Post Mortem Tissue Distribution of Synthetic Cathinones

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FSF Emerging Forensic Scientist Award
Paper Presentation

Disclosure

- There is no real or apparent conflicts of interest related to the content of this presentation
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  - Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS
- The authors declare no competing interest
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Postmortem Toxicology

Pre-analytical Factors

- Environment
- Postmortem Interval
- Drug Properties

Further Complications

- Drug Instability
- Postmortem Redistribution (PMR)

Challenging Samples

- Sample Quality
- Sample Collection
- Alternative Samples

Interpretation Challenges

- Changes in concentration
- Site to site differences

Factors Influencing PMR

- Drug Properties
  - Volume of Distribution (>3-4)
  - pKa (>7)
  - Lipophilicity
- Physio-Chemical Changes
  - Cell death
  - Blood acidification
- Drug Release
  - Organs
  - Protein bound
- Blood Movement
  - Putrefactive processes
  - Physical
- Bacteria
  - External
  - GI
- Environment
- Postmortem Interval
- Incomplete Distribution

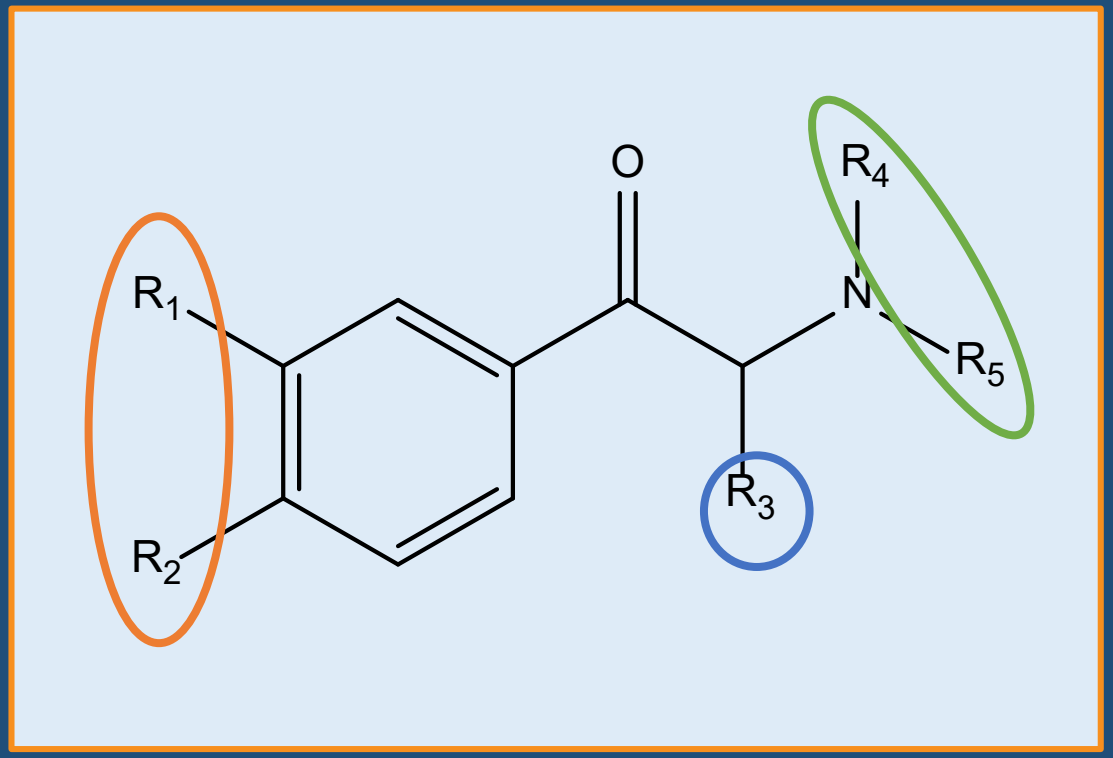
- Pre-analytical factors
  - Case, time, and site dependent variables
- Basic and lipophilic drugs more susceptible to redistribution

Assessing PMR

- Antemortem to postmortem specimen comparison
  - Possible in hospital deaths
- Cardiac to Peripheral blood (C/P) ratio
  - C/P >1
- Liver to Peripheral blood (L/P) ratio
  - L/P >20
  - L/P <5
- More reliable with large population of cases
  - Case to case variability

