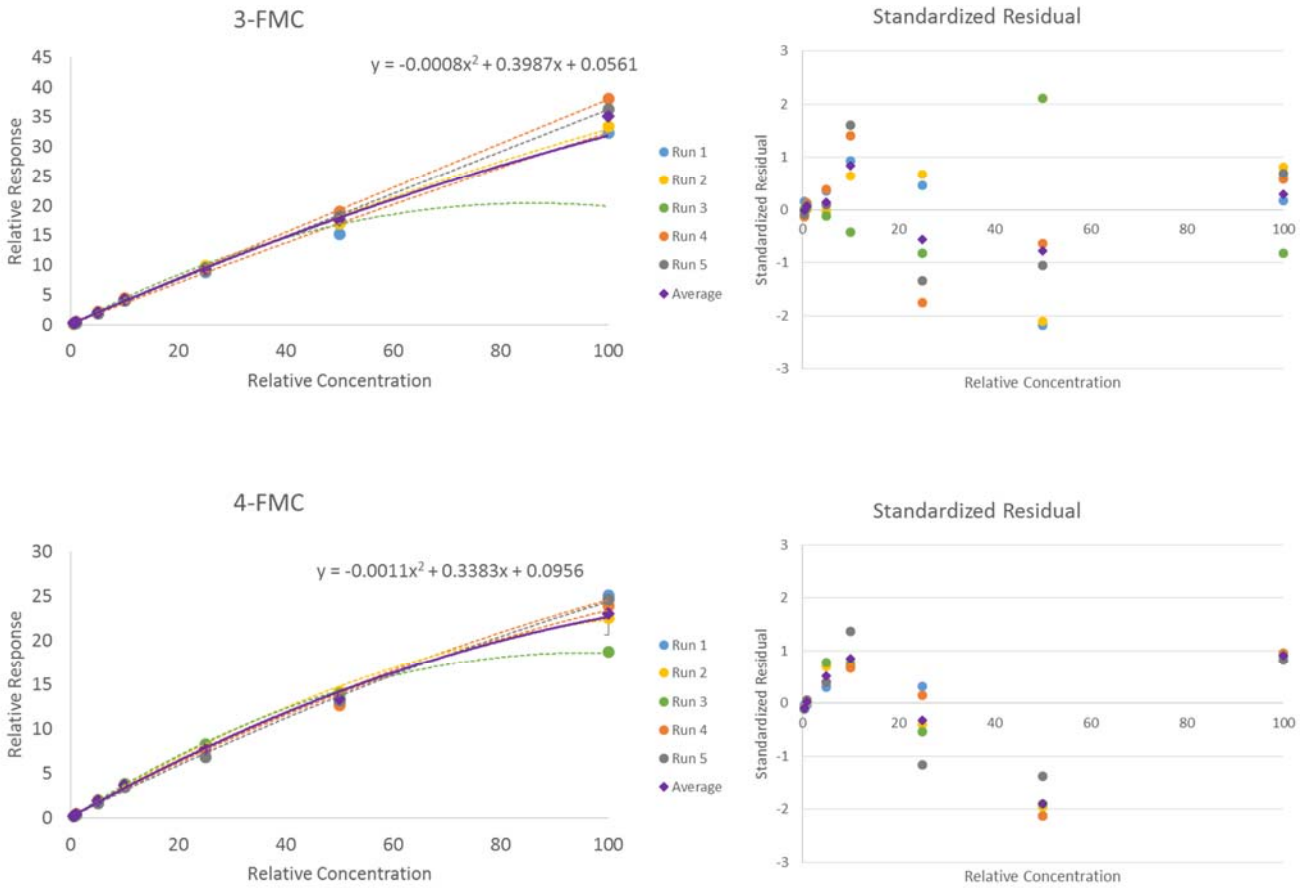

Buphedrone
y = -0.0003x² + 0.395x + 0.0921
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

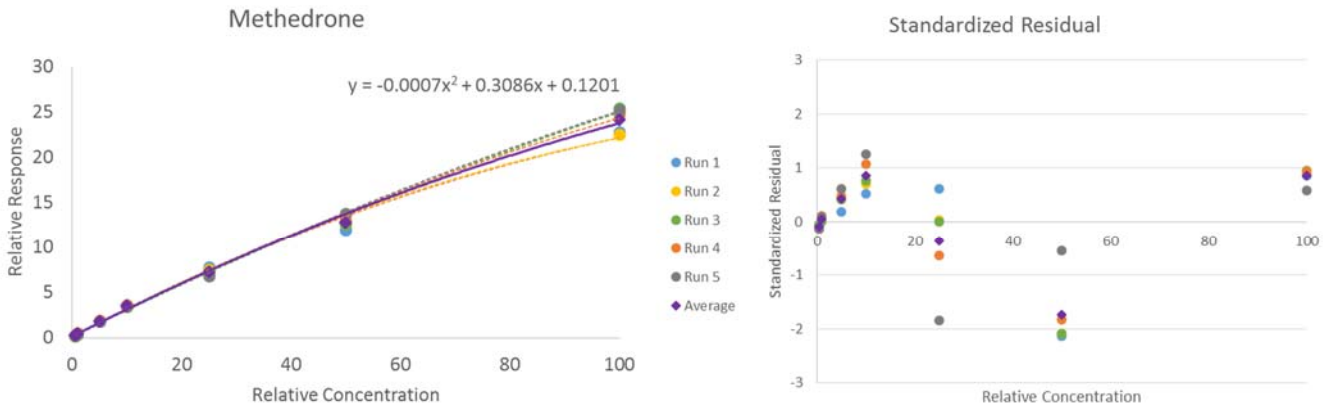

Standardized Residual
Standardized Residual
Relative Concentration

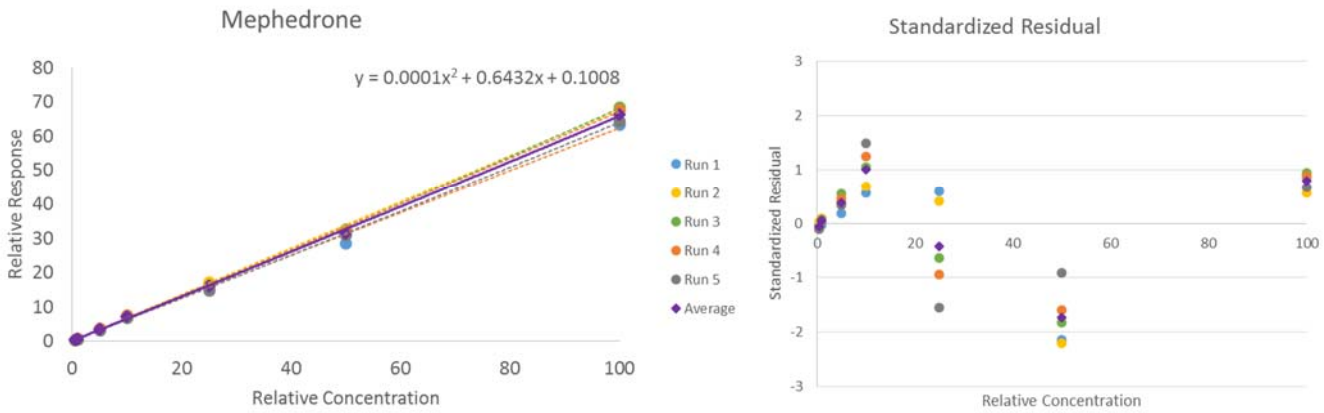

Pentedrone
y = -0.0005x² + 0.3022x + 0.1079
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

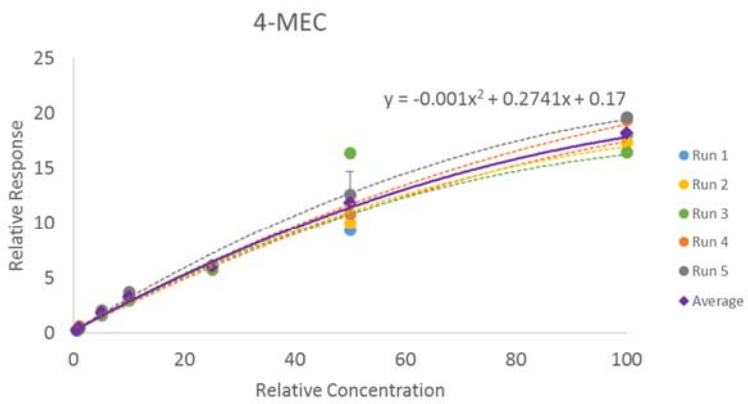

Standardized Residual
Standardized Residual
Relative Concentration

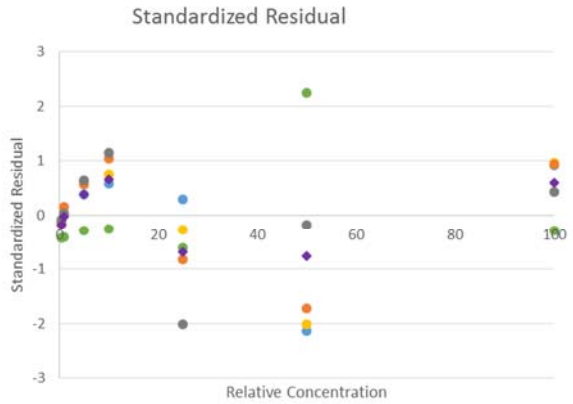
Secondary amine, substituted cathinones

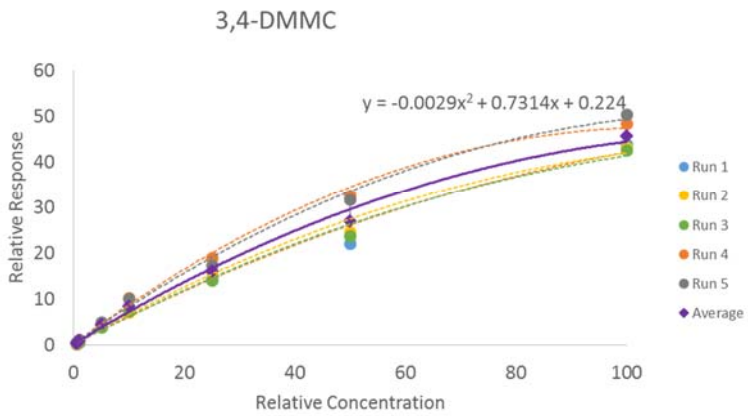


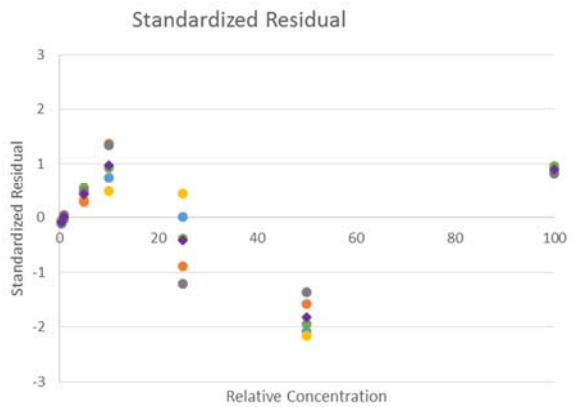

Methedrone
y = -0.0007x² + 0.3086x + 0.1201
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

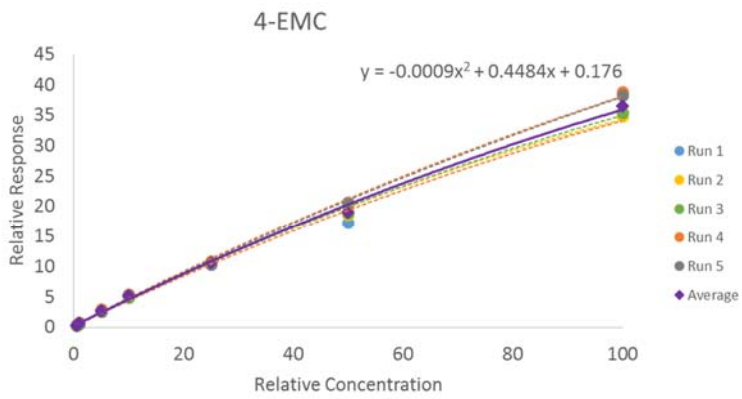

Mephedrone
y = 0.0001x² + 0.6432x + 0.1008
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

