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

Lindsay Glicksberg, BS; Sarah Kerrigan, PhD

Department of Forensic Science
Sam Houston State University
Huntsville, TX, USA

46th Annual SOFT, Dallas, 2016

Sam Houston State University

DISCLOSURE

The authors have no commercial disclosure

Project funded by NIJ Award # 2013-R2-CX-K006

SYNTHETIC CATHINONE BACKGROUND

- Derived from cathinone, a naturally occurring compound in the leaves of the *Catha edulis* shrub
- Synthetic cathinones synthesized for the same effects
  - Effects similar to methamphetamine and 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

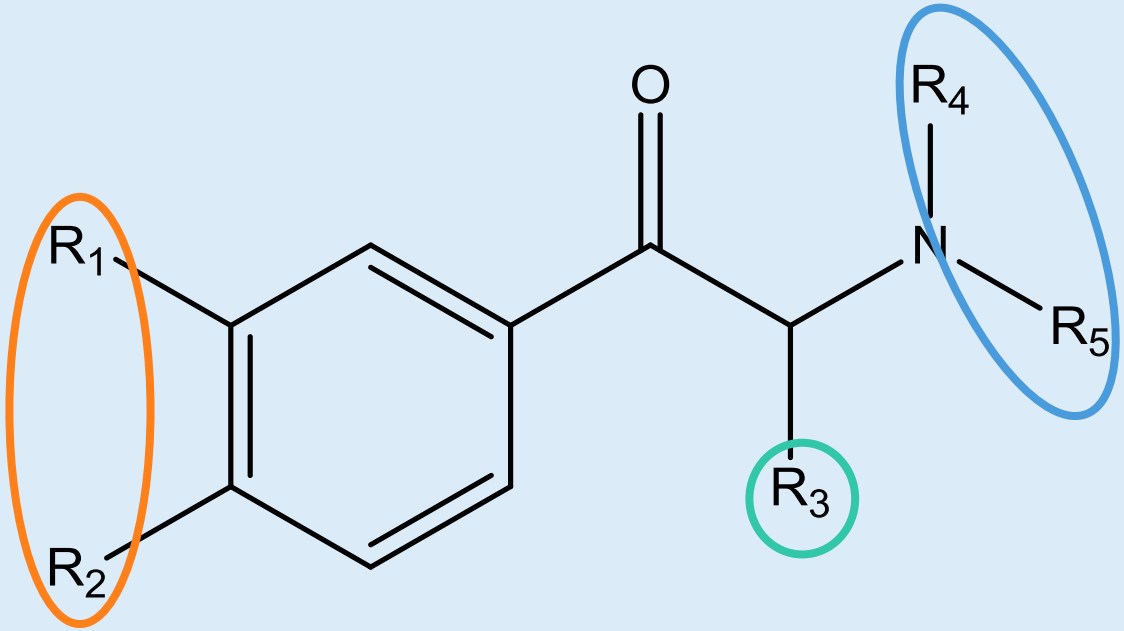
Antemortem Cases: Motor Vehicle Accidents & Driving While Impaired

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 in 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)

BIOLOGICAL MATERIAL

- Paul and Cole (2001):
  - Cathinone/Methcathinone
  - Urine
  - -18°C and 4°C
  - 3 months
  - -18°C: stable for 2 months
  - 4°C: stable for 3 days, unstable for 3 months
- 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
- Tsujikawa (2012):
  - Methcathinone, Mephedrone, 3-FMC, 4-FMC, Ethcathinone
  - Aqueous pH solutions
  - More stable at acidic pH, decomposition rate dependent upon chemical structure

BIOLOGICAL MATERIAL

- 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

Previous stability studies have been reported, however…

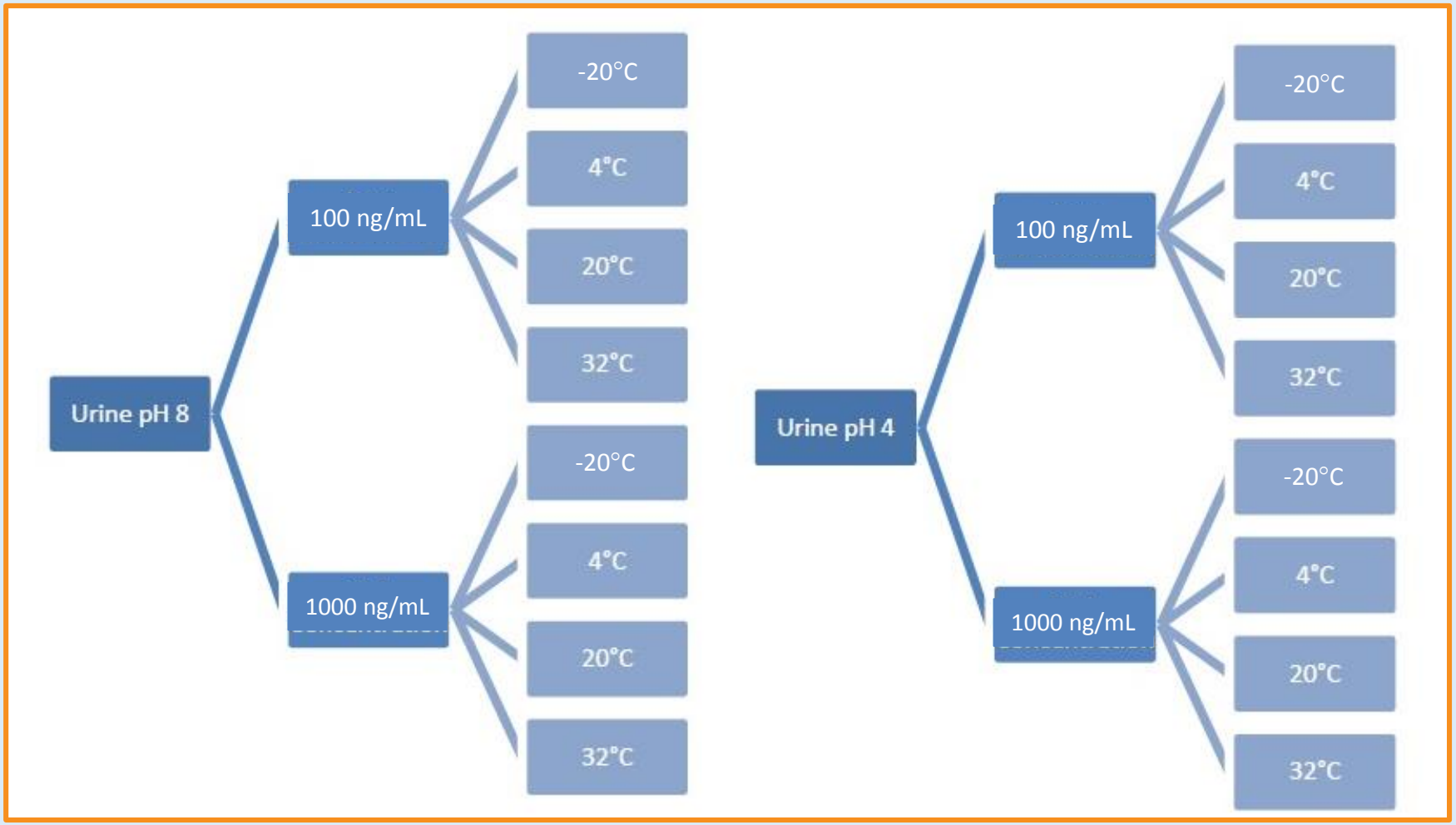
No systematic and fully comprehensive study addressing synthetic cathinone stability in biological evidence

