Synthetic Cathinone Stability in Blood Using LC/Q-TOF-MS

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Paper Presentation

Disclosure

- There is no real or apparent conflicts of interest related to the content of this presentation
- Products used:
  - Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS
- The authors declare no competing interest
- Project funded by NIJ Award # 2013-R2-CX-K006

About Me

- Originally from Chicago
- Primetime Television
  - *CSI, Law and Order: SVU, Crossing Jordan, ER*
- Medical Examiner
  - Medical School
- Forensic Science
- Bachelors in Chemistry from the University of Tulsa
- PhD in Forensic Science from Sam Houston State University
- Dallas County Southwestern Institute of Forensic Sciences (SWIFS)

Synthetic Cathinone Background

- **Derived from cathinone**
  - *Catha edulis* shrub

- **Synthesized for effects similar to methamphetamine & MDMA**
- **Available on the internet and in head shops with labels such as:**
  - "not for human consumption"
  - "bath salts"
  - "plant food"

Pharmacology and Toxicology

Desired Effects:

- stimulant and euphoric symptoms
  - Increased energy, mood enhancement, empathy, sociability, concentration, euphoria

Adverse Effects:

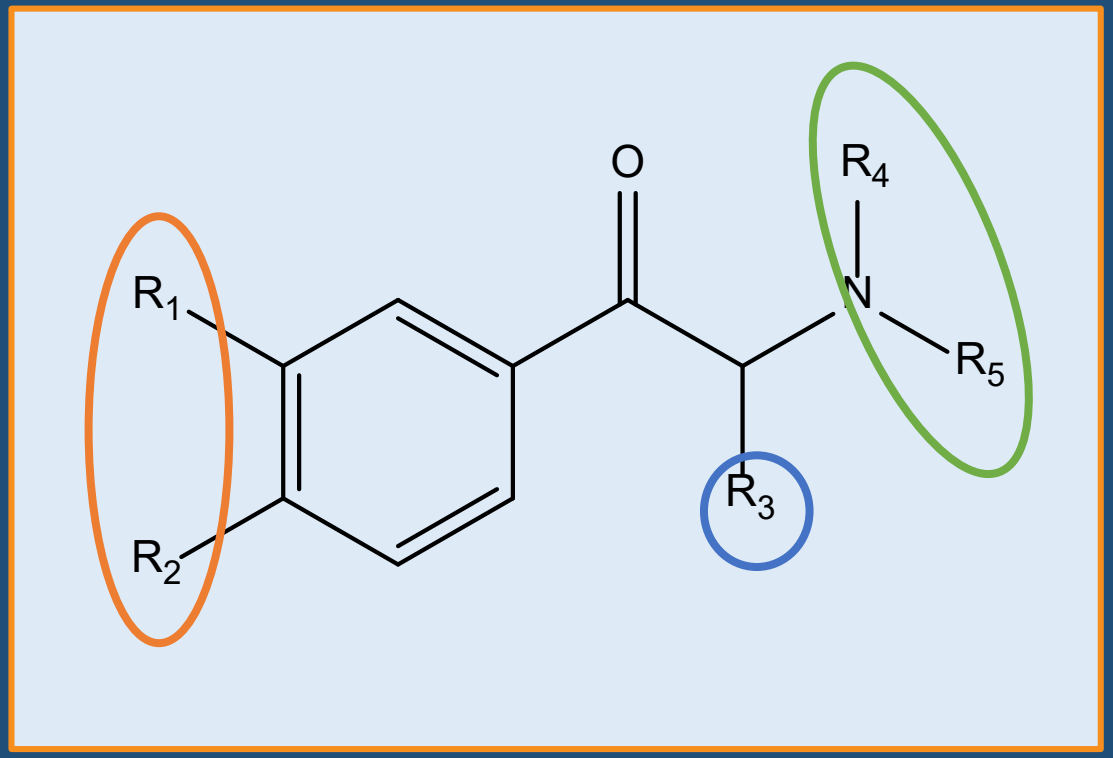
- neurological, cardiovascular, and psychopathological symptoms
  - Hallucinations, delusions, confusion, violence, homicidal tendencies, death

Antemortem Cases: Motor Vehicle Accidents & Driving While Impaired

Postmortem Cases: Overdose, Suicide, Homicide

General Structure of Cathinone

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



Importance of Stability

- Understanding the stability of a drug in biological matrices is essential
- Condition and length of storage can affect drug concentration
- Specimens stored for days, weeks, or months prior to analysis
- Subjected to various conditions during collection and shipping process

Cathinone Instability

Plant Material

- Unstable in oxygen rich conditions (Szendrei, 1980)
- Unstable in alkaline conditions (Szendrei, 1980; Berrang, 1982)
- Dimer formation (Berrang, 1982; Chappell, 2010)
- Best to air dry and refrigerate

Thermal Degradation

- Thermal degradation in GC-MS
  - Methcathinone (DeRuiter, 1994)
  - α-PVP (Tsujikawa, 2013)
  - 19 synthetic cathinones (Kerrigan, 2015)

- **Aqueous Solution (Tsujikawa, 2012)**
  - Stable at acidic pH
  - Decomposition rate dependent upon chemical structure
    - 5 synthetic cathinones