General Structure of Cathinone

- Arylaminoketones
- R groups represent positions that can be substituted to create various synthetic cathinones



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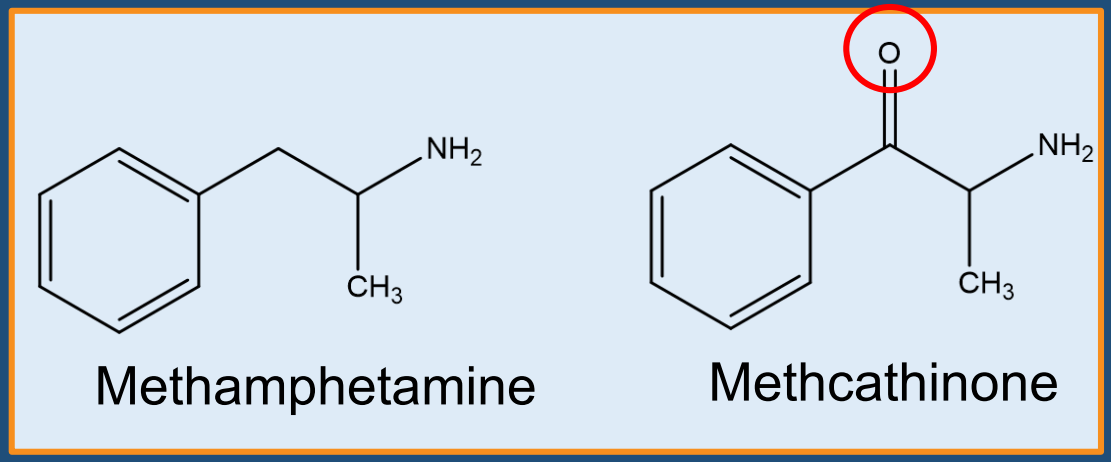


Synthetic Cathinone PMR

C/P—greater than 1; L/P—less than 5

- Individual cases
- Small sample population

Cathinone	C/P	L/P	References
Butylone	-	1.7	Rojek, 2012
Ethylone	1.0	3.6	McIntyre, 2014
MDPV	0.7 – 1.7 (1.3, n=6)	2.2 – 23 (8.9, n=7)	Marinetti, 2013; Wyman, 2013; Kesha, 2013
Methylone	1.0 – 2.1 (1.3, n=5)	1.6 – 3.2 (2.6, n=4)	Shimomura, 2016; Pearson, 2012; McIntyre, 2013; Cawrse, 2012; Barrios, 2016
Pentedrone	-	1.1	Sykutera, 2015
α-PVP	1.45	1.0 – 2.9 (1.7, n=3)	Potocka, 2017; Hasegawa, 2014; Sykutera, 2015
Pyrovalerone	1.4	3.0	Marinetti, 2013

Statement of Problem

Synthetic cathinones associated with fatal intoxications

Postmortem results require careful interpretation

Goal: Assess tissue distribution and postmortem redistribution of synthetic cathinones

Participating Laboratories

North Carolina Department of Health and Human Services,

Office of the Chief Medical Examiner

- ethylone (n=8)
- methylone (n=14)
- α-PVP (n=16)
- pentedrone (n=1)
- 4-MEC (n=1)
- pentylone (n=1)
- methedrone (n=1)
- buytlone (n=1)
- MDPV (n=4)

LA County Department of Medical Examiner-Coroner

- Ethylone (n=3)

50 cases, 139 specimens total

Specimens

Central blood sources:

- Vena cava
- Right chest cavity
- Heart blood
- Central blood
- Aorta blood

Peripheral blood sources:

- Subclavian vessel
- Peripheral blood
- Jugular vein
- Iliac vein
- Femoral vessel

Vitreous humor

Stomach contents

Urine

Liver

Optimized Extraction

Bovine blood (2 mL) (25 ng/mL I.S.)

Protein precipitation (4 mL ACN)

Centrifuge (5 min, 4,000 RPM)

1.0 M PO_4 buffer, pH 6 (6 mL)

Drug free urine (1 mL)
(25 ng/mL I.S.)