- 22 synthetic cathinones
- 1 biological matrices
- 4 temperatures
- >6 months

Comprehensive study assessing stability as it relates

1. pH
2. Concentration
3. Temperature
4. Storage time
5. Chemical properties

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

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**

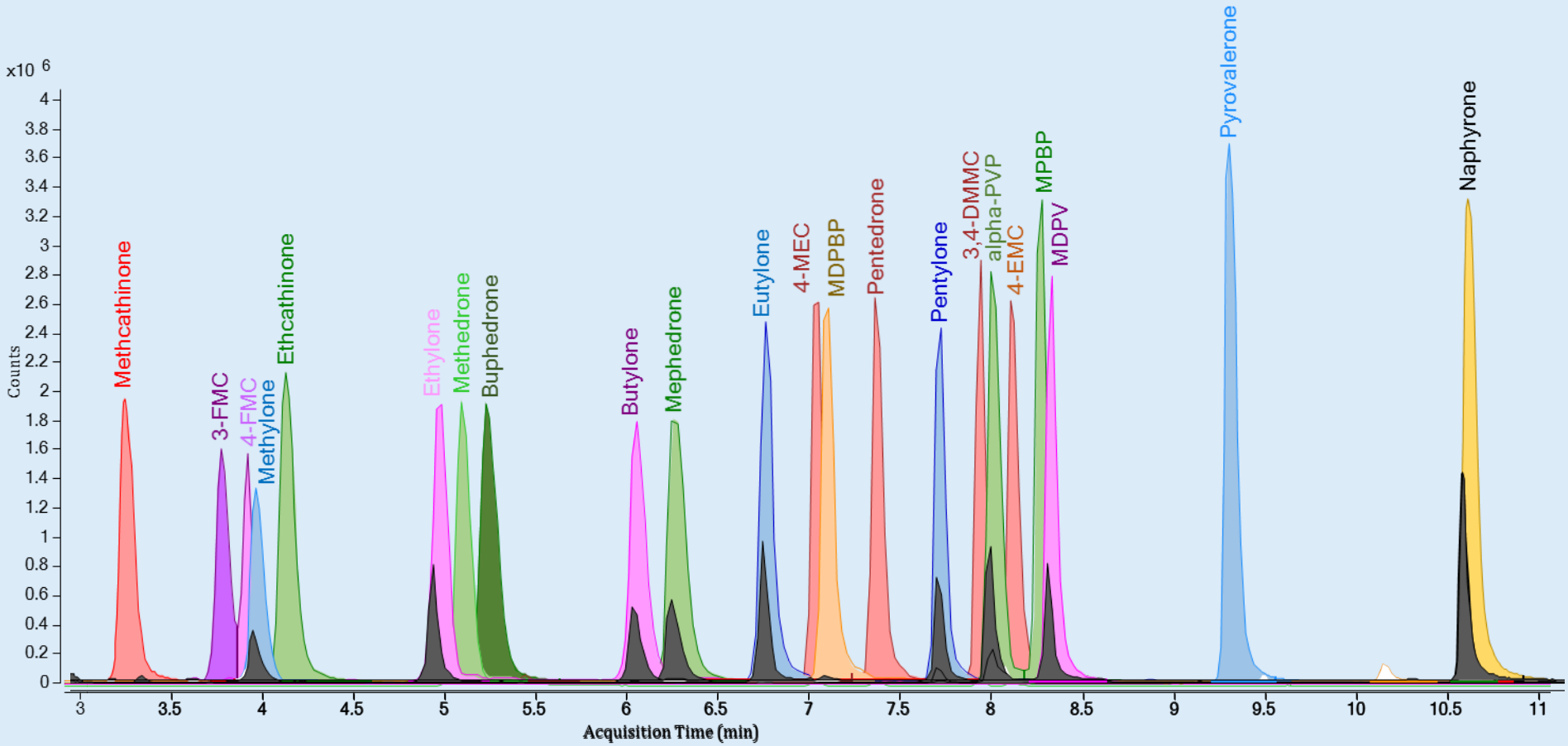
Q/TOF Parameters

- 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

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: 0.25 – 5 ng/mL
LOQ: 0.25 – 5 ng/mL
Precision: ±15%
Bias: ±15%
Accuracy: 84 – 104%
Matrix Effects: ± 20%
Dilution Integrity: 2- and 4- fold
Interferences: No interferences (>50 interferents)