Biological Material

- **Sorensen (2011):**
  - Methcathinone, Ethcathinone, Mephedrone, Flephedrone, Methedrone, Methylone, Butylone
  - Blood (pH 7.4 and 5.9)
  - 5°C and 20°C
  - 7 days
  - More stable in pH 5.9 and 5°C over 7 day period
- **Johnson and Botch-Jones (2013):**
  - MDPV/Mephedrone
  - Blood, Plasma, Urine
  - -20°C, 4°C, 22°C
  - 14 days
  - -20°C: stable in 3 matrices
  - Mephedrone unstable at 4°C and 22°C
- **Soh and Elliott (2014):**
  - 4-MEC
  - Blood and Plasma
  - 20°C and 5°C
  - 7 days
  - Unstable at both temperature
- **Busardo (2016):**
  - Mephedrone
  - Antemortem & Postmortem Blood
  - -20°C, 4°C, 20°C
  - 6 months
  - Unstable at 4°C and 20°C by 3 months
  - Stable at -20°C

Stability Studies

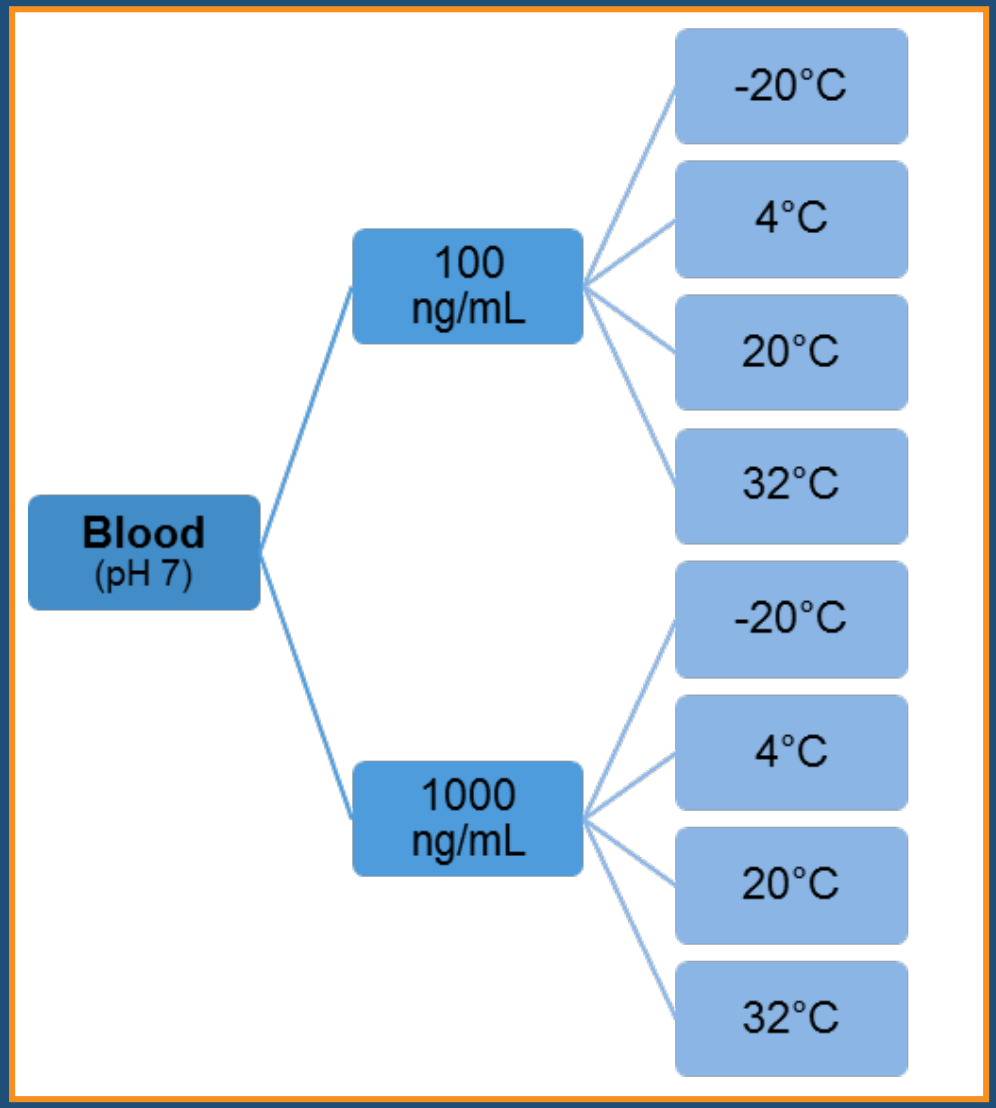
No systematic and fully comprehensive study addressing synthetic cathinone stability in biological evidence

- 22 synthetic cathinones
- 2 biological matrix
- 4 temperatures
- >6 months

Comprehensive study assessing stability as it relates to

1. Concentration
2. Temperature
3. Storage Time
4. Chemical Structure

Research Design



LC/Q-TOF-MS Conditions

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

LC Separation

- Poroshell 120 EC-C18 Column (2.1x100mm, 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