1.0 M PO_4 buffer, pH 6 (2 mL)

Add to SPE cartridges

Aqueous Washes
- DI water (1 mL)
- 1 M acetic acid (1 ml)

Vacuum Dry (5 min)

Organic Washes
- Hexane (1 mL)
- Ethyl acetate (1 mL)
- Methanol (1 mL)
- Dichloromethane (1 mL) (blood only)

Elute: 2% NH_4OH in 95 DCM: 5 IPA (1 mL)

Evaporation (50°C, N_2)

Reconstitute 50:50 MP A:B (25 µL)

Specimen Extraction

Liver

- Homogenization
  - Bead Ruptor 12
  - One part tissue (0.5 g) to two parts deionized water
  - 0.5 mL homogenate fortified and extracted
  - Blood protocol
    - 2 mL ACN protein precipitation
    - 3 mL phosphate buffer

Other Matrices

- Vitreous, stomach contents
  - Urine protocol

LC-Q/TOF MS Conditions

Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS

LC Separation

- Poroshell 120 EC-C18 Column (2.1x100 mm, 2.7 µm particle size)
- Mobile Phase A: 0.1% FA in diH_2O
- Mobile Phase B: 0.1% FA in ACN
- Flow Rate: 0.40 mL/min
- LC Gradient:
  - 96% A to 5 min, 90% A until 11 min, 60% A for 1 min, 0% A to equilibrate the column

Q/TOF Parameters

- Gas Temperature: 200°C
- Gas Flow Rate: 13 L/min
- Sheath Gas Temperature: 250°C
- Sheath Gas Flow Rate: 12 L/min
- Nebulizer Pressure: 20 psig

Mass Spectrometry

- Capillary Voltage: 4000 V
- Fragmentor Voltage: 150 V
- Nozzle Voltage: 0 V
- Collision Energy: 30 eV, 20 eV
- MS Scan Rate: 8 spectra/sec
- MS/MS Scan Rate: 3 spectra/sec
- MS Scan Range: 40-1000 m/z
- ESI Mode: Positive

Acquisition

- Minimum of two ion transitions per drug
- Run Time: 13 minutes

Validation Summary

Evaluated according to Scientific Working Group of Forensic Toxicology (SWGTOX) Standard Practices for Method Validation

Parameter	Blood	Urine
Calibration Model	Quadratic, 1/x	
Limit of Detection	1 – 5 ng/mL	0.25 – 5 ng/mL
Limit of Quantitation	1 – 5 ng/mL	0.25 – 5 ng/mL
Inter-assay Precision (n=15)	3 – 12%	2 – 12%
Intra-assay Precision (n=3)	0 – 17%	0 – 11%
Bias (n=15)	-7 – 11%	-3 – 12%
Accuracy (n=15)	93 – 111%	97 – 112%
Analytical Recovery (n=4)	81 – 91%	84 – 104%
Interference Studies	No qualitative interferences observed in either matrix	
Matrix Effects (n=10)	-15 – 3%	-21 – -1%
Dilution Integrity	2- and 4- fold	

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.