STABILITY STUDY ANALYSIS

Extraction

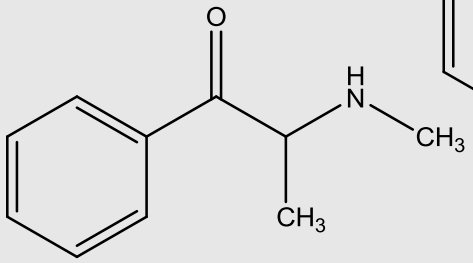
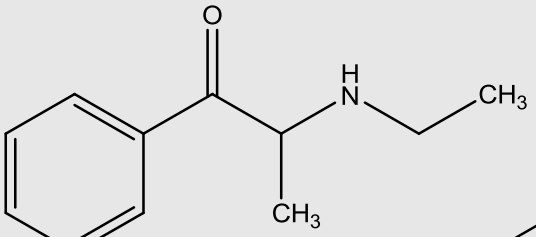
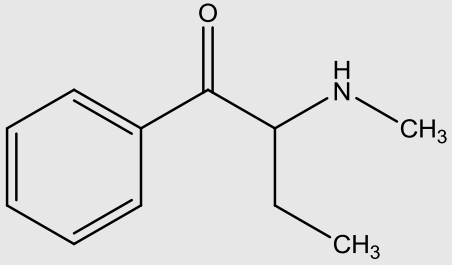
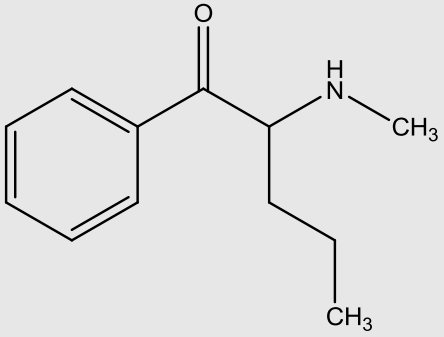
- Urine samples in duplicate (n=2)
  - 1000 ng/mL samples 1:4 dilution
- Calibrators extracted with every run
  - 10, 25, 100, 250, 350, and 500 ng/mL
- Negative and Positive (100 ng/mL) Controls

Analysis

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

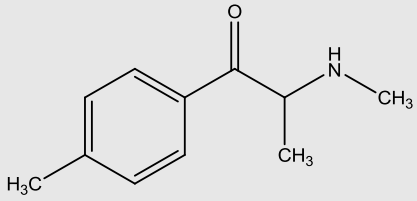
Month	Samplings/Week
1	4
2-3	2-3*
4-6	1
6-12	1/month

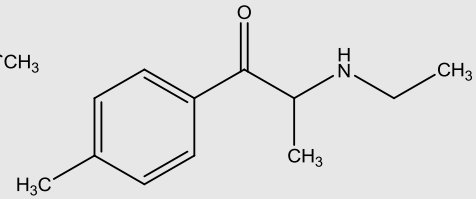
SECONDARY AMINES, NO RING SUBSTITUENTS

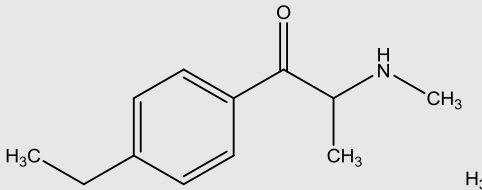
Methcathinone	Ethcathinone	Buphedrone	Pentedrone
			