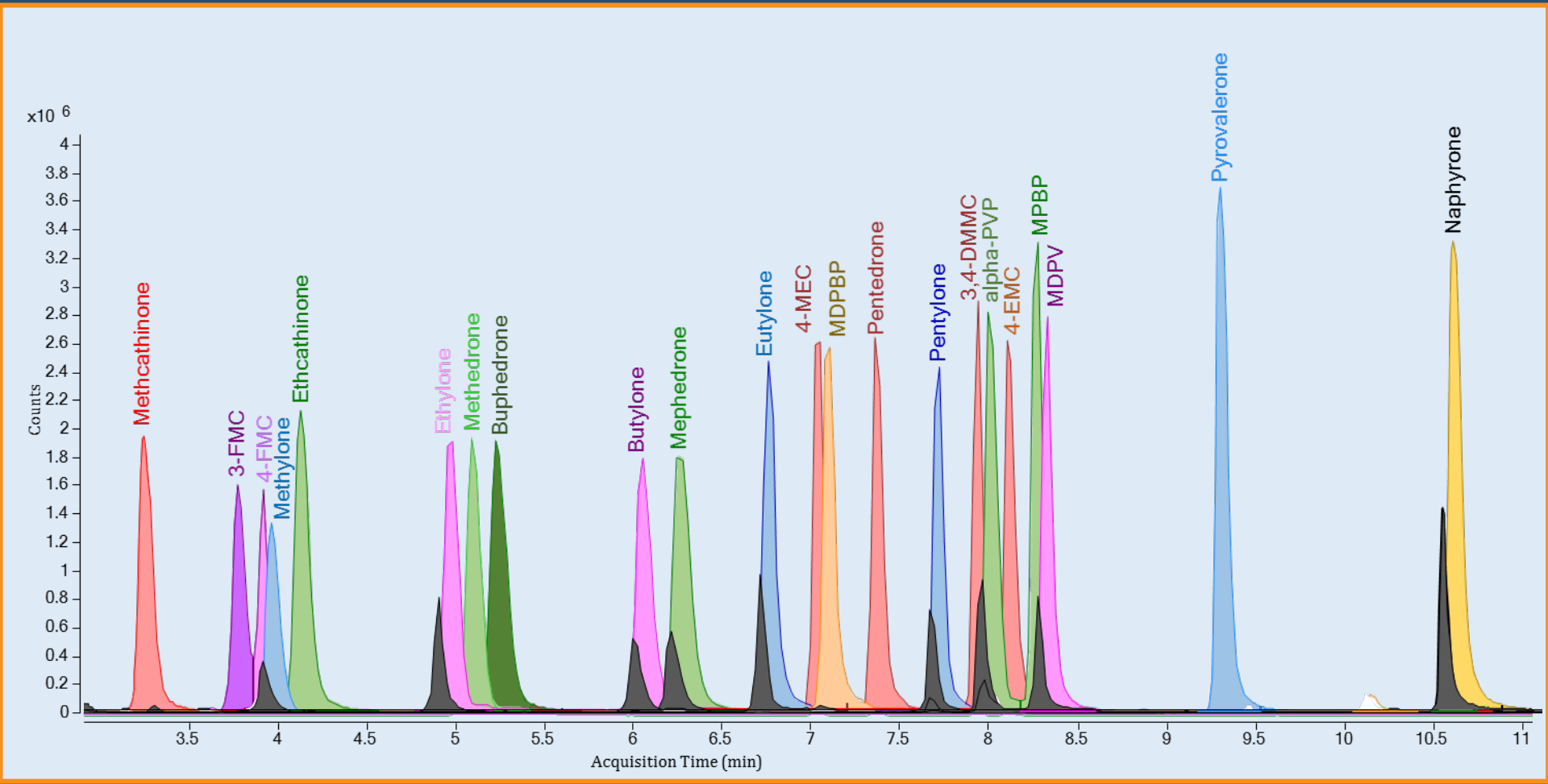
Mass Spectrometry

- 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

LC/Q-TOF EIC


x10 6
Counts
Acquisition Time (min)
Methcathinone
3-FMC
4-FMC
Methylone
Ethcathinone
Ethylone
Methedrone
Buphedrone
Butylone
Mephedrone
Eutylone
4-MEC
MDPBP
Pentedrone
Pentylone
3,4-DMMC
alpha-PVP
4-EMC
MPBP
MDPV
Pyrovalerone
Naphyrone

Validation Summary

SWGTOX Standard Practices for Method Validation

LOD: 1 – 5 ng/mL

LOQ: 1 – 5 ng/mL

Inter-assay Precision: 3 – 12%

Intra-assay Precision: 0 – 14%

Bias: -7 – 11%

Accuracy: 93 – 100%

Matrix Effects: -15 – 3%

Dilution Integrity: 2- and 4- fold

Interferences: No interferences (>50 interferents)

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.

Stability Study Analysis

Extraction

- Blood samples in duplicate (n=2)
  - 1000 ng/mL samples 1:4 dilution
- Calibrators extracted with every run
  - 10, 25, 100, 250, 350, and 500 ng/mL
- Negative and Positive (100 ng/mL) Controls

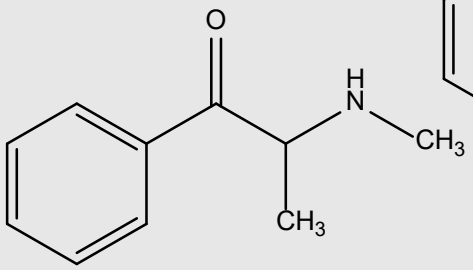
Analysis

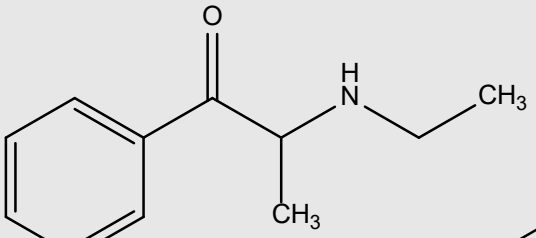
- Concentration Mean (n=2)
- Error bars emitted for clarity
- Significant >20% loss

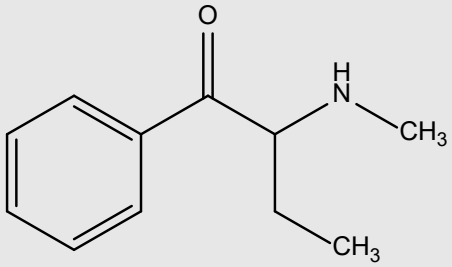
Month	Samplings/Week
1	4
2-3	2-3*
4-6	1
6-12	1/month