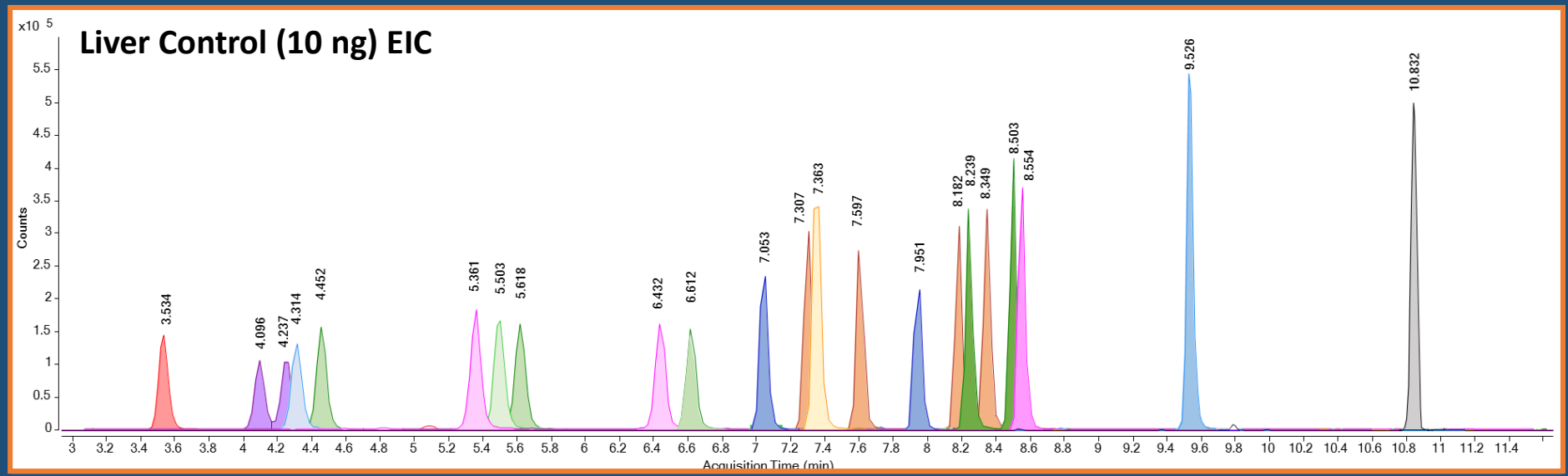
Tissue Validation

Precision and Bias (±20% CV)