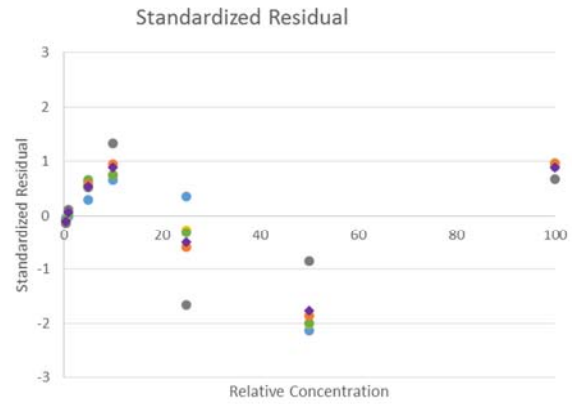

4-MEC
y = -0.001x² + 0.2741x + 0.17
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average


Standardized Residual
Standardized Residual
Relative Concentration

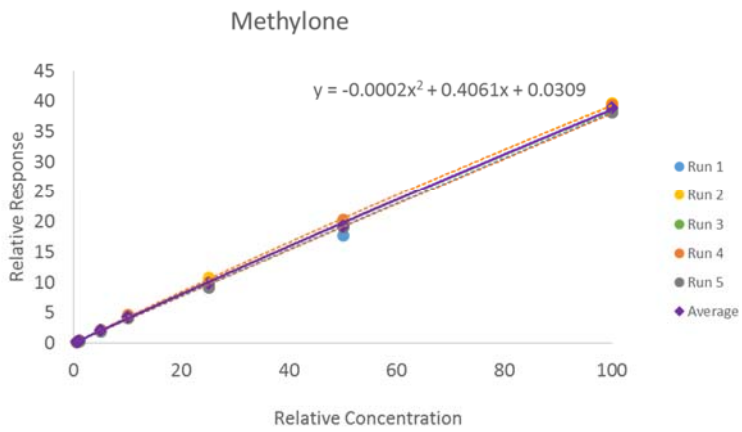

3,4-DMMC
y = -0.0029x² + 0.7314x + 0.224
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

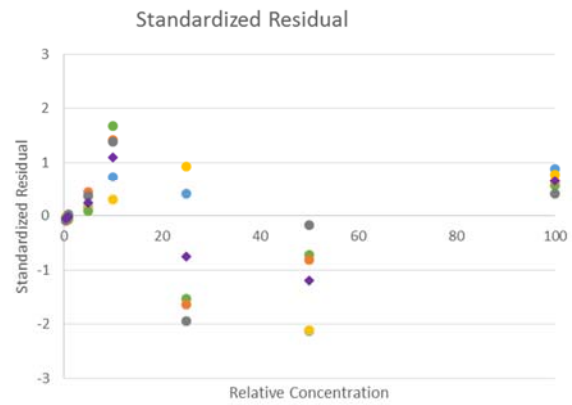

Standardized Residual
Standardized Residual
Relative Concentration

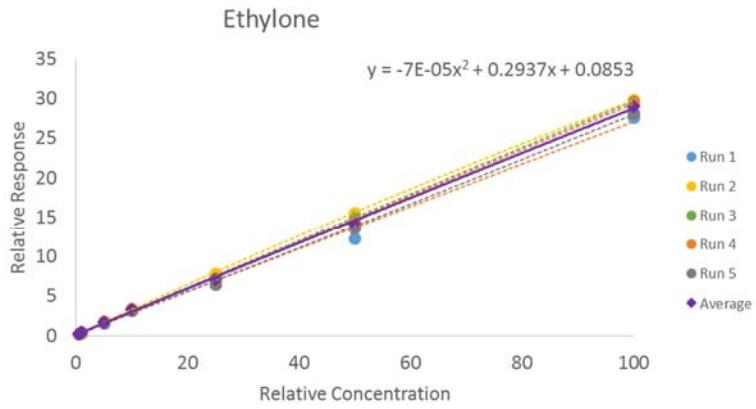


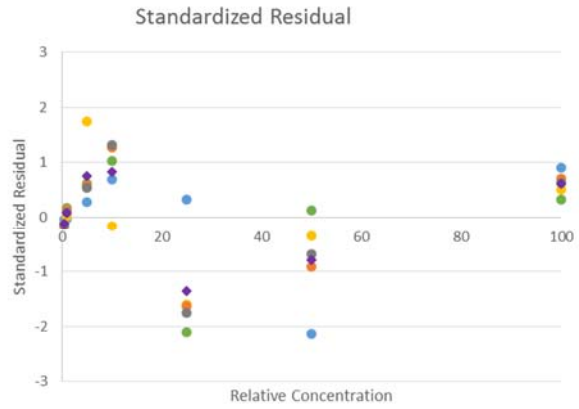


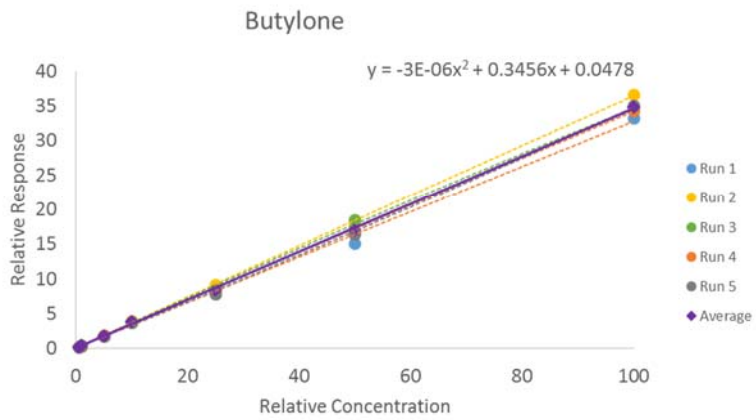
Secondary amine, methylenedioxy-type cathinones

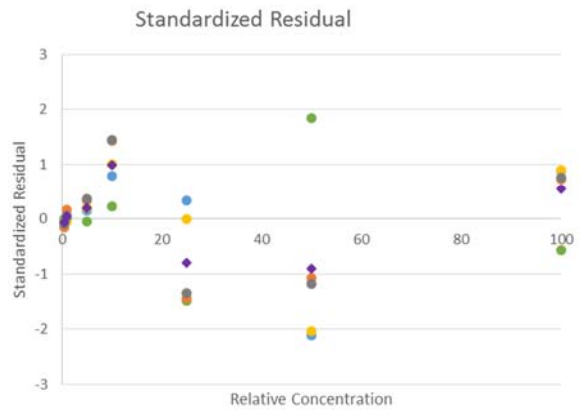


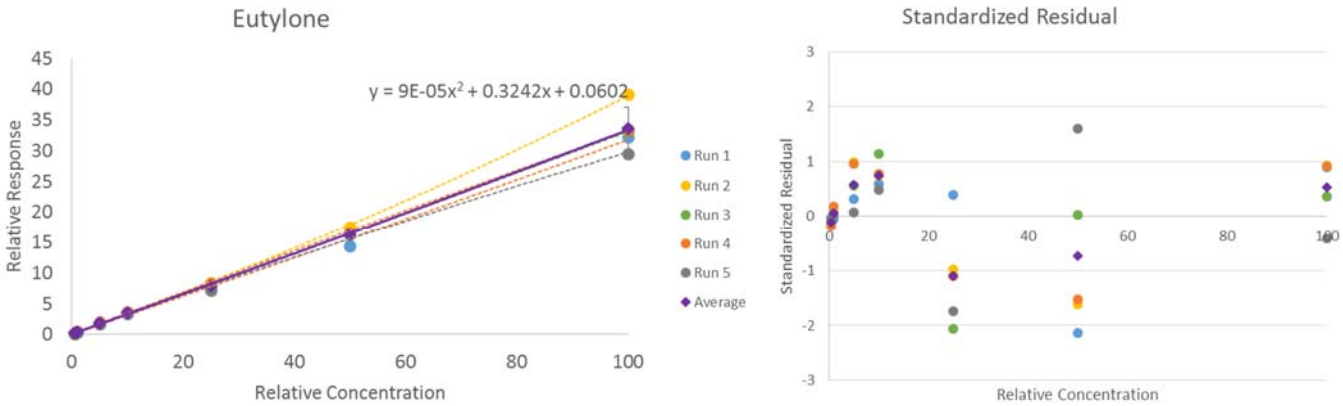


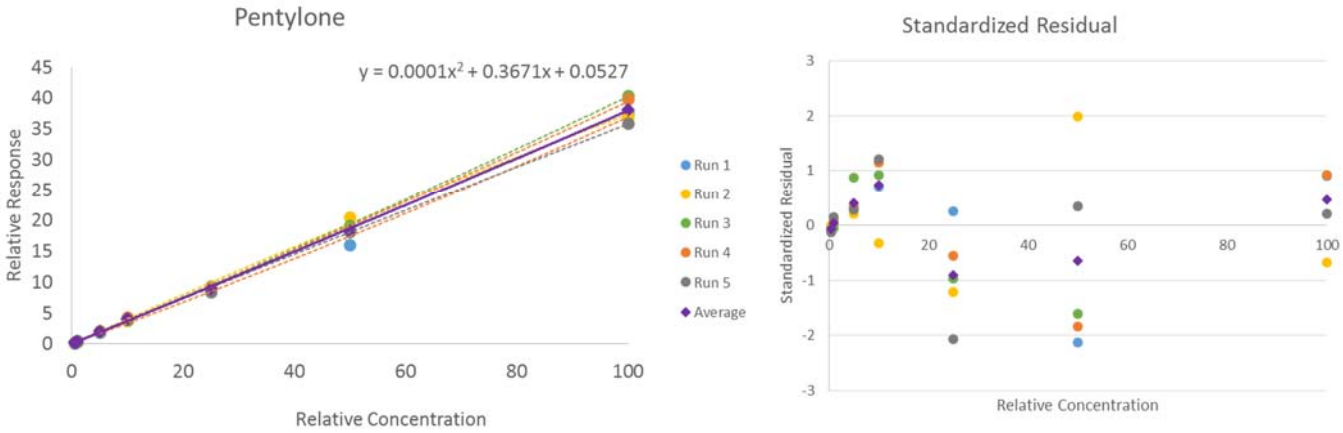

Ethylone
y = -7E-05x² + 0.2937x + 0.0853
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average


Standardized Residual
Standardized Residual
Relative Concentration

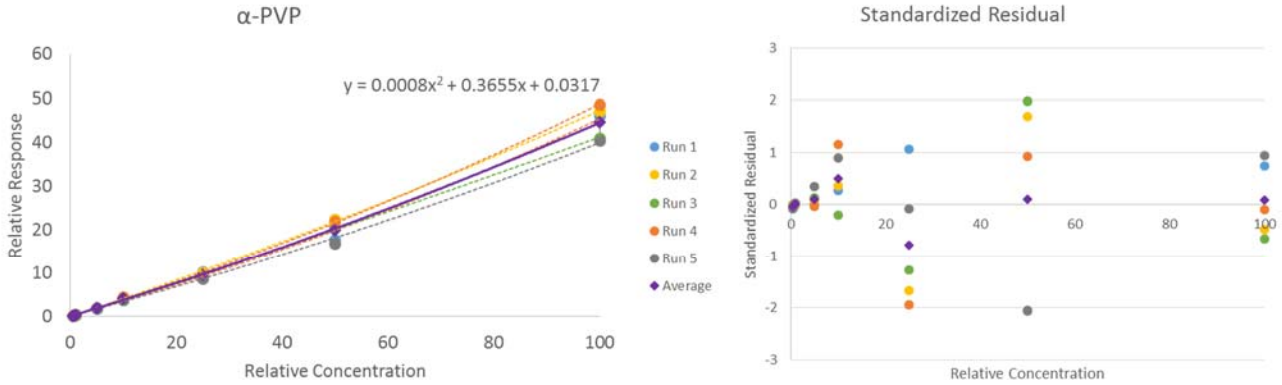

Butylone
y = -3E-06x² + 0.3456x + 0.0478
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average