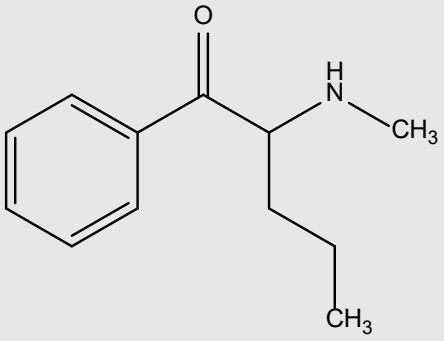
Secondary Amines, No Ring Substituents

Methcathinone	Ethcathinone	Buphedrone	Pentedrone

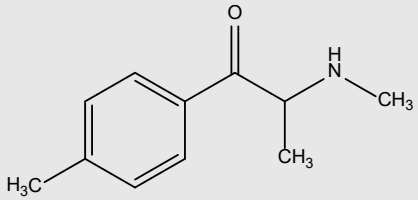
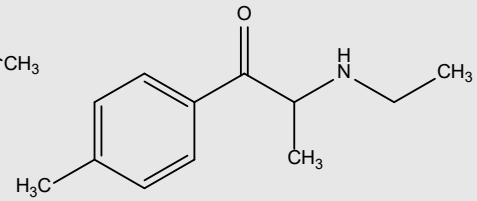
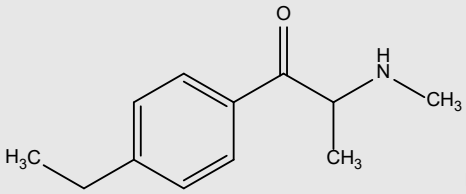
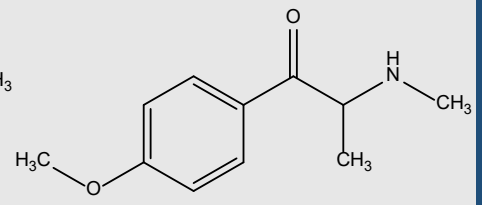


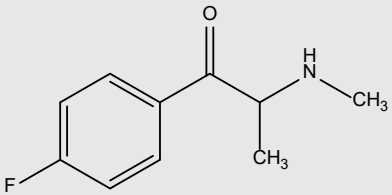
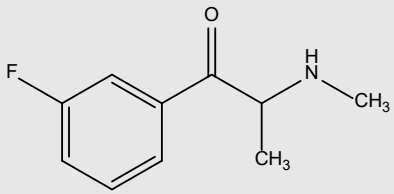
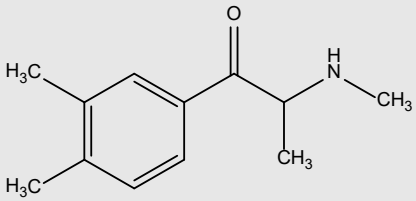




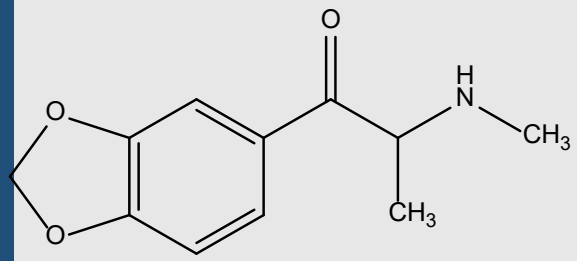
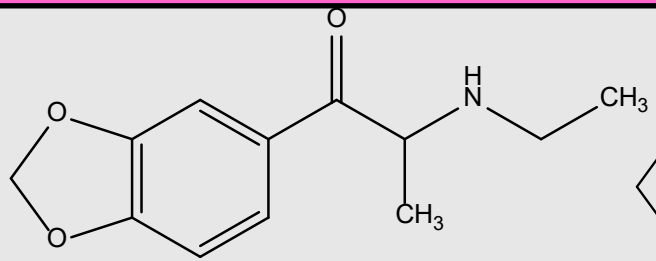
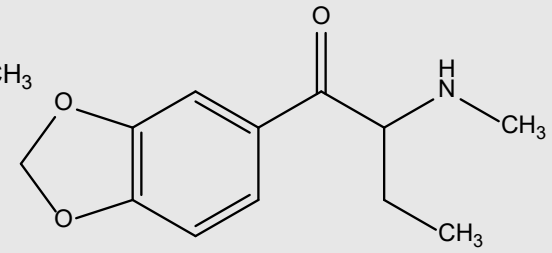


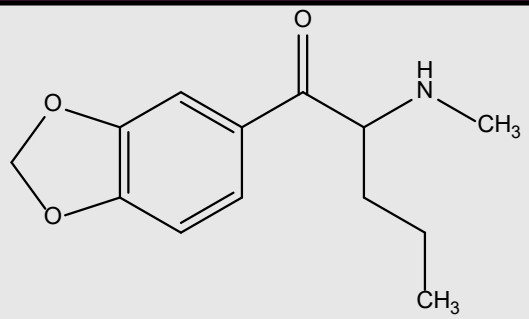
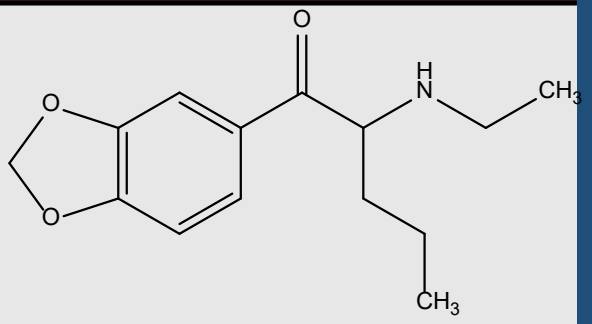
Secondary Amines, Ring Substituted