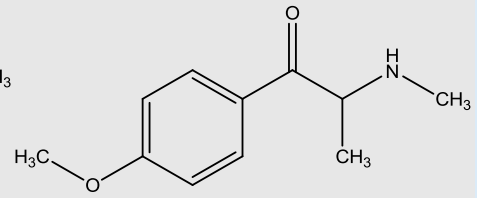
SECONDARY AMINES, RING SUBSTITUTED

Mephedrone*	4-MEC	4-EMC	Methedrone

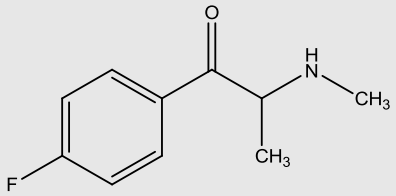


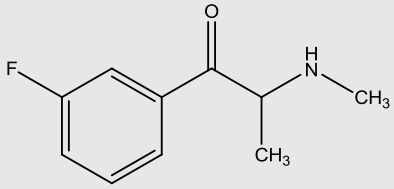


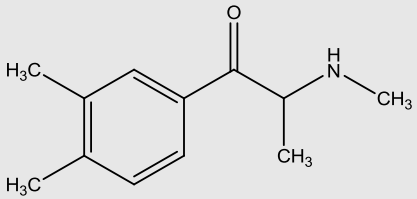




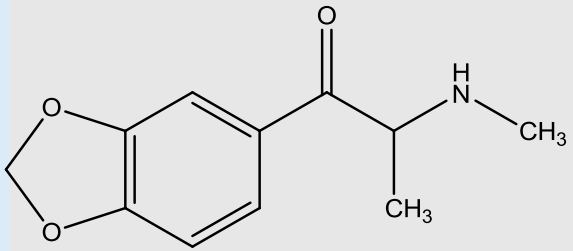
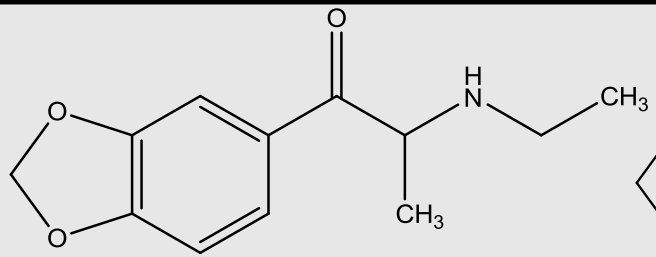
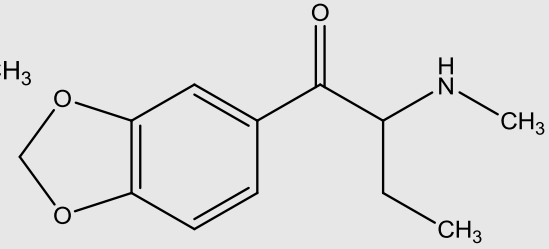
Flephedrone	3-FMC	3,4-DMMC

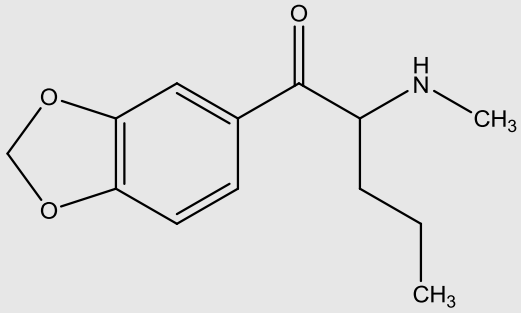
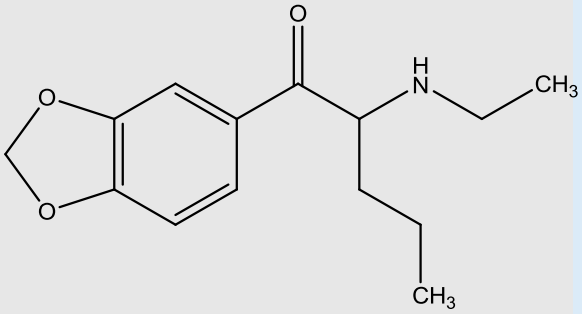




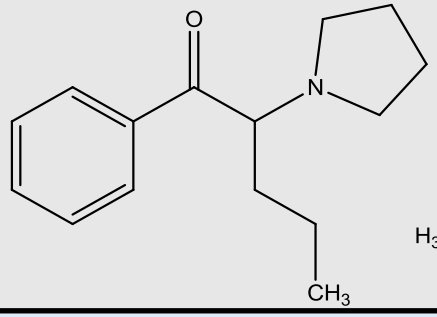
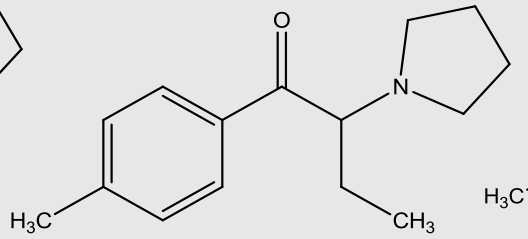
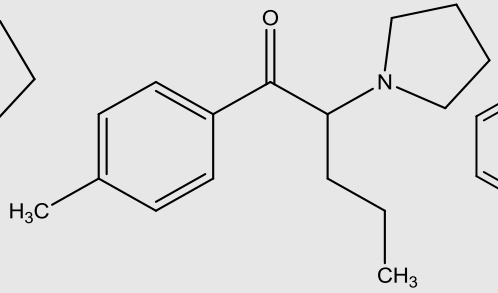
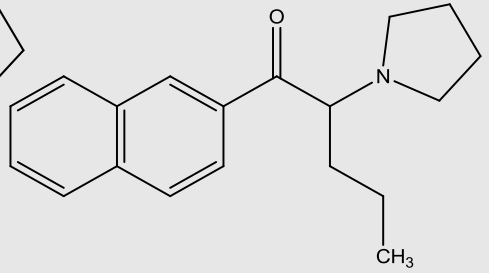


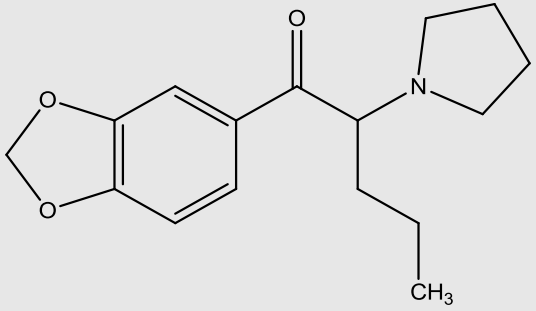
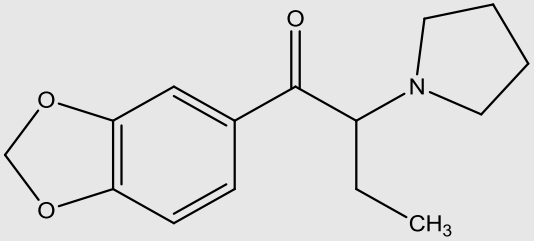
SECONDARY AMINES, METHYLENEDIOXY TYPE