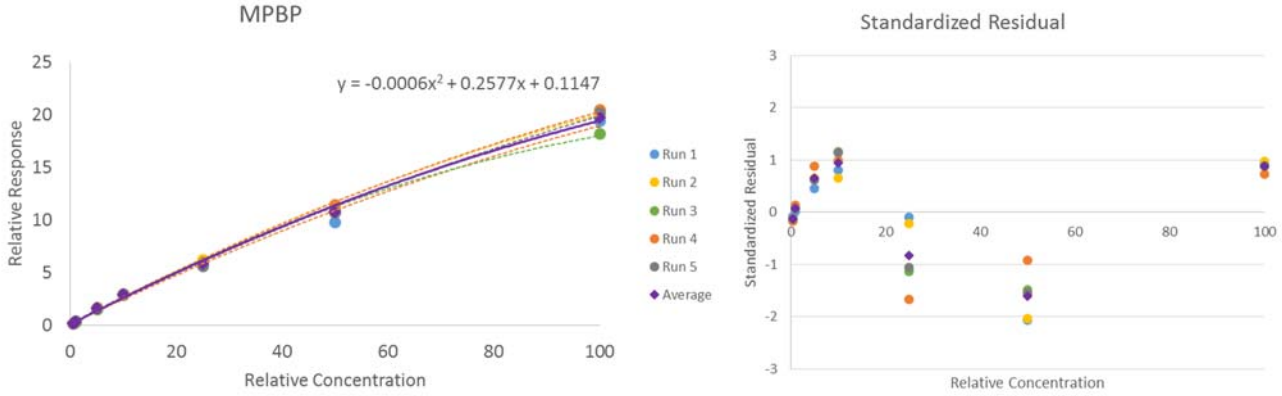

Standardized Residual
Standardized Residual
Relative Concentration

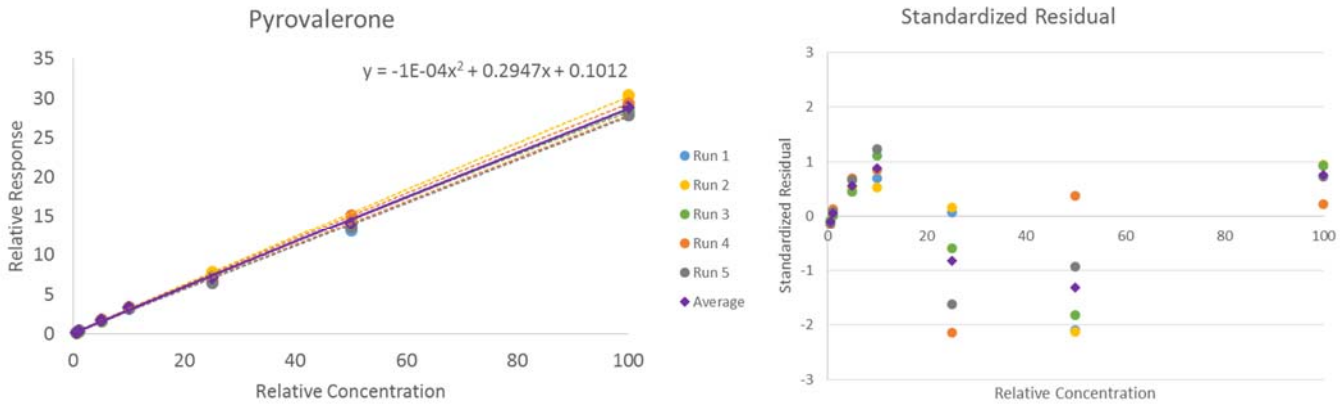

Eutylone
y = 9E-05x² + 0.3242x + 0.0602
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

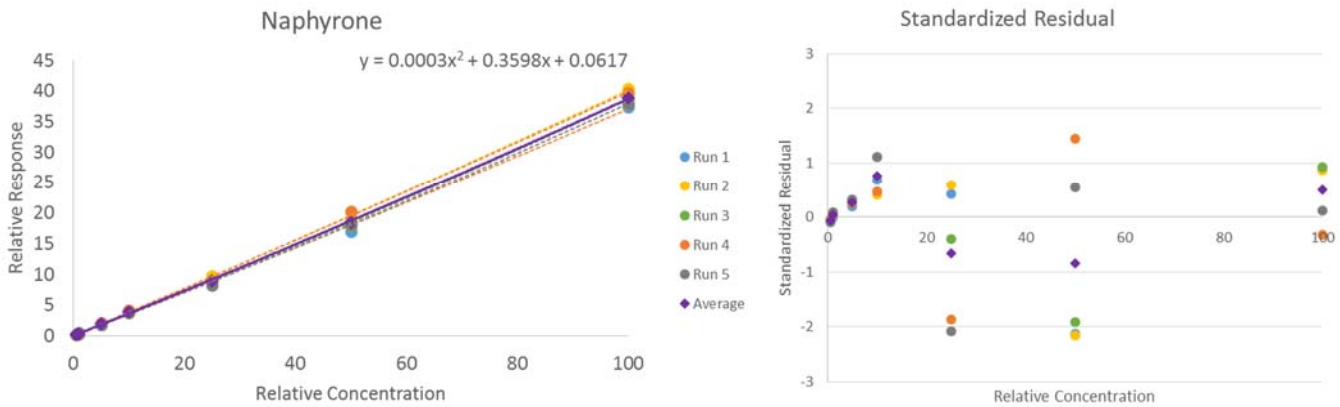

Pentylone
y = 0.0001x² + 0.3671x + 0.0527
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

Tertiary amine (pyrrolidine) cathinones

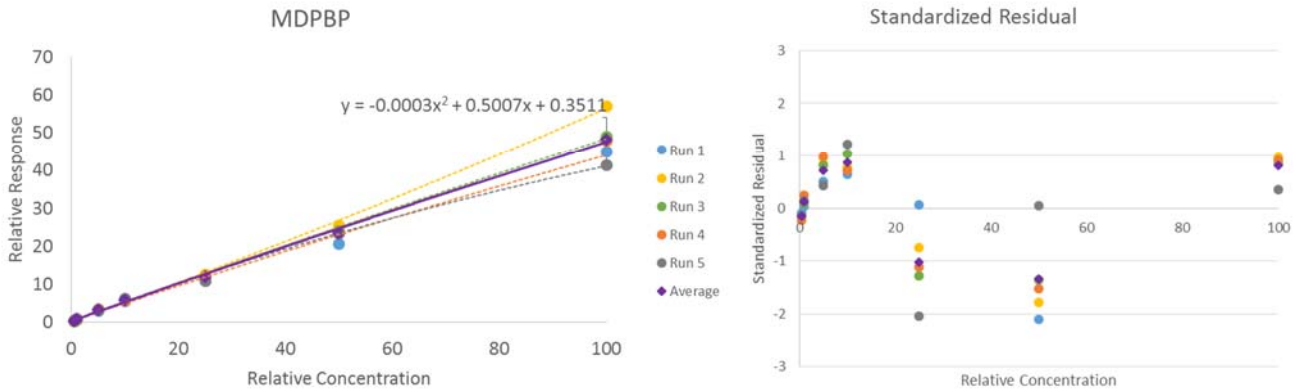


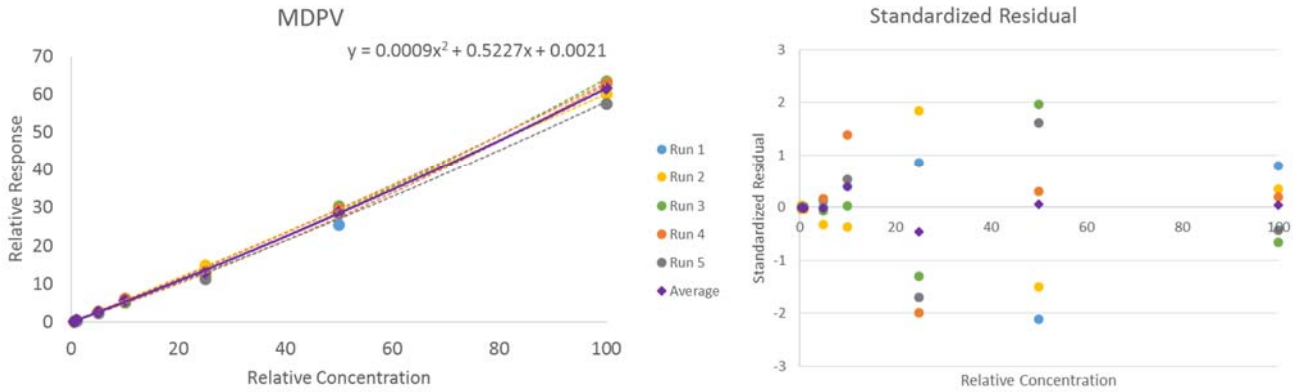



Pyrovalerone
y = -1E-04x² + 0.2947x + 0.1012
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration


Naphyrone
y = 0.0003x² + 0.3598x + 0.0617
Relative Response
Relative Concentration
Run 1
Run 2
Run 3
Run 4
Run 5
Average
Standardized Residual
Standardized Residual
Relative Concentration

Tertiary amine (pyrrolidine), methylenedioxy-type cathinones





APPENDIX D: ONE-WAY ANOVA STATISTICAL ANALYSIS FOR CONCENTRATION, TEMPERATURE, AND ANALYTE DEPENDENCE IN BLOOD

One-way ANOVA statistical analysis for stability factors in blood. Bold *p*-values indicate a significant difference.

Concentration Dependence

Concentration Dependence					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Methcathinone					
H	1	58	0.004	4.01	0.95
RT	1	57	0.001	4.01	0.97
R	1	40	1.31	4.08	0.26
F	1	29	1.97	4.18	0.17
3-FMC					
H	1	58	0.01	4.01	0.94
RT	1	58	0.02	4.01	0.89
R	1	40	0.04	4.08	0.84
F	1	35	1.20	4.12	0.28
4-FMC					
H	1	58	0.0001	4.01	0.99
RT	1	57	0.01	4.01	0.90
R	1	40	0.39	4.08	0.53
F	1	26	0.15	4.23	0.70
Methylone					
H	1	57	0.10	4.01	0.76
RT	1	50	0.28	4.03	0.60
R	1	37	0.07	4.11	0.80
F	Stable, no comparison made				
Ethcathinone					
H	1	56	0.003	4.01	0.96
RT	1	55	0.01	4.02	0.91
R	1	36	2.54	4.11	0.12
F	1	33	4.00	4.14	0.05
Ethylone					
H	1	53	0.00002	4.02	1.00

(continued)

Concentration Dependence					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
RT	1	47	0.32	4.05	0.58
R	Stable, no comparison made				
F	Stable, no comparison made				
Methedrone					
H	1	55	0.01	4.02	0.92
RT	1	53	0.01	4.02	0.91
R	1	37	1.04	4.11	0.31
F	Stable, no comparison made				
Buphedrone					
H	1	56	0.02	4.01	0.88
RT	1	56	0.001	4.01	0.97
R	1	39	1.55	4.09	0.22
F	1	29	2.48	4.18	0.13
Butylone					
H	1	54	0.17	4.02	0.69
RT	1	50	0.21	4.03	0.65
R	Stable, no comparison made				
F	Stable, no comparison made				
Mephedrone					
H	1	56	0.02	4.01	0.89
RT	1	55	0.0001	4.02	0.99
R	1	41	0.41	4.08	0.53
F	1	36	3.64	4.11	0.06
Eutylone					
H	1	51	0.02	4.03	0.89
RT	1	42	0.26	4.07	0.61
R	Stable, no comparison made				
F	Stable, no comparison made				
4-MEC					
H	1	56	0.03	4.01	0.86
RT	1	56	0.00002	4.01	1.00
R	1	39	0.13	4.09	0.72
F	Stable, no comparison made				
MDPBP					
H	1	50	0.04	4.03	0.84
RT	1	41	3.27	4.08	0.08
R	Stable, no comparison made				
F	Stable, no comparison made				

(continued)