Mephedrone*	4-MEC	4-EMC	Methedrone
			

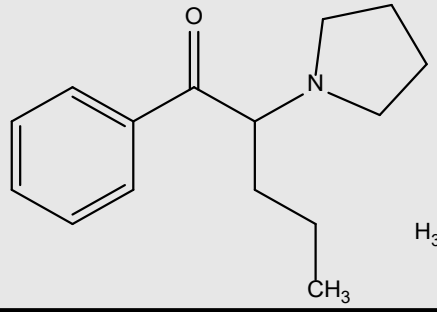
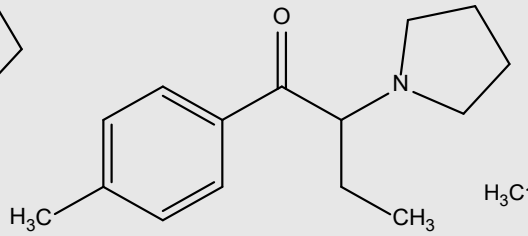
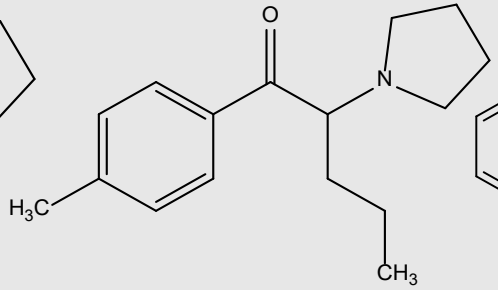
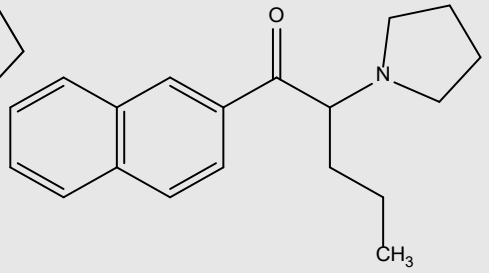
Flephedrone	3-FMC	3,4-DMMC
		

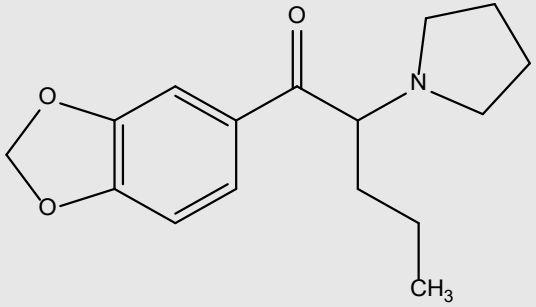
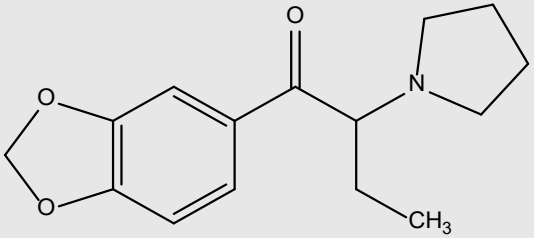
Secondary Amines, Methylenedioxy-Type