Methylone*	Ethylone*	Butylone*
		

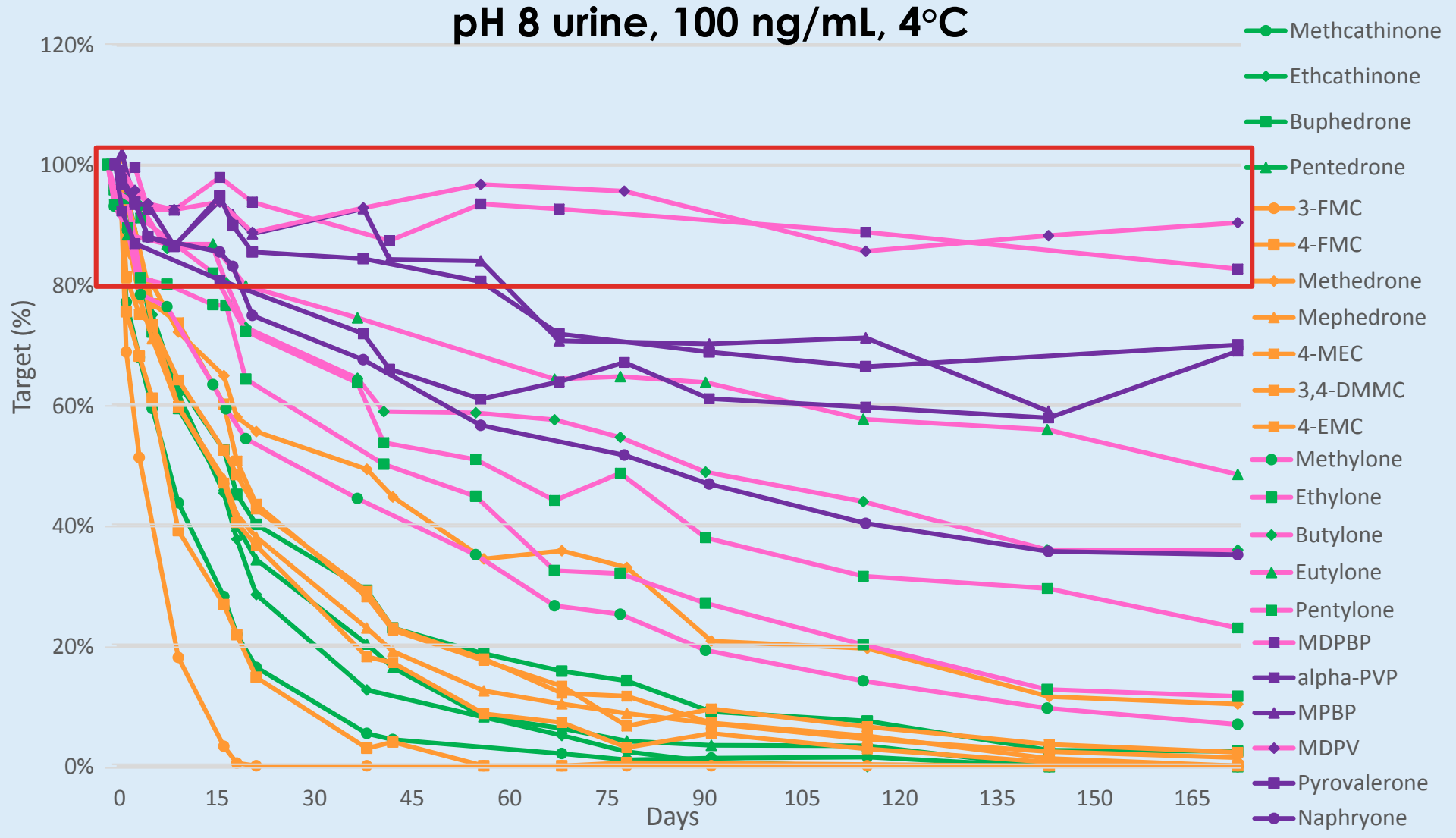
Pentylone*	Eutylone*
	

TERTIARY AMINES, PYRROLIDINE TYPE