Concentration Dependence					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Pentedrone					
H	1	56	0.01	4.01	0.91
RT	1	57	0.01	4.01	0.94
R	1	40	2.89	4.08	0.10
F	Stable, no comparison made				
Pentylone					
H	1	55	0.003	4.02	0.96
RT	1	49	0.003	4.04	0.95
R	Stable, no comparison made				
F	Stable, no comparison made				
3,4-DMMC					
H	1	56	0.03	4.01	0.87
RT	1	55	0.004	4.02	0.95
R	1	41	0.02	4.08	0.88
F	Stable, no comparison made				
α-PVP					
H	1	53	0.001	4.02	0.98
RT	1	48	0.05	4.04	0.83
R	Stable, no comparison made				
F	Stable, no comparison made				
4-EMC					
H	1	56	0.003	4.01	0.96
RT	1	57	0.003	4.01	0.96
R	1	42	0.40	4.07	0.53
F	Stable, no comparison made				
MPBP					
H	1	47	0.002	4.047	0.965
RT	1	49	0.002	4.038	0.969
R	Stable, no comparison made				
F	Stable, no comparison made				
MDPV					
H	1	52	0.01	4.03	0.91
RT	1	42	0.40	4.07	0.53
R	Stable, no comparison made				
F	Stable, no comparison made				
Pyrovalerone					
H	1	54	0.39	4.02	0.53
RT	1	49	0.53	4.04	0.47

(continued)

Concentration Dependence					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
R	Stable, no comparison made				
F	Stable, no comparison made				
Naphyrone					
H	1	56	0.10	4.01	0.75
RT	1	50	0.01	4.03	0.91
R	1	41	0.29	4.08	0.59
F	Stable, no comparison made				

Df: degrees of freedom

Temperature Dependence

Temperature Dependence (1,000 ng/mL)					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Methcathinone	3	94	23.11	2.70	**< .0001**
3-FMC	3	96	14.68	2.70	**< .0001**
4-FMC	3	91	16.15	2.70	**< .0001**
Methylone	3	93	30.51	2.70	**< .0001**
Ethcathinone	3	94	26.45	2.70	**< .0001**
Ethylone	3	90	24.56	2.71	**< .0001**
Methedrone	3	94	32.13	2.70	**< .0001**
Buphedrone	3	97	28.24	2.70	**< .0001**
Butylone	3	89	24.84	2.71	**< .0001**
Mephedrone	3	98	29.14	2.70	**< .0001**
Eutylone	3	85	24.50	2.71	**< .0001**
4-MEC	3	99	32.79	2.70	**< .0001**
MDPBP	3	84	14.86	2.71	**< .0001**
Pentedrone	3	98	29.34	2.70	**< .0001**
Pentylone	3	97	31.93	2.70	**< .0001**
3,4-DMMC	3	96	26.77	2.70	**< .0001**
α-PVP	3	91	23.11	2.70	**< .0001**
4-EMC	3	100	29.27	2.70	**< .0001**
MPBP	3	89	27.69	2.71	**< .0001**
MDPV	3	86	20.02	2.71	**< .0001**
Pyrovalerone	3	89	25.80	2.71	**< .0001**
Naphyrone	3	94	26.06	2.70	**< .0001**

Df: degrees of freedom

Analyte Dependence

Within cathinone sub-population

Within Group Significance					
Sub Group	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Secondary amine, unsubstituted Cathinones					
H	3	116	0.03	2.68	0.99
RT	3	114	0.17	2.68	0.91
R	3	84	0.90	2.71	0.45
F	3	64	1.18	2.75	0.32
Secondary amine, substituted Cathinones					
H	6	202	0.11	2.14	0.99
RT	6	200	0.55	2.14	0.77
R	6	144	4.56	2.16	**< .001**
F	6	116	2.65	2.18	**< .05**
Secondary amine, substituted (excl. 3-FMC) cathinones					
H	Difference not significant w/ 3-FMC included				
RT	Difference not significant w/ 3-FMC included				
R	5	124	1.02	2.29	0.41
F	5	99	1.60	2.31	0.17
Secondary amine, MD cathinones					
H	4	140	0.37	2.44	0.83
RT	4	121	0.44	2.45	0.78
R	4	105	3.63	2.46	**< .01**
F	4	104	7.05	2.49	**< .0001**
Tertiary amine, MD cathinones					
H	1	50	0.54	4.03	0.47
RT	1	44	0.14	4.06	0.71
R	1	41	0.40	4.08	0.53
F	1	31	0.001	4.16	0.97
Tertiary amine (excl. MD/3°) cathinones					
H	3	111	0.12	2.69	0.95
RT	3	100	0.36	2.70	0.78
R	3	84	2.79	2.71	**< 0.05**
F	3	61	3.69	2.76	**< 0.05**
Secondary amine, substituted cathinones					
H	15	458	0.55	1.69	0.91
RT	15	435	1.17	1.69	0.30
R	15	333	5.99	1.70	**< .0001**
F	15	260	2.42	1.70	**< .01**

(continued)

Within Group Significance					
Sub Group	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Secondary amine, substituted (excl. 3-FMC) cathinones					
H	Difference not significant w/ 3-FMC included				
RT	Difference not significant w/ 3-FMC included				
R	14	313	3.04	1.72	**< .001**
F	14	243	1.71	1.73	0.05
Tertiary amine cathinones					
H	5	161	2.03	2.27	0.08
RT	5	144	4.00	2.28	**< .01**
R	5	125	8.01	2.29	**< .0001**
F	5	92	7.67	2.31	**< .0001**

Df: degrees of freedom

Between cathinone sub-population

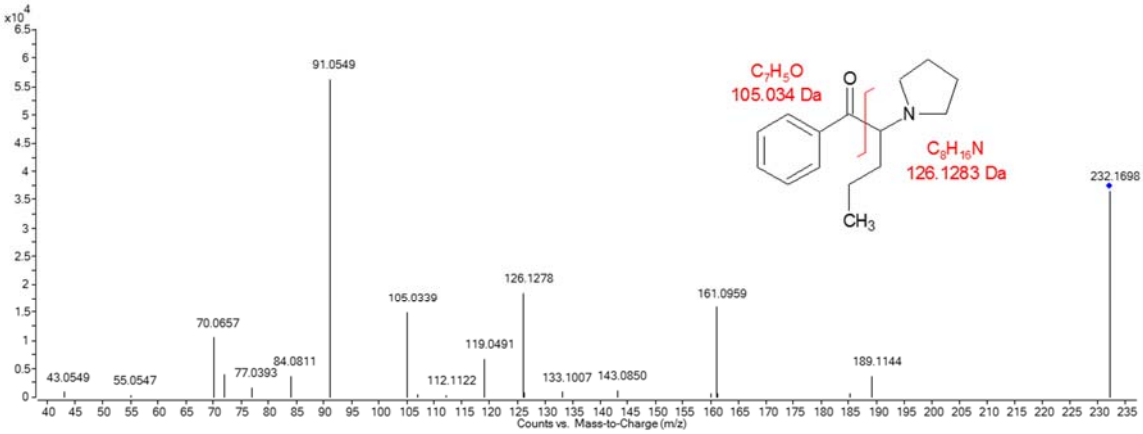
Between Group Significance					
Comparison	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Secondary amine, unsubstituted – secondary amine, substituted cathinones					
H	10	318	0.08	1.86	1.00
RT	10	314	0.38	1.86	0.95
R*	9	208	1.04	1.93	0.41
F*	9	172	1.29	1.94	0.24
Secondary amine, unsubstituted – secondary amine, MD cathinones					
H	8	256	0.65	1.97	0.73
RT	8	235	1.23	1.98	0.28
R	Significant difference *within* MD/2°				
F	Significant difference *within* MD/2°				
Secondary amine, unsubstituted – tertiary amine, MD cathinones					
H	5	166	4.04	2.27	**< .01**
RT	5	158	7.73	2.27	**< .0001**
R	5	125	4.68	2.29	**< .001**
F	5	95	0.93	2.31	0.47
Secondary amine, unsubstituted – tertiary amine, (excl. MD) cathinones					
H	7	227	0.49	2.05	0.84
RT	7	214	1.17	2.05	0.32
R	Significant difference *within* 3° (excl. MD)				
F	Significant difference *within* 3° (excl. MD)				
Secondary amine, substituted – secondary amine, MD cathinones					
H	11	342	0.67	1.82	0.77
RT	11	321	1.42	1.82	0.16
R	Significant difference *within* MD/2°				

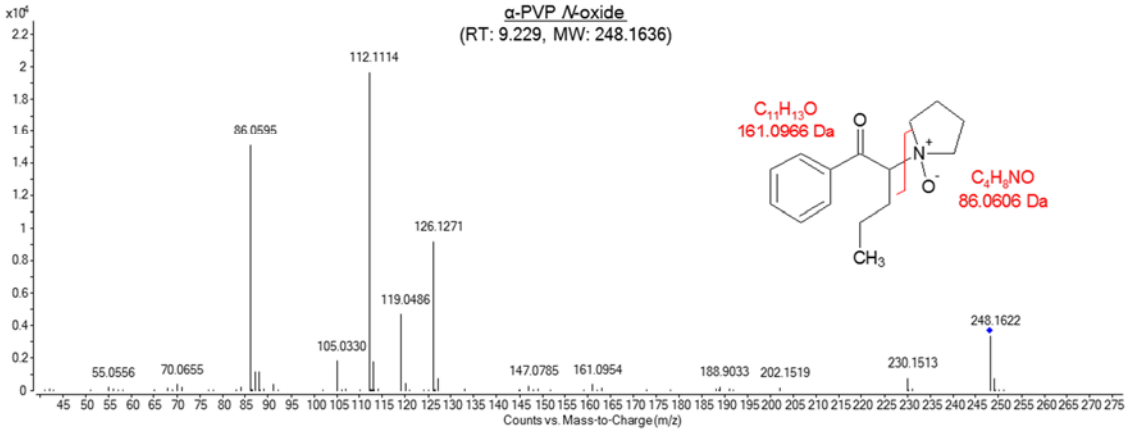
(continued)

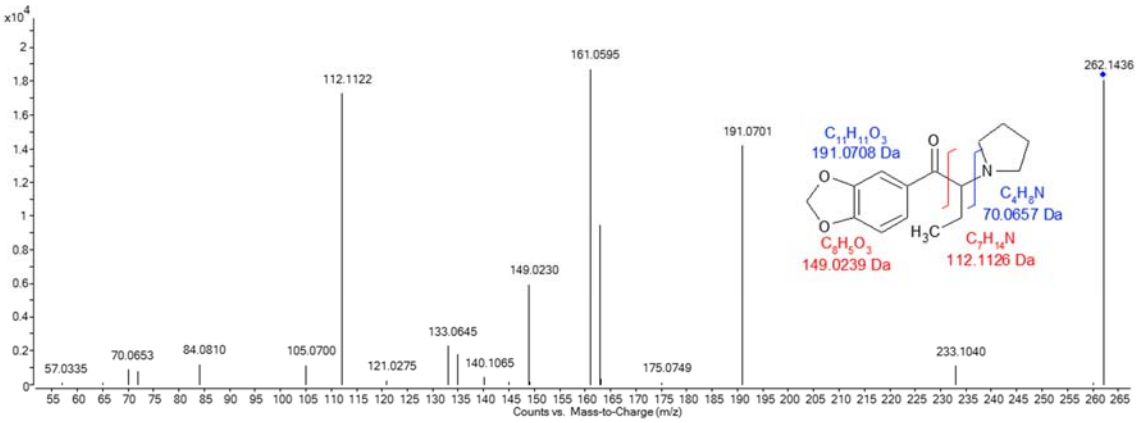
Between Group Significance					
Comparison	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
F	Significant difference *within* MD/2°				
Secondary amine, substituted – tertiary amine, MD cathinones					
H	8	252	3.12	1.98	**< .01**
RT	8	244	5.71	1.98	**< .0001**
R*	7	165	3.88	2.07	**< .001**
F*	7	130	1.40	2.08	0.21
Secondary amine, substituted – tertiary amine, (excl. MD) cathinones					
H	10	313	0.51	1.86	0.88
RT	10	300	1.33	1.86	0.21
R	Significant difference *within* 3° (excl. MD)				
F	Significant difference *within* 3° (excl. MD)				
Secondary amine, MD – tertiary amine, MD cathinones					
H	6	190	1.76	2.15	0.11
RT	6	165	2.94	2.15	**0.01**
R	Significant difference *within* MD/2°				
F	Significant difference *within* MD/2°				
Secondary amine, MD – tertiary amine (excl. MD) cathinones					
H	8	251	0.24	1.98	0.98
RT	8	221	0.36	1.98	0.94
R	Significant difference *within* MD/2° & *within* 3° (excl. MD)				
F	Significant difference *within* MD/2° & *within* 3° (excl. MD)				