Methylone*	Ethylone*	Butylone*
		

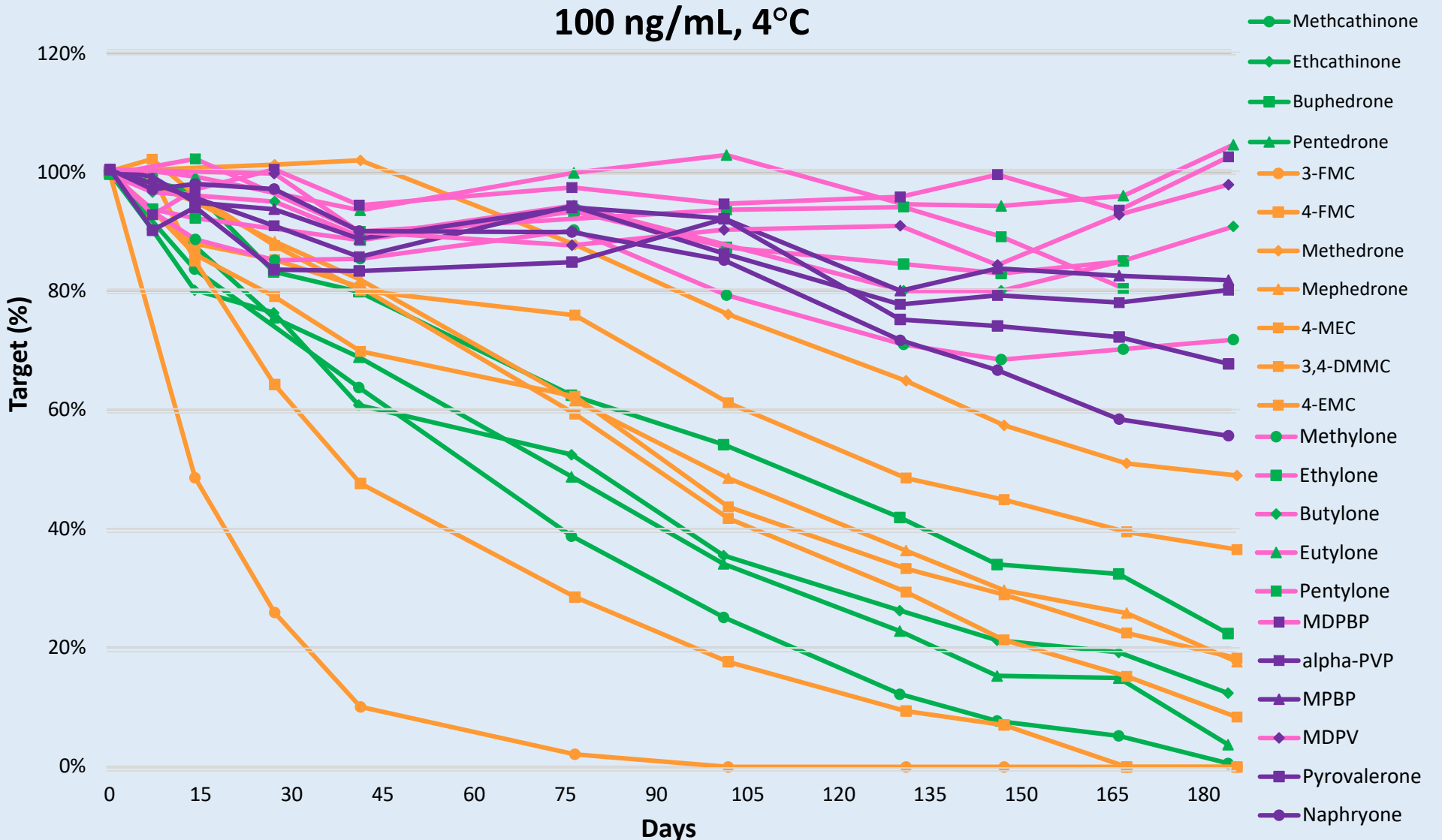
Pentylone*	Eutylone*
	

Tertiary Amines, Pyrrolidine-Type

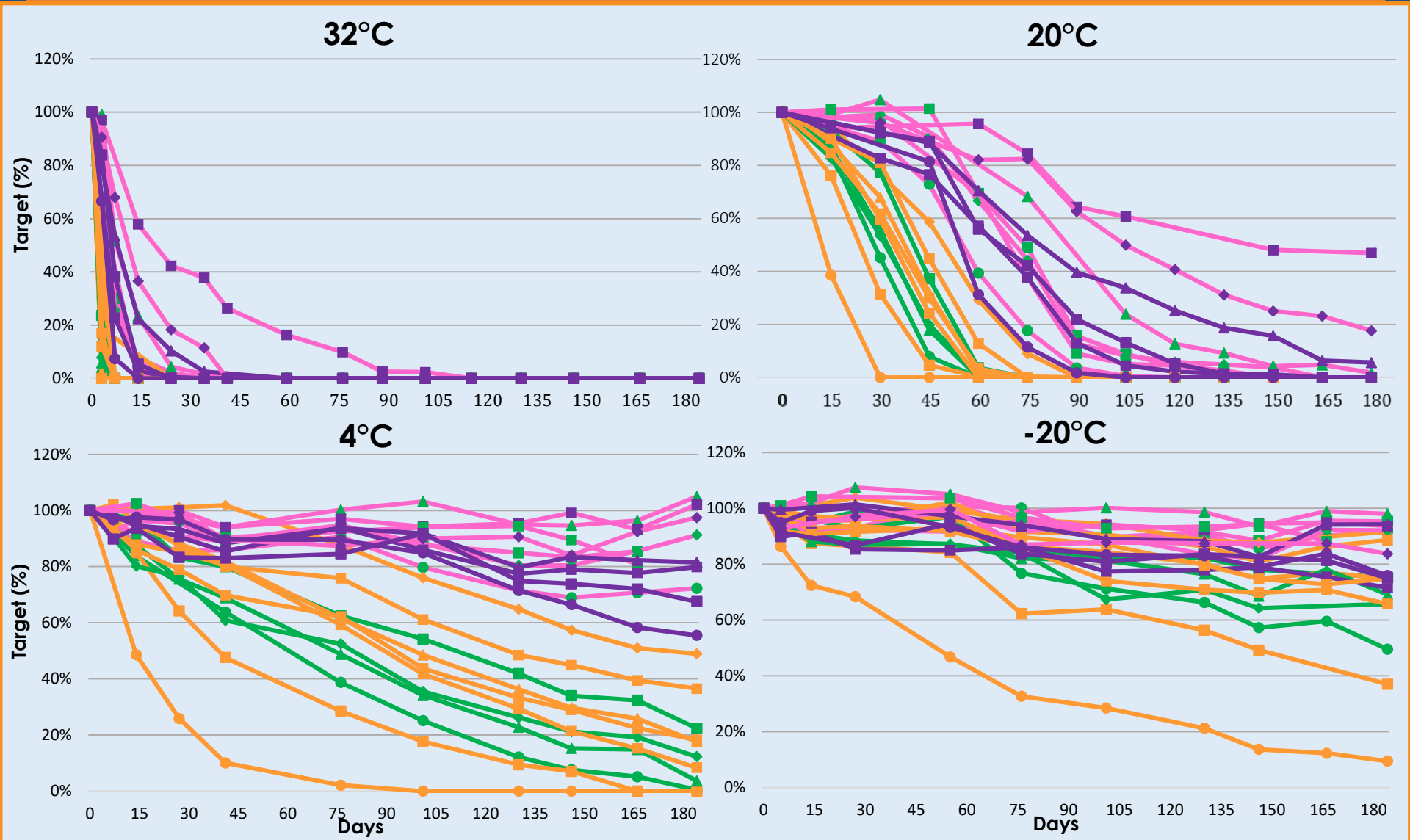