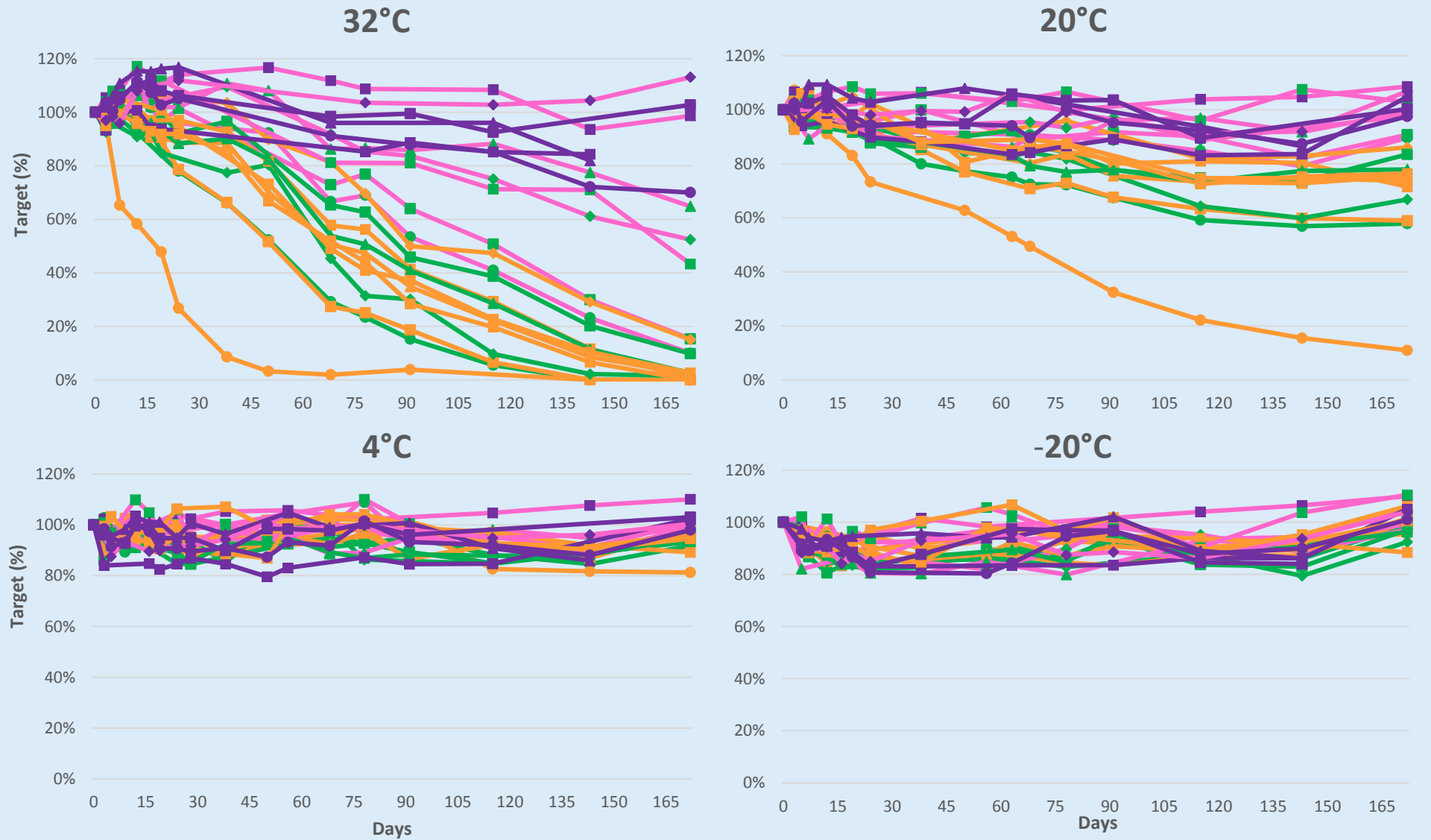
Alpha-PVP*	MPBP	Pyrovalerone	Naphyrone*
			

MDPV*	MDPBP
	

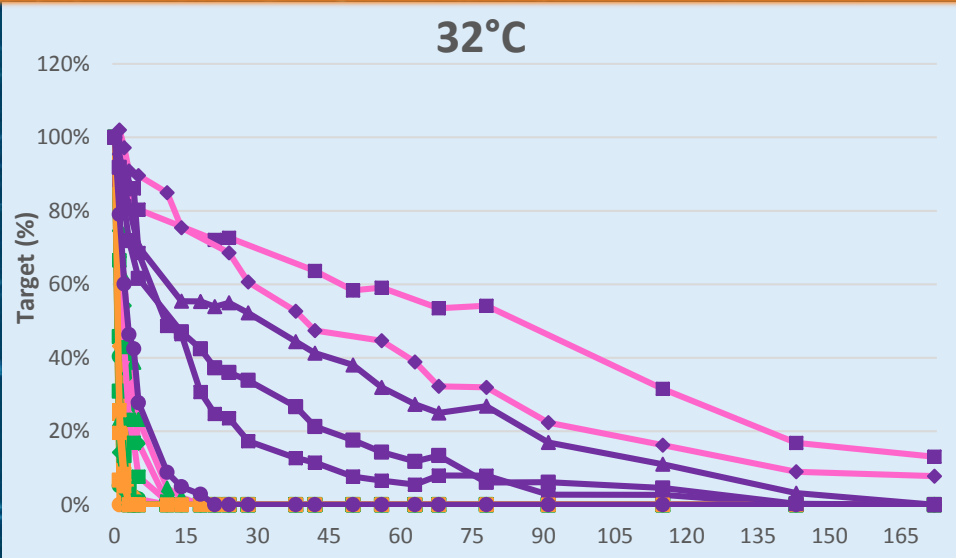
CHEMICAL STRUCTURE DEPENDENCE:

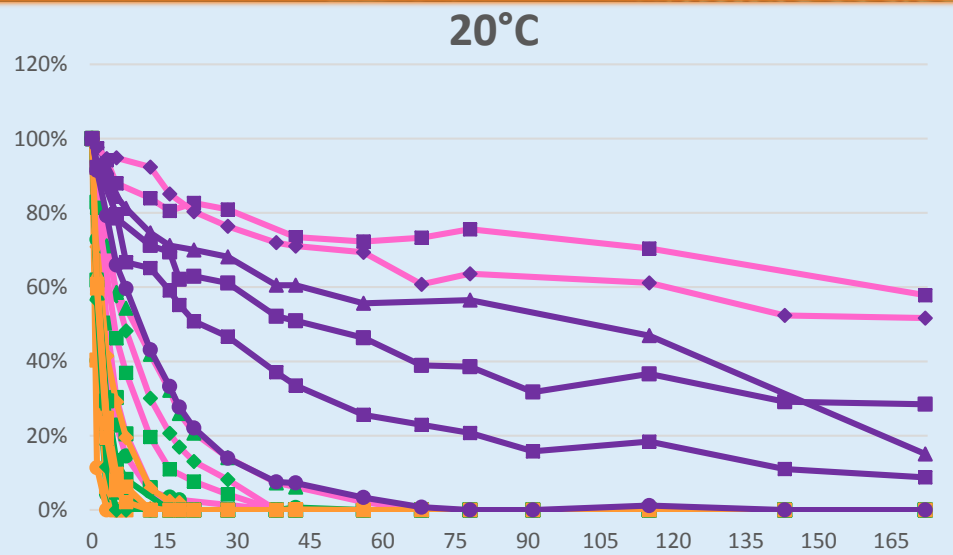


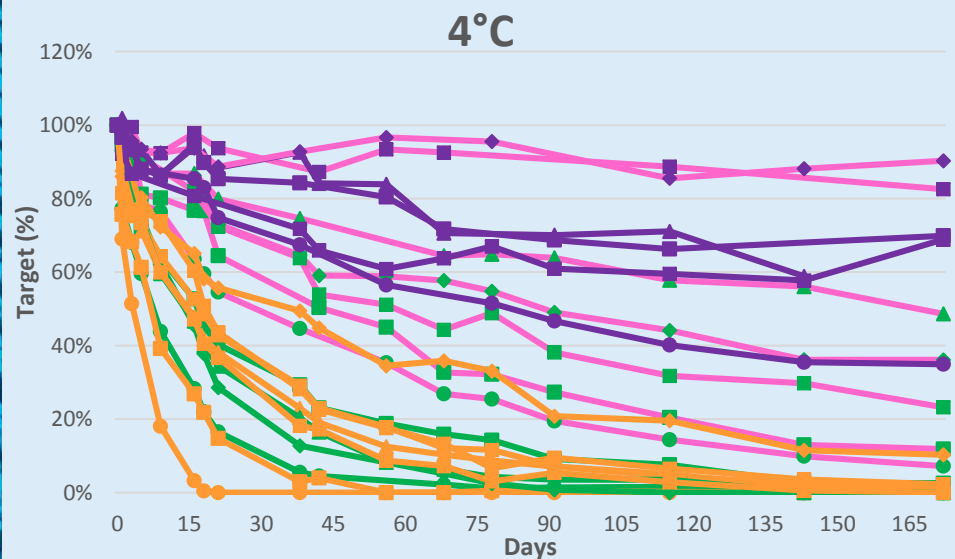
TEMPERATURE DEPENDENCE (PH 4, 100 NG/ML)

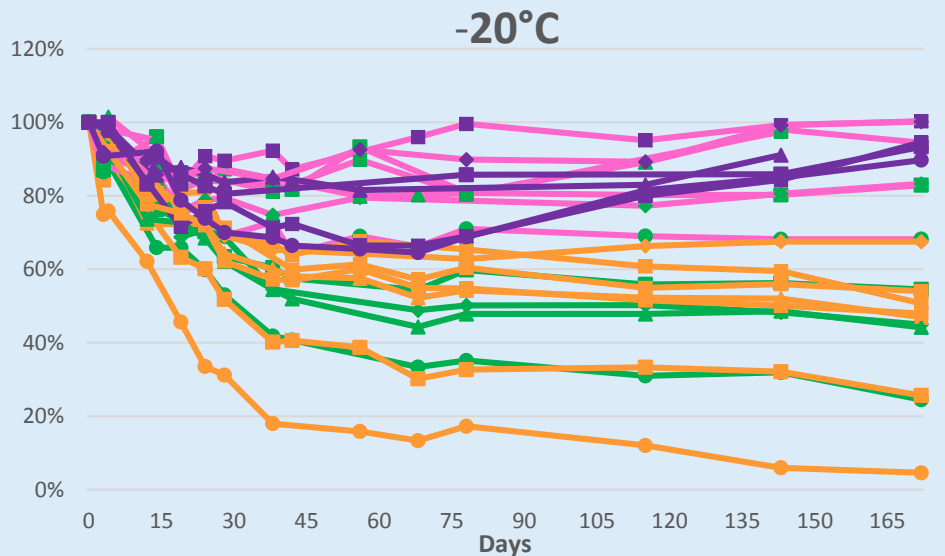


TEMPERATURE DEPENDENCE (PH 8, 100 NG/ML)