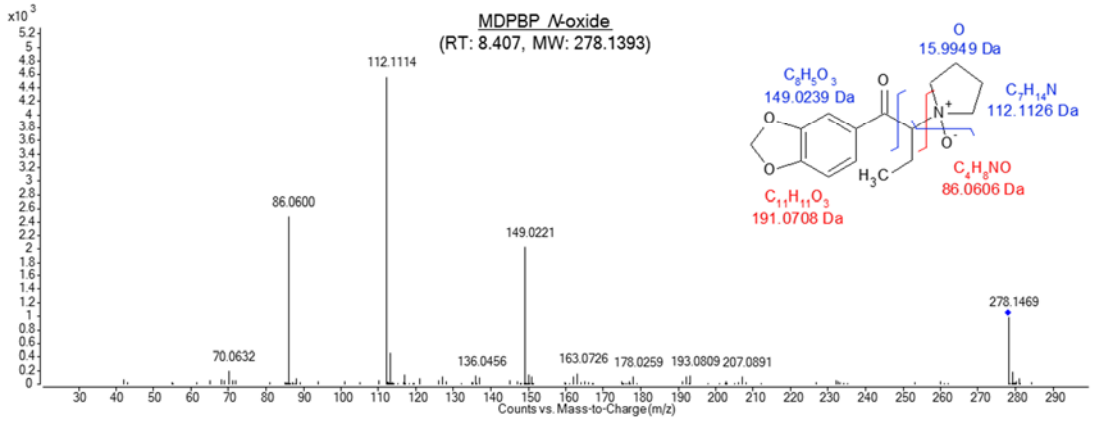
*Substituted (excluding 3-FMC) was used for statistical analysis; *Df*: degrees of freedom

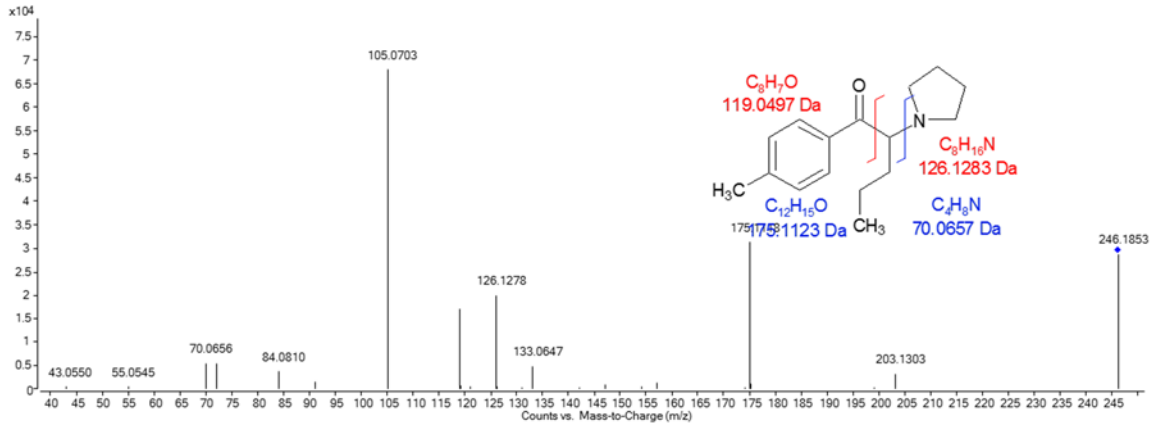
APPENDIX E: *N*-OXIDE DEGRADATION PRODUCT MS/MS SPECTRA

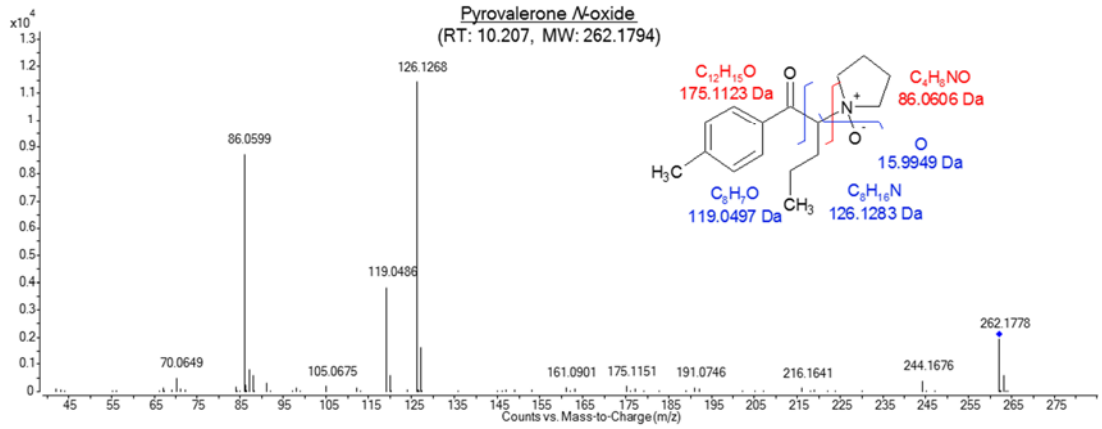


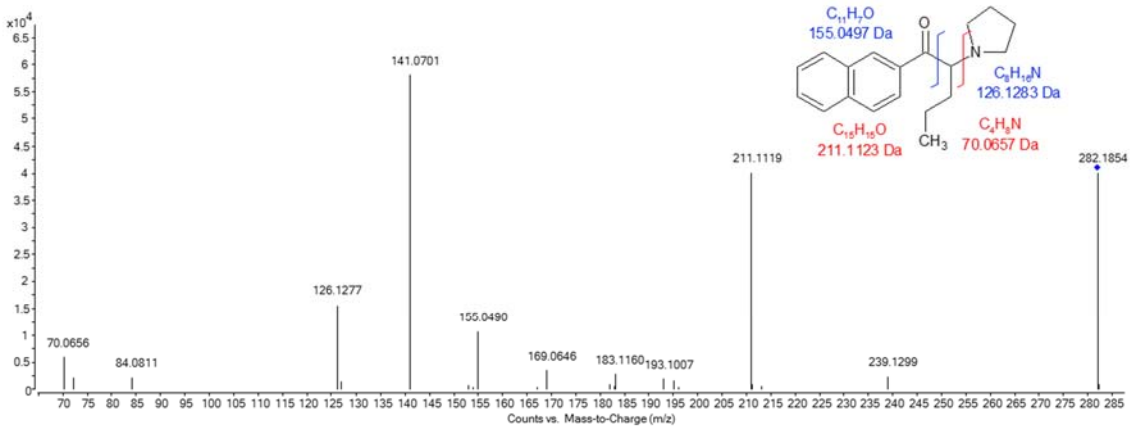


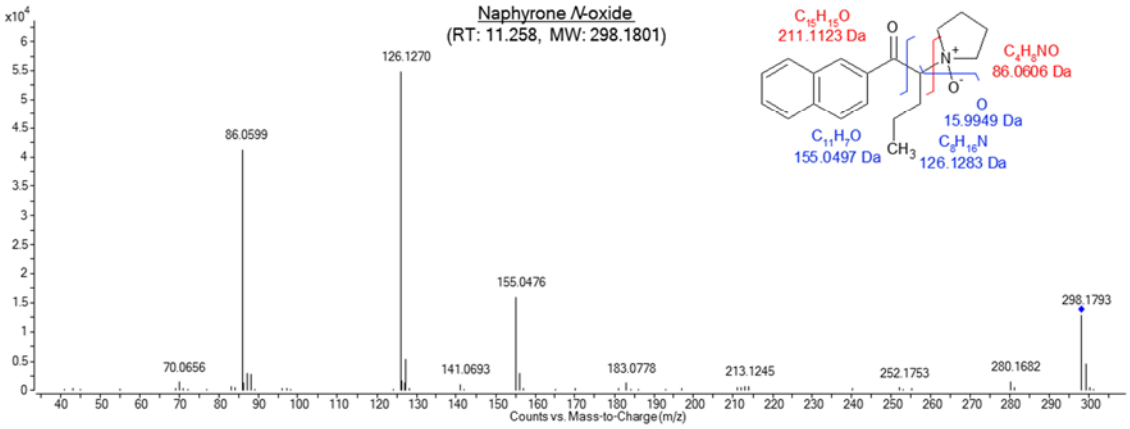

x10^4
262.1436
161.0595
112.1122
191.0701
149.0230
133.0645
57.0335
70.0653
84.0810
105.0700
121.0275
140.1065
175.0749
233.1040
C11H11O3
191.0708 Da
C4H8N
70.0657 Da
C8H5O3
149.0239 Da
C7H14N
112.1126 Da
Counts vs. Mass-to-Charge (m/z)


MDPBP N-oxide
(RT: 8.407, MW: 278.1393)
x10^3
112.1114
86.0600
149.0221
278.1469
70.0632
136.0456
163.0726
178.0259
193.0809
207.0891
O
15.9949 Da
C8H5O3
149.0239 Da
C7H14N
112.1126 Da
C4H8NO
86.0606 Da
C11H11O3
191.0708 Da
Counts vs. Mass-to-Charge (m/z)


x104
105.0703
126.1278
133.0647
175.1123
246.1853
203.1303
70.0656
84.0810
43.0550
55.0545
Counts vs. Mass-to-Charge (m/z)
C8H7O
119.0497 Da
C8H16N
126.1283 Da
C12H15O
175.1123 Da
C4H8N
70.0657 Da
H3C
CH3


Pyrovalerone N-oxide
(RT: 10.207, MW: 262.1794)
x10 4
126.1268
86.0599
119.0486
262.1778
70.0649
105.0675
161.0901
175.1151
191.0746
216.1641
244.1676
Counts vs. Mass-to-Charge (m/z)
C12H15O
175.1123 Da
C4H8NO
86.0606 Da
O
15.9949 Da
C8H7O
119.0497 Da
C8H16N
126.1283 Da
H3C
CH3


x10^4
141.0701
126.1277
155.0490
70.0656
84.0811
169.0646
183.1160
193.1007
211.1119
239.1299
282.1854
Counts vs. Mass-to-Charge (m/z)
C11H7O
155.0497 Da
C8H16N
126.1283 Da
C15H15O
211.1123 Da
C4H8N
70.0657 Da
CH3


Naphyrone N-oxide
(RT: 11.258, MW: 298.1801)
x10^4
126.1270
86.0599
155.0476
298.1793
70.0656
141.0693
183.0778
213.1245
252.1753
280.1682
Counts vs. Mass-to-Charge (m/z)
C15H15O
211.1123 Da
C4H8NO
86.0606 Da
O
15.9949 Da
C11H7O
155.0497 Da
C8H16N
126.1283 Da
CH3

APPENDIX F: ONE-WAY ANOVA STATISTICAL ANALYSIS FOR CONCENTRATION, TEMPERATURE, PH, AND ANALYTE DEPENDENCE IN URINE

One-way ANOVA statistical analysis for stability factors in urine. Bold *p*-values indicate a significant difference.