Alpha-PVP*	MPBP	Pyrovalerone	Naphyrone*
			

MDPV*	MDPBP
	

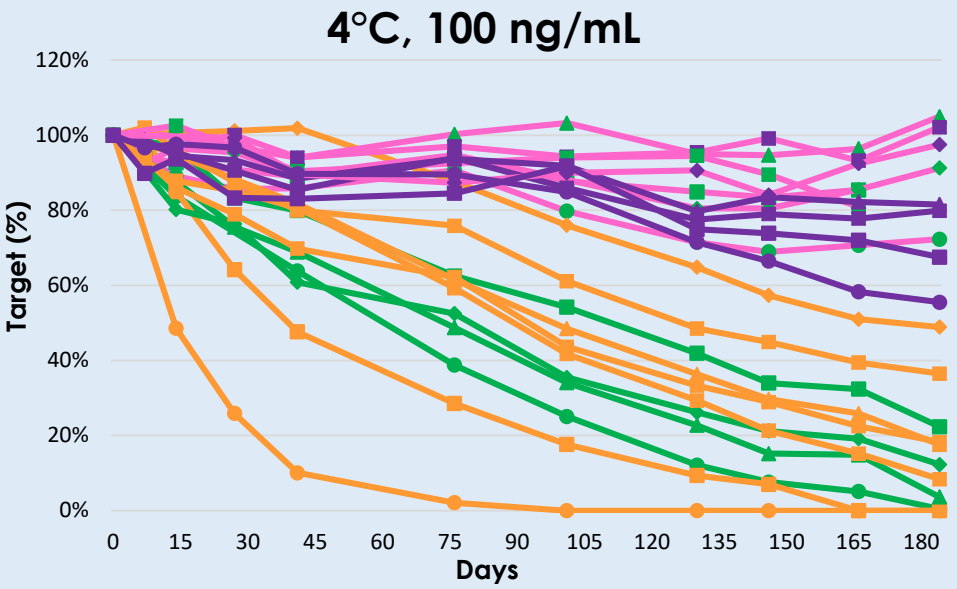
Chemical Structure Dependence:

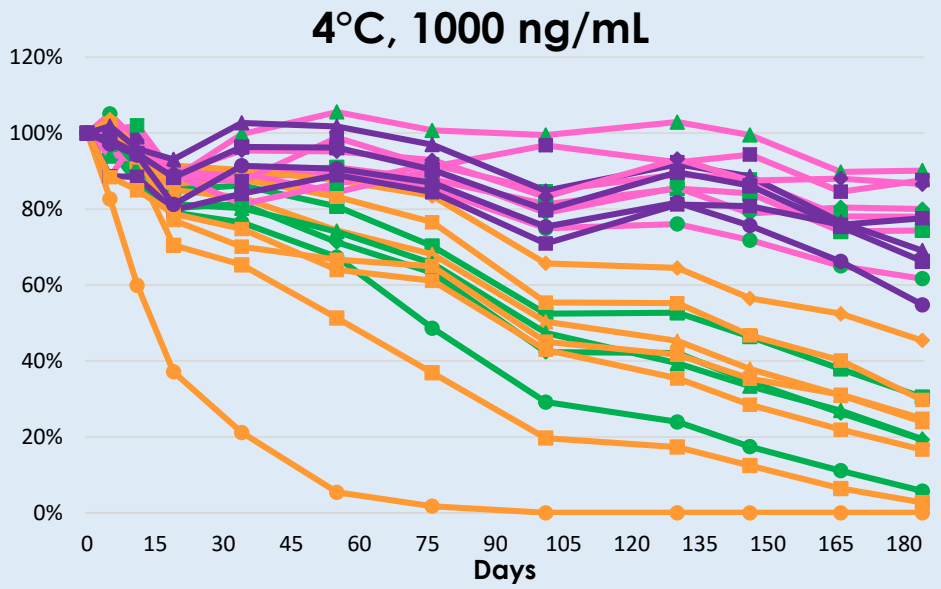


Temperature Dependence (100 ng/mL)