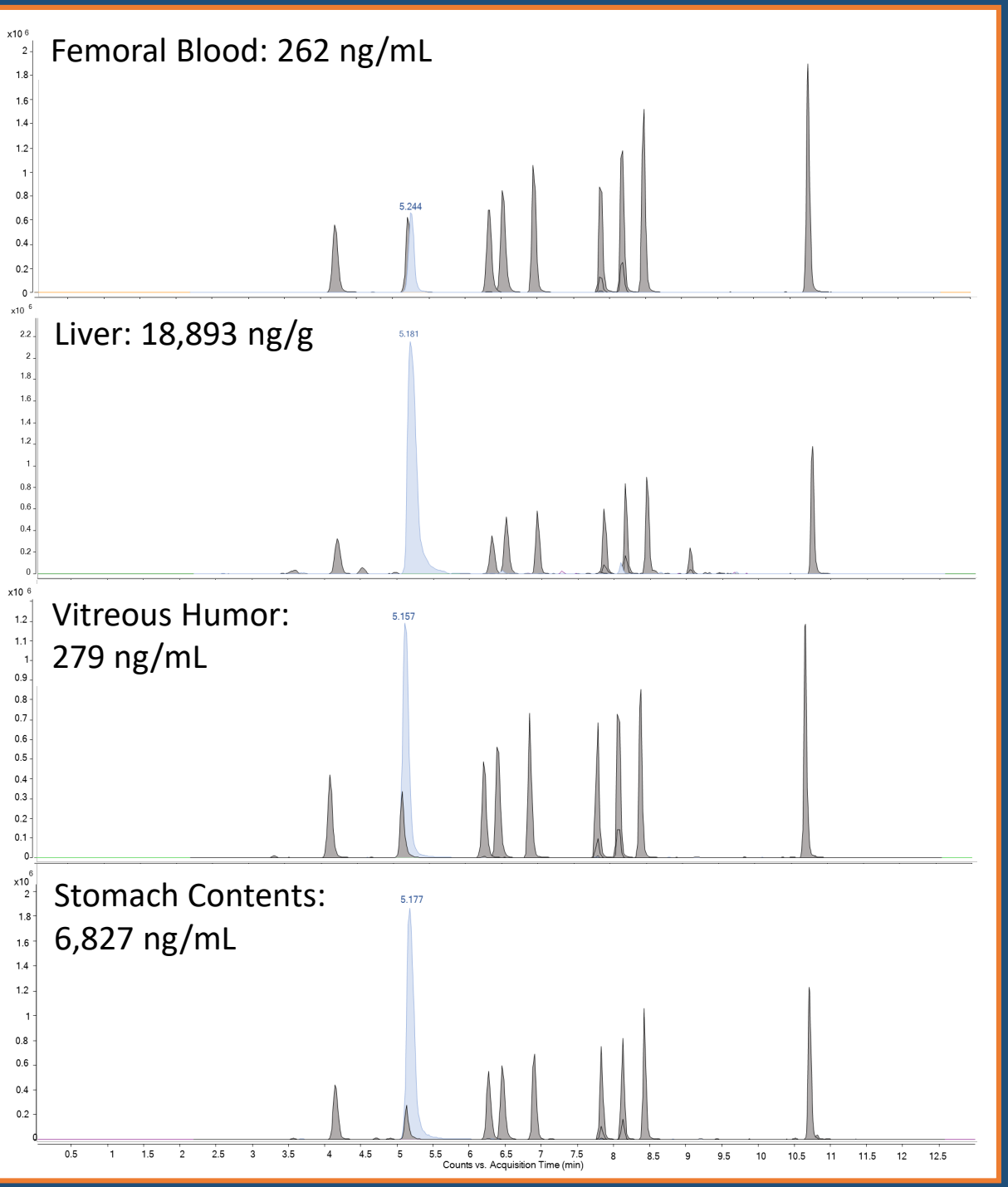
- Low: 10 ng
- Mid: 25 ng
- High: 50 ng

Results

- All within 15%
- Exceptions:
  - 3-FMC (0.3-26.4% CV)
  - Ethcathinone (0.7-20.9% CV)
  - MDPBP (2.1-20.7% CV)
  - MPBP (0.7-24.7% CV)



Case #48 Ethylone Positive



Cathinone-Positive Cases

50 positive cases

Cathinones identified:

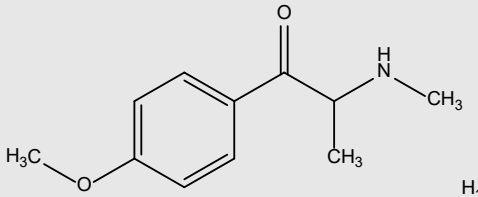
- α-PVP (n=19)
- Methylone (n=18)
- Ethylone: (n=15)
- MDPV: (n=6)
- Pentylone: (n=3)
- Methedrone: (n=2)
- Butylone, 4-MEC, MDPBP: (n=1)

7 cases involved 2 or more cathinones

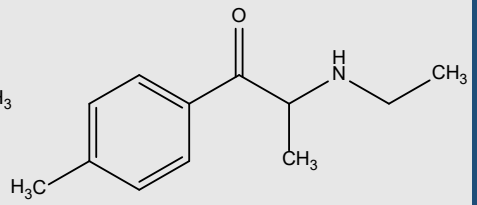
Vitreous (n=1), stomach contents (n=1)

Synthetic Cathinones Identified

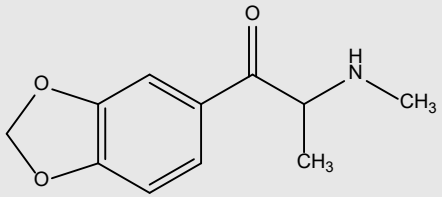
Methedrone (n=2)



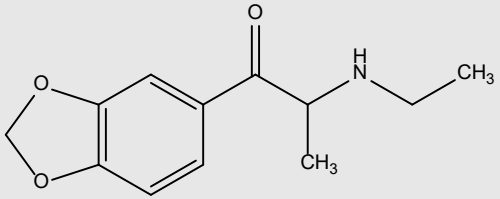
4-MEC (n=1)



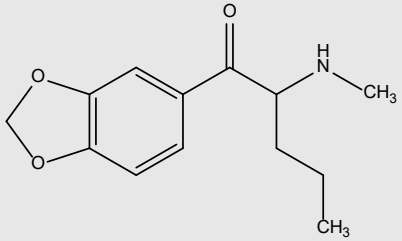
Methylone (n=18)



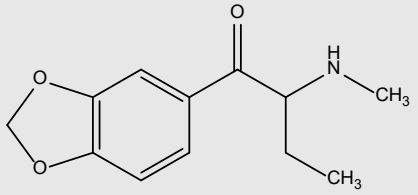
Ethylone (n=15)



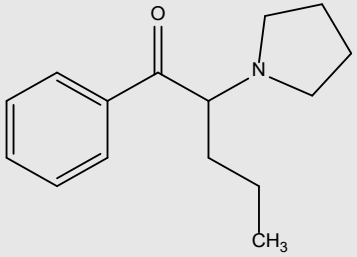
Pentylone (n=3)



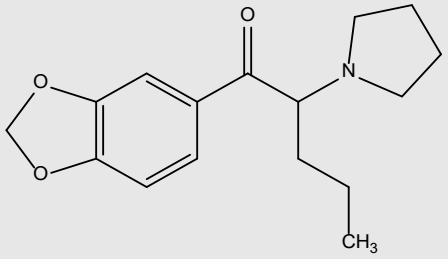
Butylone (n=1)