Concentration Dependence

		pH 4			
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Methcathinone					
H	1	53	0.04	4.02	0.84
RT	1	52	0.003	4.03	0.95
R	Stable, no comparison made				
F	Stable, no comparison made				
3-FMC					
H	1	45	0.01	4.06	0.91
RT	1	42	0.08	4.07	0.77
R	Stable, no comparison made				
F	Stable, no comparison made				
4-FMC					
H	1	54	0.0002	4.02	0.99
RT	1	50	0.07	4.03	0.79
R	Stable, no comparison made				
F	Stable, no comparison made				
Methylone					
H	1	49	0.28	4.04	0.60
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				
Ethcathinone					
H	1	45	0.23	4.06	0.63
RT	1	43	0.07	4.07	0.79
R	Stable, no comparison made				
F	Stable, no comparison made				
Ethylone					
H	1	45	0.01	4.06	0.92
RT	Stable, no comparison made				

(continued)

pH 4					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
R	Stable, no comparison made				
F	Stable, no comparison made				
Methedrone					
H	1	47	0.02	4.05	0.88
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				
Buphedrone					
H	1	50	0.19	4.03	0.67
RT	1	53	0.16	4.02	0.69
R	Stable, no comparison made				
F	Stable, no comparison made				
Butylone					
H	1	46	0.45	4.05	0.50
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				
Mephedrone					
H	1	53	0.07	4.02	0.79
RT	1	49	1.00	4.04	0.32
R	Stable, no comparison made				
F	Stable, no comparison made				
Eutylone					
H	1	47	0.74	4.05	0.40
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				
4-MEC					
H	1	50	0.02	4.03	0.88
RT	1	48	0.10	4.04	0.75
R	Stable, no comparison made				
F	Stable, no comparison made				
MDPBP					
H	1	40	0.21	4.08	0.65
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				

(continued)

pH 4					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Pentedrone					
H	1	48	0.01	4.04	0.90
RT	1	53	0.66	4.02	0.42
R	Stable, no comparison made				
F	Stable, no comparison made				
Pentylone					
H	1	68	0.17	3.98	0.68
RT	1	68	1.23	3.98	0.27
R	Stable, no comparison made				
F	Stable, no comparison made				
3,4-DMMC					
H	1	46	0.08	4.05	0.78
RT	1	49	1.46	4.04	0.23
R	Stable, no comparison made				
F	Stable, no comparison made				
α-PVP					
H	Stable, no comparison made				
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				
4-EMC					
H	1	47	0.01	4.05	0.94
RT	1	51	0.34	4.03	0.56
R	Stable, no comparison made				
F	Stable, no comparison made				
MPBP					
H	Stable, no comparison made				
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				
MDPV					
H	Stable, no comparison made				
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				
Pyrovalerone					
H	1	44	0.25	4.06	0.62
RT	1	50	0.36	4.03	0.55

(continued)

pH 4					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
R	Stable, no comparison made				
F	Stable, no comparison made				
Naphyrone					
H	1	45	0.30	4.06	0.59
RT	Stable, no comparison made				
R	Stable, no comparison made				
F	Stable, no comparison made				

Df: degrees of freedom

pH 8					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Methcathinone					
H	1	65	5.07E-05	3.99	0.99
RT	1	66	0.01	3.99	0.93
R	1	63	0.26	3.99	0.61
F	1	52	0.20	4.03	0.66
3-FMC					
H	1	56	0.0	4.0	1.0
RT	1	57	0.0	4.0	1.0
R	1	48	0.6	4.0	0.4
F	1	48	0.3	4.0	0.6
4-FMC					
H	1	64	4.0E-05	3.99	0.99
RT	1	64	0.00	3.99	0.99
R	1	57	0.02	4.01	0.90
F	1	52	0.07	4.03	0.80
Methylone					
H	1	65	0.01	3.99	0.91
RT	1	64	0.04	3.99	0.85
R	1	60	0.19	4.00	0.67
F	1	52	0.21	4.03	0.65
Ethcathinone					
H	1	61	0.0003	4.00	0.99
RT	1	63	0.03	3.99	0.86
R	1	55	1.08	4.02	0.30
F	1	46	0.04	4.05	0.84

(continued)

pH 8					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Ethylone					
H	1	65	0.001	3.99	0.98
RT	1	66	0.002	3.99	0.97
R	1	58	0.07	4.01	0.80
F	1	51	0.31	4.03	0.58
Methedrone					
H	1	63	0.01	3.99	0.94
RT	1	64	0.01	3.99	0.91
R	1	56	0.26	4.01	0.61
F	1	46	0.00	4.05	0.96
Buphedrone					
H	1	65	0.004	3.99	0.95
RT	1	64	0.02	3.99	0.89
R	1	55	21.17	4.02	**< .0001**
F	1	55	14.73	4.02	**< .001**
Butylone					
H	1	65	0.002	3.99	0.96
RT	1	64	0.001	3.99	0.98
R	1	55	0.001	4.02	0.97
F	1	42	0.75	4.07	0.39
Mephedrone					
H	1	65	0.002	3.99	0.96
RT	1	66	0.003	3.99	0.96
R	1	61	0.38	4.00	0.54
F	1	52	0.01	4.03	0.91
Eutylone					
H	1	66	0.02	3.99	0.88
RT	1	63	0.31	3.99	0.58
R	1	54	0.52	4.02	0.47
F	Stable, no comparison made				
4-MEC					
H	1	65	0.002	3.99	0.96
RT	1	66	0.01	3.99	0.91
R	1	60	0.001	4.00	0.98
F	1	48	0.42	4.04	0.52
MDPBP					
H	1	44	1.14	4.06	0.29
RT	1	42	10.17	4.07	**< .01**

(continued)

pH 8					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
R	1	40	0.83	4.08	0.37
F	Stable, no comparison made				
Pentedrone					
H	1	65	0.0003	3.99	0.99
RT	1	66	0.003	3.99	0.95
R	1	61	0.02	4.00	0.89
F	1	50	0.002	4.03	0.97
Pentylone					
H	1	65	0.01	3.99	0.90
RT	1	64	0.01	3.99	0.94
R	1	56	0.15	4.01	0.70
F	1	47	1.55	4.05	0.22
3,4-DMMC					
H	1	63	9E-05	3.99	0.99
RT	1	64	0.05	3.99	0.83
R	1	51	0.01	4.03	0.91
F	1	48	0.44	4.04	0.51
α-PVP					
H	1	60	0.02	4.00	0.89
RT	1	58	0.003	4.01	0.96
R	1	48	3.41	4.04	0.07
F	Stable, no comparison made				
4-EMC					
H	1	63	0.001	3.99	0.98
RT	1	64	0.002	3.99	0.96
R	1	59	0.23	4.00	0.63
F	1	49	0.07	4.04	0.79
MPBP					
H	1	50	1.32	4.03	0.26
RT	1	50	6.37	4.03	**0.01**
R	1	47	0.39	4.05	0.54
F	1	41	1.65	4.08	0.21
MDPV					
H	1	55	0.004	4.02	0.95
RT	1	49	0.003	4.04	0.95
R	Stable, no comparison made				
F	Stable, no comparison made				

(continued)

pH 8					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Pyrovalerone					
H	1	61	0.24	4.00	0.63
RT	1	54	0.23	4.02	0.64
R	1	53	2.04	4.02	0.16
F	Stable, no comparison made				
Naphyrone					
H	1	66	0.05	3.99	0.82
RT	1	63	0.002	3.99	0.96
R	1	58	0.004	4.01	0.95
F	1	48	3.17	4.04	0.08

Df: degrees of freedom

Temperature Dependence

pH 4; 1,000 ng/mL					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Methcathinone	3	103	23.72	2.69	**< .0001**
3-FMC	3	86	31.75	2.71	**< .0001**
4-FMC	3	97	20.48	2.70	**< .0001**
Methylone	3	103	9.89	2.69	**< .0001**
Ethcathinone	3	90	14.00	2.71	**< .0001**
Ethylone	3	101	15.31	2.69	**< .0001**
Methedrone	3	97	15.65	2.70	**< .0001**
Buphedrone	3	103	12.05	2.69	**< .0001**
Butylone	3	100	5.27	2.70	**< .01**
Mephedrone	3	103	13.30	2.69	**< .0001**
Eutylone	3	96	4.28	2.70	**< .01**
4-MEC	3	88	10.27	2.71	**< .0001**
MDPBP	3	80	1.33	2.72	0.27
Pentedrone	3	100	20.27	2.70	**< .0001**
Pentylone	3	99	10.60	2.70	**< .0001**
3,4-DMMC	3	93	20.69	2.70	**< .0001**
α-PVP	3	90	5.12	2.71	**< .01**
4-EMC	3	95	15.77	2.70	**< .0001**

(continued)

pH 4; 1,000 ng/mL					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
MPBP	3	90	2.45	2.71	0.07
MDPV	3	103	46.49	2.69	**< .0001**
Pyrovalerone	3	98	3.06	2.70	**< .05**
Naphyrone	3	102	5.18	2.69	**< .01**

Df: degrees of freedom

pH 8; 1,000 ng/mL					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Methcathinone	3	125	25.55	2.68	**< .0001**
3-FMC	3	101	9.83	2.69	**< .0001**
4-FMC	3	121	25.02	2.68	**< .0001**
Methylone	3	123	59.82	2.68	**< .0001**
Ethcathinone	3	112	35.49	2.69	**< .0001**
Ethylone	3	121	68.77	2.68	**< .0001**
Methedrone	3	113	46.48	2.68	**< .0001**
Buphedrone	3	119	37.65	2.68	**< .0001**
Butylone	3	113	57.13	2.68	**< .0001**
Mephedrone	3	124	39.00	2.68	**< .0001**
Eutylone	3	120	69.45	2.68	**< .0001**
4-MEC	3	119	35.83	2.68	**< .0001**
MDPBP	3	78	38.87	2.72	**< .0001**
Pentedrone	3	121	31.29	2.68	**< .0001**
Pentylone	3	119	56.15	2.68	**< .0001**
3,4-DMMC	3	117	42.79	2.68	**< .0001**
α-PVP	3	103	28.39	2.69	**< .0001**
4-EMC	3	119	29.09	2.68	**< .0001**
MPBP	3	92	37.38	2.70	**< .0001**
MDPV	3	98	18.76	2.70	**< .0001**
Pyrovalerone	3	106	47.37	2.69	**< .0001**
Naphyrone	3	116	48.17	2.68	**< .0001**

Df: degrees of freedom

pH Dependence

	1,000 ng/mL				
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Methcathinone					
H	1	60	34.31	4.00	**< .0001**
RT	1	59	177.28	4.00	**< .0001**
R	1	59	151.82	4.00	**< .0001**
F	1	50	50.94	4.03	**< .0001**
3-FMC					
H	1	51	11.26	4.03	**< .001**
RT	1	49	36.01	4.04	**< .0001**
R	1	43	164.39	4.07	**< .0001**
F	1	44	86.33	4.06	**< .0001**
4-FMC					
H	1	59	30.40	4.00	**< .0001**
RT	1	56	144.46	4.01	**< .0001**
R	1	54	173.56	4.02	**< .0001**
F	1	49	53.92	4.04	**< .0001**
Methylone					
H	1	60	57.23	4.00	**< .0001**
RT	1	59	319.88	4.00	**< .0001**
R	1	57	75.64	4.01	**< .0001**
F	1	50	42.24	4.03	**< .0001**
Ethcathinone					
H	1	55	36.12	4.02	**< .0001**
RT	1	53	186.40	4.02	**< .0001**
R	1	51	153.66	4.03	**< .0001**
F	1	43	32.94	4.07	**< .0001**
Ethylone					
H	1	57	49.81	4.01	**< .0001**
RT	1	58	286.28	4.01	**< .0001**
R	1	57	70.88	4.01	**< .0001**
F	1	50	38.03	4.03	**< .0001**
Methedrone					
H	1	55	44.80	4.02	**< .0001**
RT	1	57	277.38	4.01	**< .0001**

(continued)

		1,000 ng/mL			
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
R	1	52	78.75	4.03	**< .0001**
F	1	46	29.17	4.05	**< .0001**
Buphedrone					
H	1	60	53.70	4.00	**< .0001**
RT	1	59	296.93	4.00	**< .0001**
R	1	55	111.85	4.02	**< .0001**
F	1	48	42.74	4.04	**< .0001**
Butylone					
H	1	58	69.11	4.01	**< .0001**
RT	1	58	182.92	4.01	**< .0001**
R	1	52	48.20	4.03	**< .0001**
F	1	45	13.83	4.06	**< .001**
Mephedrone					
H	1	61	50.08	4.00	**< .0001**
RT	1	58	263.83	4.01	**< .0001**
R	1	58	128.86	4.01	**< .0001**
F	1	50	48.25	4.03	**< .0001**
Eutylone					
H	1	58	72.74	4.01	**< .0001**
RT	1	58	120.83	4.01	**< .0001**
R	1	55	47.92	4.02	**< .0001**
F	1	45	9.36	4.06	**< .01**
4-MEC					
H	1	58	41.93	4.01	**< .0001**
RT	1	56	185.98	4.01	**< .0001**
R	1	52	83.24	4.03	**< .0001**
F	1	41	24.96	4.08	**< .0001**
MDPBP					
H	1	39	74.09	4.09	**< .0001**
RT	1	40	96.67	4.08	**< .0001**
R	1	41	23.57	4.08	**< .0001**
F	1	38	5.37	4.10	**< 0.05**
Pentedrone					
H	1	57	40.05	4.01	**< .0001**

(continued)