Concentration Dependence

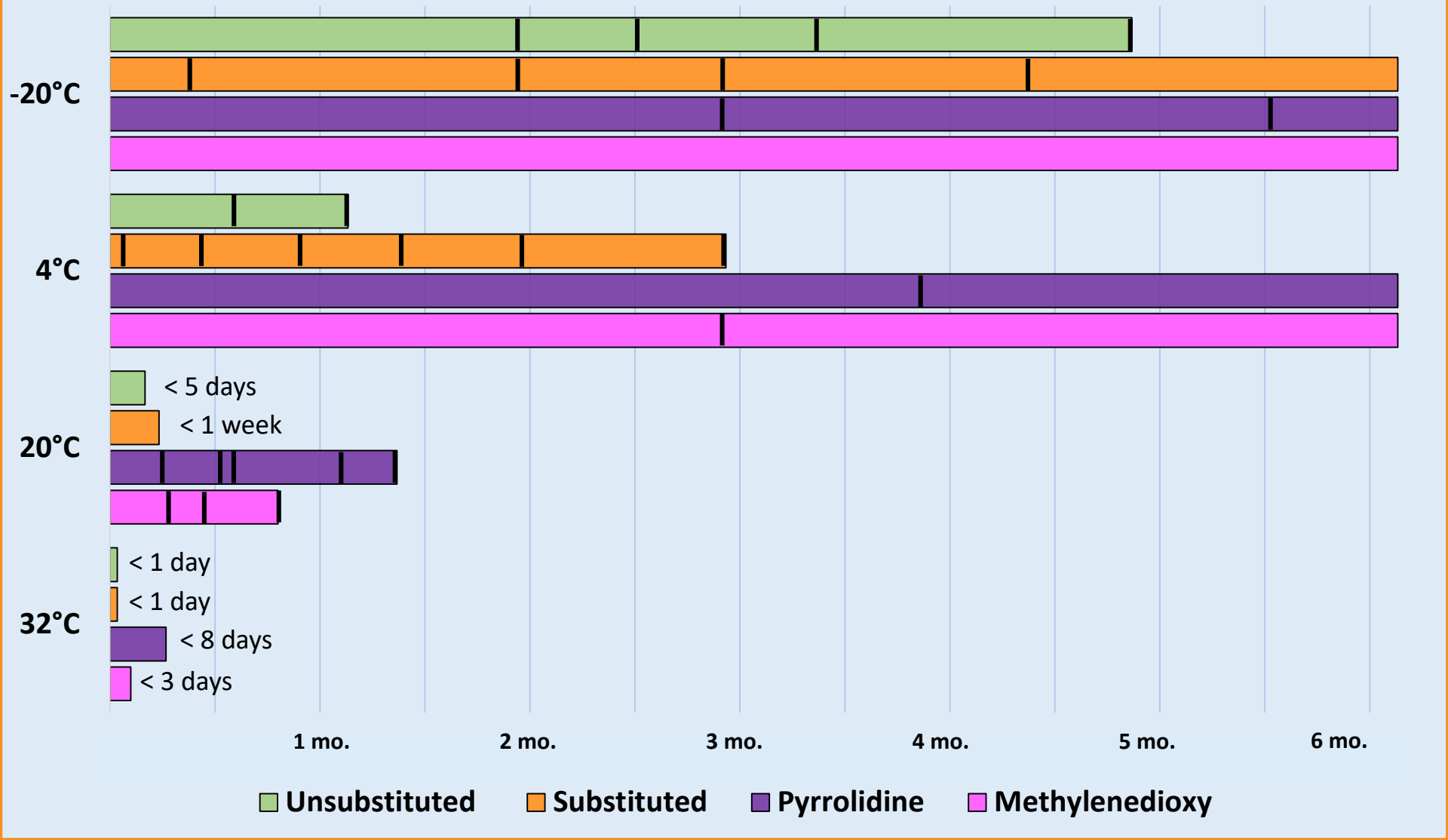




Stability Range
(in days)

Cathinone Structural Group	32°C	20°C	4°C	-20°C
Unsubstituted	<1	2 – 5	19 – 34	76 – 146
Ring Substituted	<1	<1 – 7	3 – 88	14 - >184
Methylenedioxy	1 – 3	9 – 24	88 - >184	>184
Pyrrolidine	2 – 8	8 – 34	115 - >184	88 - >184

Stability Range



Detection Window
(in days)

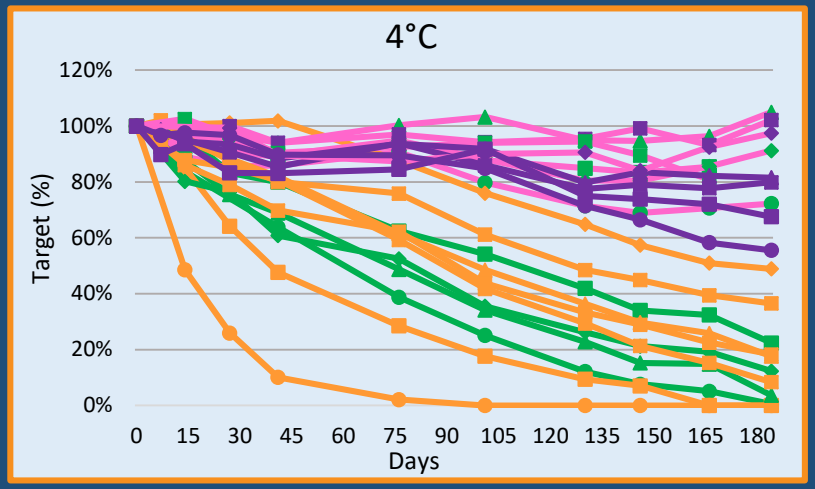
Cathinone Structural Group	32°C	20°C	4°C	-20°C
Unsubstituted	3 – 4	19 - 24	≥184	>184
Ring Substituted	2 – 7	7 – 55	88 - >184	>184
Methylenedioxy	14 – 27	76 – 184	>184	>184
Pyrrolidine	11 – 115	55 - >184	>184	>184

Conclusions

- **Temperature Dependence**
  -20°C > 4°C > 20°C > 32°C
- **No Concentration Dependence**
- **Significant Structural Dependence**
  MD/PYR > PYR > MD > Ring Substituted > Unsubstituted > 3-FMC

Conclusions Cont.

- **Significant loss on the order of hours**
  - 3-FMC: 32°C—undetectable after 24 hours
  - Substituted and Unsubstituted
    - 32°C: >20% loss at 48 hours
    - 20°C: >20% loss at 48 hours
- **Instability at common storage conditions (4°C)**



- **Significant Structural Influence**

Acknowledgements

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FSF Emerging Forensic Scientist Award
Paper Presentation

Questions?

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Friday, February 23rd @ --
Post Mortem Distribution of Synthetic Cathinones

Sam Houston State University