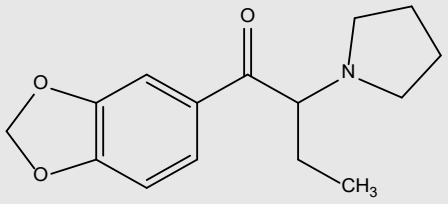
α-PVP (n=19)



MDPV (n=6)



MDPBP (n=1)



Quantitative Results

Cathinone	Central Blood	Peripheral Blood	Liver	Urine
α-PVP	<2 – 1,090 ng/mL (n=17)	2 – 1,019 ng/mL (n=14)	<60 – 169 ng/g (n=5)	33 – 7,580 ng/mL (n=11)
Methylone	<2 – 202 ng/mL (n=19)	<2 – 28 ng/mL (n=7)	<60 – 1,347 ng/g (n=8)	2 – 38,064 ng/mL (n=11)
Ethylone	3 – 2,743 ng/mL (n=12)	<2 – 780 ng/mL (n=9)	<60 – 5,196 ng/g (n=6)	32 – 8,743 ng/mL (n=9)
MDPV	4 – 80 ng/mL (n=4)	3 – 80 ng/mL (n=2)	<60 – 223 ng/g (n=2)	4 – 5,210 ng/mL (n=3)
Pentylone	<5 – 323 ng/mL (n=2)	<5 – 160 ng/mL (n=2)		122 - >5,000 ng/mL (n=2)
Methedrone	79 ng/mL	70 ng/mL	720 ng/g	4 – 1,213 ng/mL (n=2)
Butylone	6 ng/mL	8 ng/mL	116 ng/g	934 ng/mL
4-MEC		57 ng/mL		
MDPBP				111 ng/mL

Postmortem Redistribution

Determine C/P and L/P ratios for 7 cathinones over 21 specimens

Cathinone	C/P	L/P
α-PVP	0.5 – 1.9 (1.1, n=9)	<1.4 (n=3)
MDPV	1.0 (n=1)	-
Methedrone	1.1 (n=1)	10 (n=1)
Butylone	0.7 (n=1)	14 (n=1)
Ethylone	0.5 – 9.2 (2.9, n=6)	0.2 – 20 (7.2, n=4)
Methylone	1.5 – 6.1 (4.0, n=5)	3.1 – 40 (n=2)
Pentylone	2.0 (n=1)	-

C/P Ratios

Determine C/P and L/P ratios for 7 cathinones

Cathinone	C/P	L/P
α-PVP	0.5 – 1.9 (1.1, n=9)	<1.4 (n=3)
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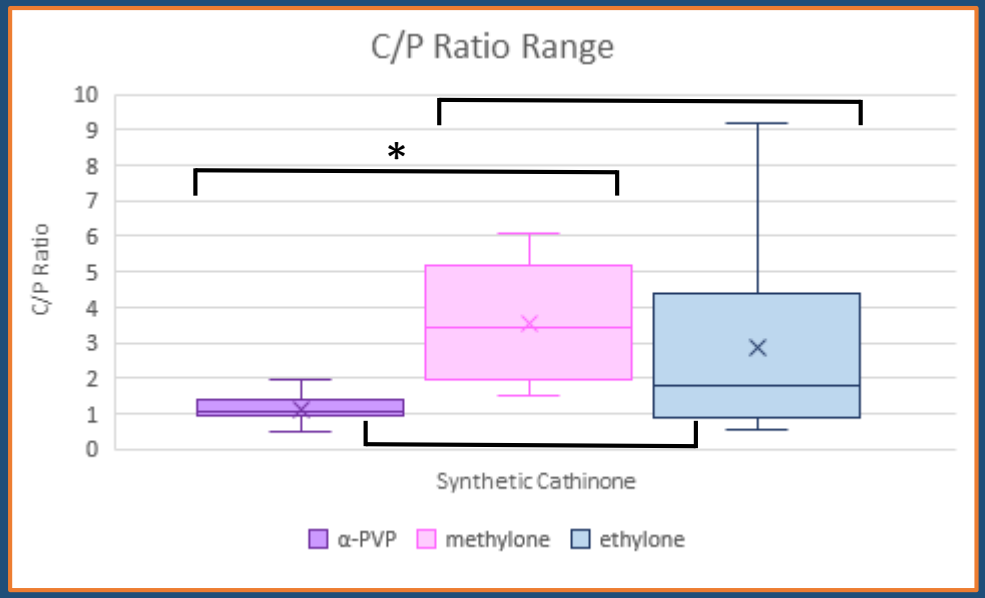