		1,000 ng/mL			
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
RT	1	60	288.98	4.00	**< .0001**
R	1	58	129.85	4.01	**< .0001**
F	1	46	40.90	4.05	**< .0001**
Pentylone					
H	1	54	49.55	4.02	**< .0001**
RT	1	59	194.47	4.00	**< .0001**
R	1	58	63.63	4.01	**< .0001**
F	1	47	16.94	4.05	**< .0001**
3,4-DMMC					
H	1	54	38.10	4.02	**< .0001**
RT	1	56	227.89	4.01	**< .0001**
R	1	52	115.15	4.03	**< .0001**
F	1	48	49.35	4.04	**< .0001**
α-PVP					
H	1	48	77.74	4.04	**< .0001**
RT	1	56	76.14	4.01	**< .0001**
R	1	50	58.10	4.03	**< .0001**
F	1	39	5.74	4.09	**< .05**
4-EMC					
H	1	55	35.15	4.02	**< .0001**
RT	1	58	232.32	4.01	**< .0001**
R	1	56	95.82	4.01	**< .0001**
F	1	45	43.92	4.06	**< .0001**
MPBP					
H	1	46	84.50	4.05	**< .0001**
RT	1	50	82.03	4.03	**< .0001**
R	1	46	28.81	4.05	**< .0001**
F	1	40	2.20	4.08	0.14
MDPV					
H	1	47	61.11	4.05	**< .0001**
RT	1	54	26.05	4.02	**< .0001**
R	1	53	26.63	4.02	**< .0001**
F	1	47	1.85	4.05	0.18

(continued)

1,000 ng/mL					
Synthetic Cathinone	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Pyrovalerone					
H	1	53	100.08	4.02	**< .0001**
RT	1	50	77.72	4.03	**< .0001**
R	1	54	57.82	4.02	**< .0001**
F	1	47	5.02	4.05	**< 0.05**
Naphyrone					
H	1	56	73.52	4.01	**< .0001**
RT	1	57	140.36	4.01	**< .0001**
R	1	59	65.49	4.00	**< .0001**
F	1	46	15.89	4.05	**< .0001**

Analyte Dependence

Within sub-population

Within Group Significance (pH 4)					
Sub Group	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Secondary amine, unsubstituted cathinones					
H	3	76	1.25	2.72	0.30
RT	3	77	1.31	2.72	0.28
R	3	80	2.25	2.72	0.09
F	3	69	0.19	2.74	0.90
Secondary amine, substituted cathinones					
H	6	130	3.24	2.17	**< .01**
RT	6	127	12.11	2.17	**< .0001**
R	6	131	3.31	2.17	**< .01**
F	6	119	5.78	2.18	**< .0001**
Secondary amine, substituted (excl. 3-FMC) cathinones					
H	5	112	0.85	2.30	0.52
RT	5	111	3.94	2.30	**< .01**
R	5	113	3.53	2.29	**< .01**
F	5	104	7.41	2.30	**< .0001**
Secondary amine, MD cathinones					
H	4	91	2.07	2.47	0.09
RT	4	102	0.15	2.46	0.96
R	4	108	1.41	2.46	0.24
F	4	84	2.73	2.48	**< 0.05**

(continued)

Within Group Significance (pH 4)					
Sub Group	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Tertiary amine, MD cathinones					
H	1	33	0.39	4.14	0.53
RT	1	41	13.66	4.08	**< .001**
R	1	40	12.68	4.08	**< .001**
F	1	32	26.22	4.15	**< .0001**
Tertiary amine (excl. tertiary amine, MD) cathinones					
H	3	64	4.64	2.75	**< .01**
RT	3	85	1.60	2.71	0.19
R	3	87	3.02	2.71	**< .05**
F	3	66	1.03	2.74	0.38
Secondary amine cathinones					
H	15	297	4.85	1.70	**< .0001**
RT	15	306	11.22	1.70	**< .0001**
R	15	319	3.39	1.70	**< .0001**
F	15	272	4.29	1.70	**< .0001**
Secondary amine (excl. 3-FMC) cathinones					
H	14	279	3.19	1.73	**< .0001**
RT	14	290	5.86	1.73	**< .0001**
R	14	301	3.78	1.72	**< .0001**
F	14	257	4.66	1.73	**< .0001**
Tertiary amine cathinones					
H	5	97	2.68	2.31	**< .05**
RT	5	126	3.23	2.29	**< .01**
R	5	127	4.74	2.29	**< .001**
F	5	98	8.71	2.31	**< .0001**

Df: degrees of freedom

Within Group Significance (pH 8)					
Sub Group	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Secondary amine, unsubstituted cathinones					
H	3	106	0.02	2.69	1.00
RT	3	106	0.13	2.69	0.94
R	3	97	0.58	2.70	0.63
F	3	73	1.33	2.73	0.27
Secondary amine, substituted cathinones					
H	6	182	0.04	2.15	1.00

(continued)

Within Group Significance (pH 8)					
Sub Group	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
RT	6	182	0.41	2.15	0.87
R	6	160	2.13	2.16	0.05
F	6	130	7.38	2.17	**< .0001**
Secondary amine, substituted (excl. 3-FMC) cathinones					
H	Difference not significant w/ 3-FMC included				
RT	Difference not significant w/ 3-FMC included				
R	5	144	1.85	2.28	0.11
F	5	111	3.17	2.30	**< .01**
Secondary amine, MD cathinones					
H	4	135	0.71	2.44	0.58
RT	4	134	2.02	2.44	0.10
R	4	113	4.38	2.45	**< .01**
F	4	96	3.70	2.47	**< .01**
Tertiary amine, MD cathinones					
H	1	36	2.73	4.11	0.11
RT	1	34	14.78	4.13	**< .001**
R	1	34	0.01	4.13	0.91
F	1	33	7.16	4.14	**< .01**
Tertiary amine, (excl. tertiary amine, MD) cathinones					
H	3	98	3.04	2.70	**< .05**
RT	3	88	4.35	2.71	**< .01**
R	3	82	6.64	2.72	**< .001**
F	3	67	4.05	2.74	**< .01**
Secondary amine cathinones					
H	15	423	0.58	1.69	0.89
RT	15	422	2.38	1.69	**< .01**
R	15	370	7.74	1.69	**< .0001**
F	15	314	9.54	1.70	**< .0001**
Secondary amine (excl. 3-FMC) cathinones					
H	Difference not significant w/ 3-FMC included				
RT	14	399	2.33	1.72	**< .01**
R	14	354	7.71	1.72	**< .0001**
F	14	280	6.59	1.73	**< .0001**
Tertiary amine cathinones					
H	5	134	7.50	2.28	**< .0001**
RT	5	122	8.28	2.29	**< .0001**
R	5	116	8.22	2.29	**< .0001**
F	5	100	5.71	2.31	**< .0001**

Df: degrees of freedom

Between Sub-population

Between Group Significance (pH 4)					
Comparison	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Secondary amine, unsubstituted – secondary amine, substituted					
H *	9	188	0.90	1.93	0.53
RT	Significant difference *within* Sub even when 3-FMC excluded				
R	Significant difference *within* Sub even when 3-FMC excluded				
F	Significant difference *within* Sub even when 3-FMC excluded				
Secondary amine, unsubstituted - secondary amine, MD					
H	8	167	4.77	1.99	**< .0001**
RT	8	179	8.58	1.99	**< .0001**
R	8	188	2.43	1.99	**< .05**
F	Significant difference *within* MD/2°				
Secondary amine, unsubstituted - tertiary amine, MD					
H	5	109	9.34	2.30	**< .0001**
RT	Significant difference *within* MD/3°				
R	Significant difference *within* MD/3°				
F	Significant difference *within* MD/3°				
Secondary amine, unsubstituted - tertiary amine (excl. MD) cathinones					
H	Significant difference *within* 3°				
RT	7	162	11.41	2.07	**< .0001**
R	Significant difference *within* 3°				
F	7	135	0.47	2.08	0.85
Secondary amine, substituted - secondary amine, MD cathinones					
H *	10	203	3.55	1.88	**< .0001**
RT	Significant difference *within* Sub even when 3-FMC excluded				
R	Significant difference *within* Sub even when 3-FMC excluded				
F	Significant difference *within* Sub even when 3-FMC excluded & *within* MD/2°				
Secondary amine, substituted - tertiary amine, MD cathinones					
H *	7	145	5.87	2.07	**< .0001**
RT	Significant difference *within* Sub even when 3-FMC excluded & *within* MD/3°				
R	Significant difference *within* Sub even when 3-FMC excluded & *within* MD/3°				
F	Significant difference *within* Sub even when 3-FMC excluded & *within* MD/3°				

(continued)

Between Group Significance (pH 4)					
Comparison	*Df-Between*	*Df-Within*	*F*	*F crit*	*P-value*
Secondary amine, substituted - tertiary amine, (excl. MD) cathinones					
H	Significant difference *within* 3° (excl. MD)				
RT	Significant difference *within* Sub even when 3-FMC excluded				
R	Significant difference *within* Sub even when 3-FMC excluded & *within* 3° (excl. MD)				
F	Significant difference *within* Sub even when 3-FMC excluded				
Secondary amine, MD – tertiary amine, MD cathinones					
H	6	124	4.37	2.17	**< .001**
RT	Significant difference *within* MD/3°				
R	Significant difference *within* MD/3°				
F	Significant difference *within* MD/3° & *within* MD/2°				
Secondary amine, MD – tertiary amine (excl. MD) cathinones					
H	Significant difference *within* 3° (excl. MD)				
RT	8	187	1.66	1.99	0.11
R	Significant difference *within* 3° (excl. MD)				
F	Significant difference *within* MD/2°				

*Substituted (excluding 3-FMC) was used for comparison; *Df*: degrees of freedom

APPENDIX G: QUANTITATION RESULTS FOR REDWOOD TOXICOLOGY LABORATORY (RTL) CASES

α-PVP (n=92)					
Case Number	**RTL Quant**	**pH**	**SHSU Quant**	**% Remaining**	**Date Difference (m)**
R054	9523	4.5	7189	75%	15
R046	11943	5	10826	91%	17
R063	12314	5	10765	87%	17
R079	16316	5	11921	73%	17
R121	7435	5	446	6%	15
R123	67	5	70	104%	15
R135	7465	5	7580	102%	14
R003	729	5.5	688	94%	40
R065	500	5.5	593	119%	15
R086	1427	5.5	1671	117%	15
R012	1429	5.57	1301	91%	13
2R004	100	6	110	110%	8
R039	5480	6	5057	92%	15
R055	18094	6	19926	110%	17
R070	5396	6	4838	90%	15
R111	2887	6	3261	113%	15
R119	4328	6	4711	109%	15
R139	540	6	72	13%	14
2R015	68	6.5	0	0%	5
2R020	101	6.5	103	102%	6
2R021	87	6.5	100	115%	5
R011	2114	6.5	2014	95%	13
R058	7396	6.5	6734	91%	15
R004	2347	7	2201	94%	14
R029	7277	7	4766	65%	15
R031	1094	7	1058	97%	15
R033	270	7	257	95%	15
R036	330	7	318	96%	15
R041	93	7	99	106%	15
R052	2948	7	2782	94%	15

(continued)

α-PVP (n=92)					
Case Number	**RTL Quant**	**pH**	**SHSU Quant**	**% Remaining**	**Date Difference (m)**
R081	11187	7	0	0%	17
R084	2501	7	1562	62%	15
R088	14112	7	7644	54%	17
R094	129	7	135	105%	15
R110	7774	7	5473	70%	15
R113 ReExt	13292	7	7838	59%	16
R137	3524	7	3805	108%	14
R147	95	7	103	108%	14
R014	4599	7.5	2866	62%	13
R017	797	7.5	31	4%	13
R032	2407	7.5	145	6%	15
R059	10627	7.5	450	4%	17
R069	13703	7.5	2206	16%	17
R030	147	8	0	0%	15
R051	352	8	0	0%	15
R053	329	8	10	3%	15
R064	7679	8	8221	107%	15
R083	375	8	7	2%	15
R091	26	8	0	0%	15
R097	324	8	287	89%	15
R115	25	8	9	35%	15
R116	11095	8	144	1%	16
R117	7255	8	6368	88%	15
R120	471	8	2	0%	15
R146	12311	8	755	6%	16
R150	1013	8	5	0%	14
R151	40	8	10	26%	14
R157	23792	8	14802	62%	16
R009	200	8.5	7	3%	13
R034	77	8.5	0	0%	15
R035	36	8.5	1	3%	15
R037	1105	8.5	74	7%	15
R038	375	8.5	18	5%	15

(continued)

α-PVP (n=92)					
Case Number	**RTL Quant**	**pH**	**SHSU Quant**	**% Remaining**	**Date Difference (m)**
R049	1686	8.5	45	3%	15
R050	520	8.5	5	1%	15
R056	1770	8.5	80	5%	15
R061	131	8.5	6	5%	15
R062	37	8.5	5	13%	15
R068	114	8.5	5	4%	15
R107	703	8.5	22	3%	15
R010	31	9	2	5%	13
R018	2126	9	37	2%	13
R066	1042	9	61	6%	15
R078	4091	9	24	1%	15
R096	85	9	2	3%	15
R099	247	9	0	0%	15
R101	1477	9	0	0%	15
R102	2466	9	49	2%	15
R109	285	9	16	6%	15
R114	1628	9	16	1%	15
R129	46	9	1	2%	14
R133	6864	9	246	4%	14
R136	392	9	58	15%	14
R148	12333	9	231	2%	16
R152	26	9	12	45%	14
R159	764	9	107	14%	14
R016	1201	9.29	45	4%	13
R015	450	9.5	71	16%	13
R122	27	10	1	4%	15
R124	1348	10	65	5%	14
R125	570	10	5	1%	14
R138	392	10	3	1%	14

Ethylone (n=55)					
Case Number	**RTL Quant**	**pH**	**SHSU Quant**	**% Remaining**	**Date Difference (m)**
2R006	36	4.5	22	61%	7
2R001	379	4.5	255	67%	8
2R011	1542	5	1162	75%	7
R156	252	5	171	68%	14
R027	127	5	69	55%	15
R104	74050	5	56418	76%	17
2R018	189	5.5	150	79%	6
2R002	98	5.5	66	67%	8
R045	1312	5.5	23	2%	15
R080	97	5.5	88	91%	15
R085	1059	5.5	1037	98%	15
R106	308	5.5	273	89%	15
R077	38	6	21	56%	15
R090	195	6	198	102%	15
R040	131	6	132	101%	15
R023	206	6	144	70%	15
R025	110	6	68	61%	15
R026	75	6	22	30%	15
R103 ReExt	167973	6	146124	87%	17
R105 ReExt	9584	6	9368	98%	17
R089	32661	6	12284	38%	17
R008	275	6.5	240	87%	13
2R005	91	7	0	0%	7
R140	312	7	7	2%	14
R131	59	7	5	9%	14
R071	433	7	3	1%	14
R118	2788	7	153	6%	15
R095	42	7	31	74%	15
R092	195	7	94	48%	15
R043	272	7	0	0%	15
R022	2257	7	493	22%	15
R028	6416	7.5	0	0%	15
R087	22512	7.5	0	0%	17

(continued)

Ethylone (n=55)					
Case Number	**RTL Quant**	**pH**	**SHSU Quant**	**% Remaining**	**Date Difference (m)**
R073	119535	7.5	0	0%	17
R149	30	8	0	0%	14
R154	41	8	0	0%	14
R155	35	8	0	0%	14
R145	105	8	0	0%	14
R126	39	8	0	0%	14
R072	237	8	0	0%	15
R112	110	8	6	6%	15
R047	1144	8	0	0%	15
R024	62	8	0	0%	15
R013	642	8.5	0	0%	13
R005	487	8.5	0	0%	13
R006	121	8.5	0	0%	13
R075	37080	8.5	0	0%	17
R141	123	9	0	0%	14
R144	33	9	0	0%	14
R100	69	9	0	0%	14
R098	695	9	0	0%	15
R076	8423	9	17	0%	15
R130	102	9.5	0	0%	14
R108	6311	9.5	0	0%	15
R153	61	10	0	0%	14

Methylone (n=9)					
Case Number	**RTL Quant**	**pH**	**SHSU Quant**	**% Remaining**	**Date Difference (m)**
2R001	87	4.5	56	64%	7.9
2R011	175	5	110	63%	6.5
2R016	1535	5	922	60%	5.9
2R002	32	5.5	26	81%	7.8
2R018	246	5.5	191	78%	5.7
2R004	75	6	9	12%	7.5
2R020	84	6.5	15	18%	5.6
2R021	56	6.5	12	21%	5.4
R002	7316	9.32	2	0.03%	56.7

Case Number	**Cathinone**	**RTL Quant**	**pH**	**SHSU Quant**	**% Remaining**	**Date Difference**
R049	MDPV	6626	8.5	479	7%	15
R001	butylone	385	6.5	50	13%	59
2R010	pentylone	585	6.5	434	74%	7

VITA

Lindsay Glicksberg

Relevant Professional Experience

Sam Houston State University

August 2013- Present

- Graduate Assistant
- Aided in laboratory preparation, instrument troubleshooting, mentoring students, inventory, and administrative duties.
- Teaching Assistant for Forensic Toxicology Lab.

Federal Bureau of Investigation (FBI) Honors Internship Program

June 2014-May 2015

- Interned in the Explosives Unit/Forensic Operational Unit of the Terrorist Explosive Device Analytical Center (TEDAC) at the FBI Laboratory in Quantico, VA.
- Obtained Top Secret Clearance.
- Performed limit of detection studies on the following instruments:
    - Gas Chromatography-Electron Capture Detection (GC-ECD), Gas Chromatography/Mass Spectrometry (GC/MS), Ion Chromatography (IC), Liquid Chromatography/Mass Spectrometry (LC/MS)
- Gained experience using a variety of software including:
    - ChemStation, Chromeleon, Xcalibur, PDXL, Omnic.
- Observed casework for the analysis of bulk explosives and explosive residues,
- Completed a general unknown mock case using the following instrumentation:
    - X-Ray Diffraction (XRD), Scanning Electron Microscopy-Energy Dispersive X-Ray Spectrometry (SEM-EDS), FT-IR.
- Maintained FBI clearance interning at the Houston FBI Field Office during the academic year.

Tulsa Police Crime Lab Internship

September 2012-April 2013

- Assisted in building the FT-IR Library of controlled and non-controlled substances.

Education

Sam Houston State University **August 2013-Present**

- Pending Doctor of Philosophy in Forensic Science
- GPA: 4.0
- Expected Graduation: August 2017
- Dissertation: "Identification and Stability of Synthetic Cathinones in Biological Samples"

The University of Tulsa **August 2009-May 2013**

- B.S. in Chemistry with a minor in Anthropology
- GPA: 3.84
- Graduated Magna Cum Laude

Relevant Educational Experience

Sam Houston State University

- Forensic Toxicology, Forensic Instrumental Analysis, Advanced Instrumental Analysis, Quality Assurance and Ethics, Forensic Statistics and Evidence Interpretation, Law and Forensic Science, Forensic Biology, Advanced DNA Analysis, Trace Evidence and Microscopic Analysis, Controlled Substances,

The University of Tulsa

- Participated in research with Professors in Chemistry and Biochemistry Department since 2010
- Presented at the Spring 2013 American Chemical Society (ACS) meeting
- Analytical Chemistry I (Qualitative and Quantitative Analysis), Analytical Chemistry II (Instrumental Analysis), Analytical Forensic Toxicology, Introduction to Statistics

Skills and Qualifications

Extraction and Screening Techniques

- Proficient with Solid-Phase Extraction (urine and blood) and Liquid-Liquid Extraction (urine)
- Enzyme Linked Immunosorbent Assay (ELISA) and Color Tests

Instrumentation

- Proficient using Liquid Chromatography-Quadrupole/Time-of-Flight-Mass Spectrometry (LC-Q/TOF-MS), Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), UV-Vis Spectrophotometer

- Experience using Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR), Ion Mobility Spectroscopy (IMS), Headspace Gas Chromatography (HS-GC), High Performance Liquid Chromatography (HPLC), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Gas Chromatography-Electron Capture Detection (GC-ECD).

Software

- Proficient using Agilent MassHunter Acquisition, Qualitative Analysis, and Quantitative Analysis, ChemStation, ACD/Spectrus Platform.
- Experience using Chromeleon, Xcalibur, PDXL, Omnic.

Peer-Reviewed Publications

Glicksberg L, Bryand, K, Kerrigan S. Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry. *Journal of Chromatography B* 2016; 1035:91-103.

Glicksberg L, Kerrigan S. Synthetic cathinone stability in blood. *Journal of Analytical Toxicology* 2017; https://doi.org/10.1093/jat/bkx071.

Glicksberg L, Kerrigan S. Synthetic cathinone stability in urine. *Journal of Analytical Toxicology* 2017 (in press).

Glicksberg L, Rana S, Kerrigan S. Cathinone stability in authentic urine specimens. *Forensic Science International* (in review).

Technical Reports

Kerrigan S, **Glicksberg L**. Long-Term Stability of Synthetic Cathinones in Forensic Toxicology Samples. Technical Report, U.S. Department of Justice, Award Number 2012-R2-CX-K003, 2015.

Peer-Reviewed Presentations and Posters

Glicksberg L, Kerrigan S. Synthetic Cathinone Stability in Blood Using LC/Q-TOF-MS. ORAL PRESENTATION. American Academy of Forensic Science Annual Meeting. New Orleans, LA. February 2017.

Glicksberg L. Stability of Synthetic Cathinones in Biological Evidence. ORAL PRESENTATION. NIJ Forensic Science R&D Symposium, American Academy of Forensic Science Annual Meeting. New Orleans, LA. February 2017.

Glicksberg L, Kerrigan S. Synthetic Cathinone Stability in Urine Using LC/Q-TOF-MS. ORAL PRESENTATION. Society of Forensic Toxicologist Annual Meeting. Dallas, TX. October 2016.

Glicksberg L. Short-Term Stability of Synthetic Cathinones in Urine. ORAL PRESENTATION. Sam Houston State University Graduate Research Symposium. The Woodlands, TX. April 2016.

Glicksberg L, Bryand, K, Kerrigan S. Fragmentation Pathways and Structural Characterization of Synthetic Cathinones Using Electrospray Ionization and High Resolution Mass Spectrometry. POSTER. American Academy of Forensic Sciences Annual Conference. Las Vegas, NV. February 2016.

Glicksberg L, Kerrigan S. Simultaneous Identification of Twenty-Two Synthetic Cathinones in Urine using LC/Q-TOF-MS. POSTER. Society of Forensic Toxicologists Annual Meeting. Atlanta, GA. October 2015.

Glicksberg L, Ponsini R, Savage M, Cavazos C, Kerrigan S. Identification of Synthetic Cathinones from Electron Impact Mass Spectra. POSTER. American Academy of Forensic Sciences Annual Conference. Orlando, FL. February 2015.

Professional Affiliation and Memberships

- Member of the Society of Forensic Toxicologists (SOFT).2015-Present
- Member of the American Academy of Forensic Sciences (AAFS). 2014-Present
- Member of Sam Houston State University's Society of Forensic Science. August 2013-Present
    - PresidentJanuary 2014-December 2014
    - Vice PresidentJanuary 2016-Present
- President of the University of Tulsa's chapter of Iota Sigma Pi.May 2012-May 2013
- Secretary of the University of Tulsa's chapter of Mortar Board.May 2012-May 2013

Awards

- 2017 Forensic Sciences Foundation (FSF) Emerging Forensic Scientist Award.
- Institute for Forensic Research, Training and Innovation (IFRTI) Scholarship. Summer 2016

Continuing Education

- OSHA Certification in Blood Borne Pathogens and Laboratory Standard.
- Completed the following trainings offered by RTI International Forensic Science Education
    - Answering the NAS: The Ethics of Leadership and the Leadership of Ethics
    - Introduction to Uncertainty in Forensic Chemistry and Toxicology
    - Standard Operating Procedure (SOP) Writing for ISO 17025 Accreditation
    - To Hell and Back: The Ethics of Stewardship and the Stewardship of Ethics
    - Applications of Higher Resolution Mass Spectrometry in Drug Testing
    - Fundamentals of Chromatography used in Toxicology
- Attended Short Course "High Resolution Mass Spectrometry for Qualitative and Quantitative Analysis: An Introduction" at the American Society for Mass Spectrometry Annual Meeting in San Antonio, TX, June 2015.
- Attended LC/MS Master Class offered by Agilent Technologies in Austin, TX, Spring 2014.