- Welch Test
  - methylone to ethylone
  - α-PVP to methylone
  - α-PVP to ethylone

Comparison to Literature

			Previously Published Values	
Cathinone	**C/P**	**L/P**	**C/P**	**L/P**
α-PVP	0.5 – 1.9 (1.1, n=9)	<1.4 (n=3)	1.5 (n=1)	1.1 – 2.9 (1.5, n=3)
MDPV	1.0 (n=1)	-	0.7 – 1.7 (1.3, n=6)	2.2 – 23 (8.9, n=7)
Methedrone	1.1 (n=1)	10 (n=1)	-	-
Butylone	0.7 (n=1)	14 (n=1)		1.7 (n=1)
Ethylone	0.5 – 9.2 (2.9, n=6)	0.2 – 20 (7.2, n=4)	1.0 (n=1)	3.6 (n=1)
Methylone	1.5 – 6.1 (4.0, n=5)	3.1 – 40 (n=2)	1.0 – 2.1 (1.3, n=5)	1.6 – 3.2 (2.6, n=4)
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Methylone	1.5 – 6.1 (4.0, n=5)	3.1 – 40 (n=2)	1.0 – 2.1 (1.3, n=5)	1.6 – 3.2 (2.6, n=4)
Pentylone	2.0 (n=1)	-	-	-

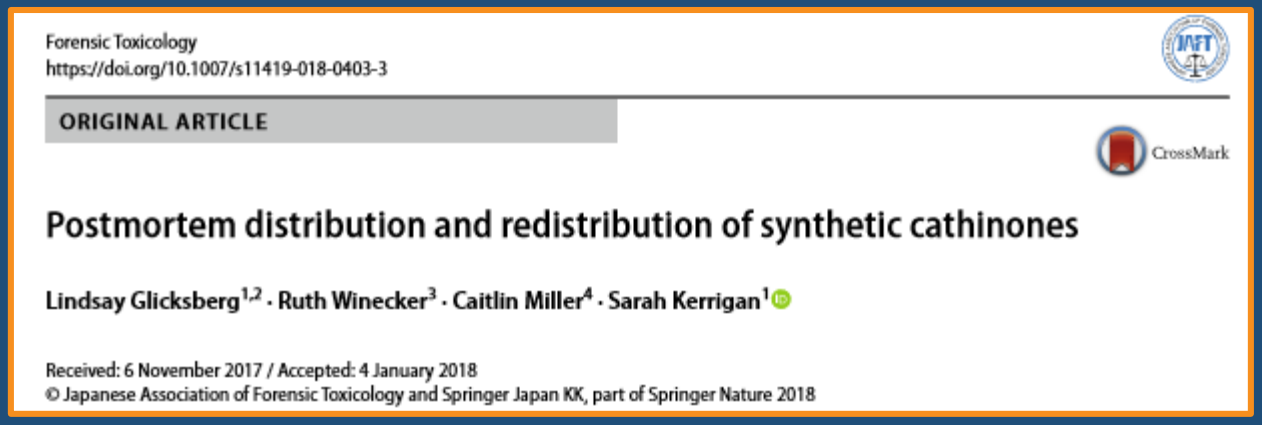
Non Cathinones
MDMA
C/P: 0.9 – 4.6 (2.7, n=7)
L/P: 3.1 – 8.5 (6.5, n=5)
Methamphetamine
C/P: 0.9 – 5.8 (2.3, n=39)
L/P: 1.7 – 9.1 (5.5, n=19)

Conclusions

- Cathinones may exhibit low to moderate PMR
- Highest C/P ratios observed for methylone and ethylone (secondary amine, methylenedioxy-substituted cathinones)
- Lowest C/P ratios observed for pyrrolidine-type cathinones

Limitations:

- Site and time dependent variables
- Further complicated by stability



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ORIGINAL ARTICLE

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Paper Presentation

Questions?

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