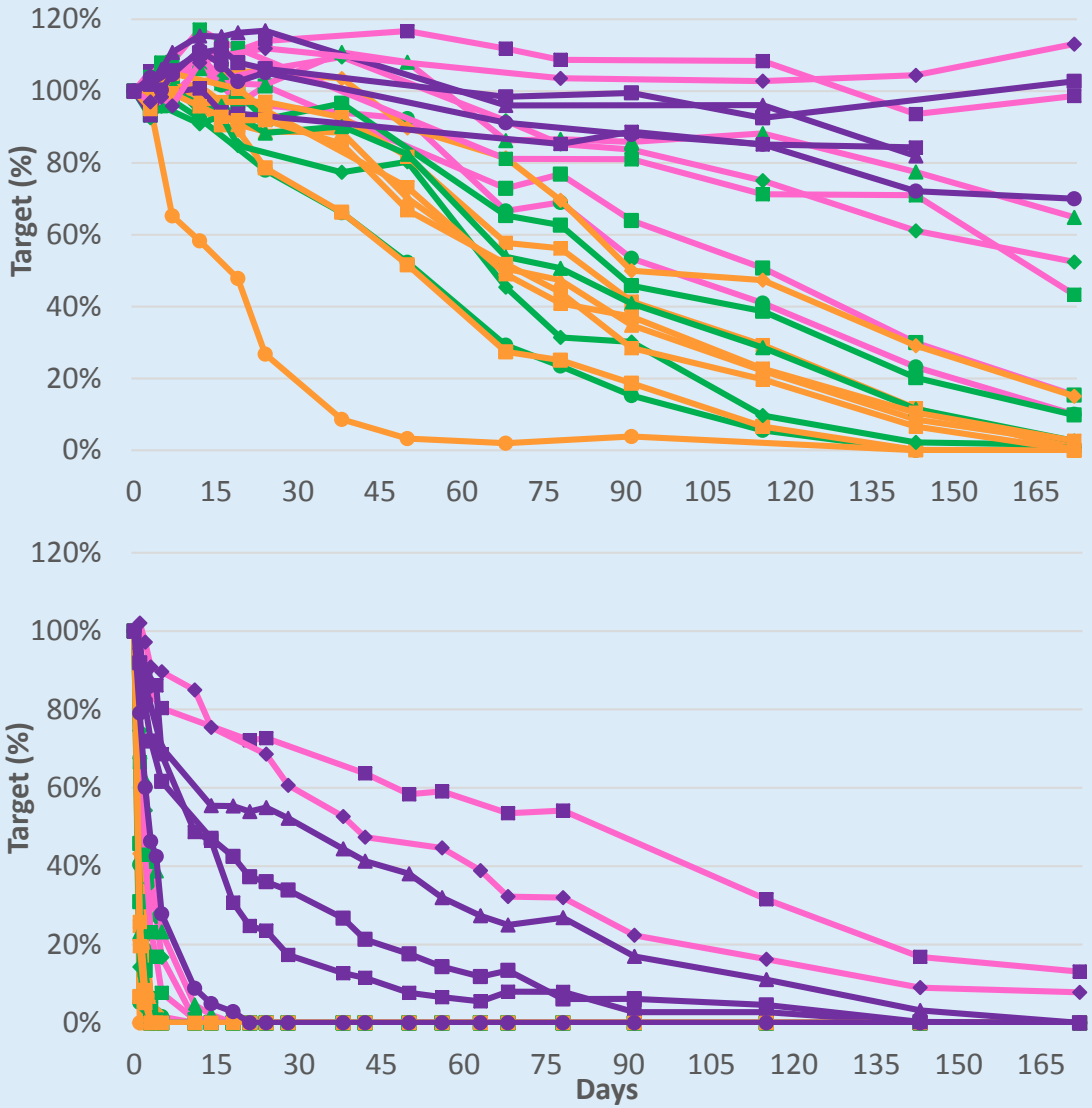


PH DEPENDENCE (100 NG/ML)

32°C

pH 4

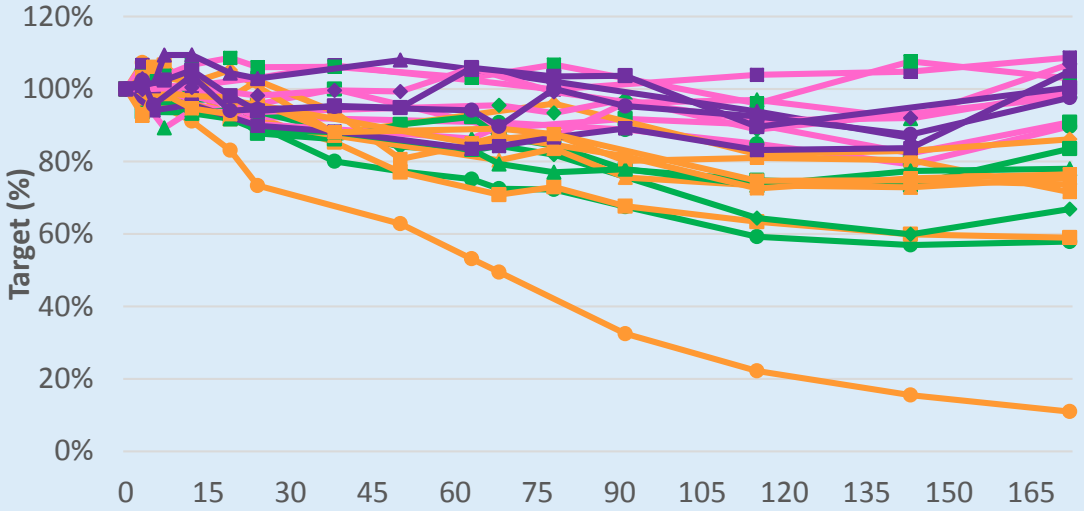
pH 8



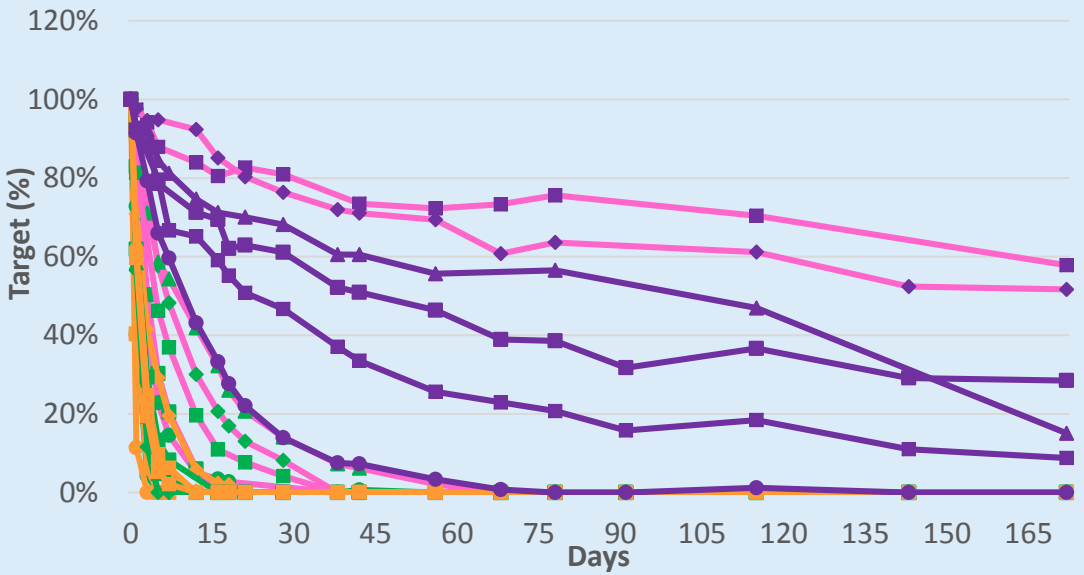
PH DEPENDENCE (100 NG/ML)

20°C

pH 4



pH 8



PH DEPENDENCE (100 NG/ML)

4°C

pH 4

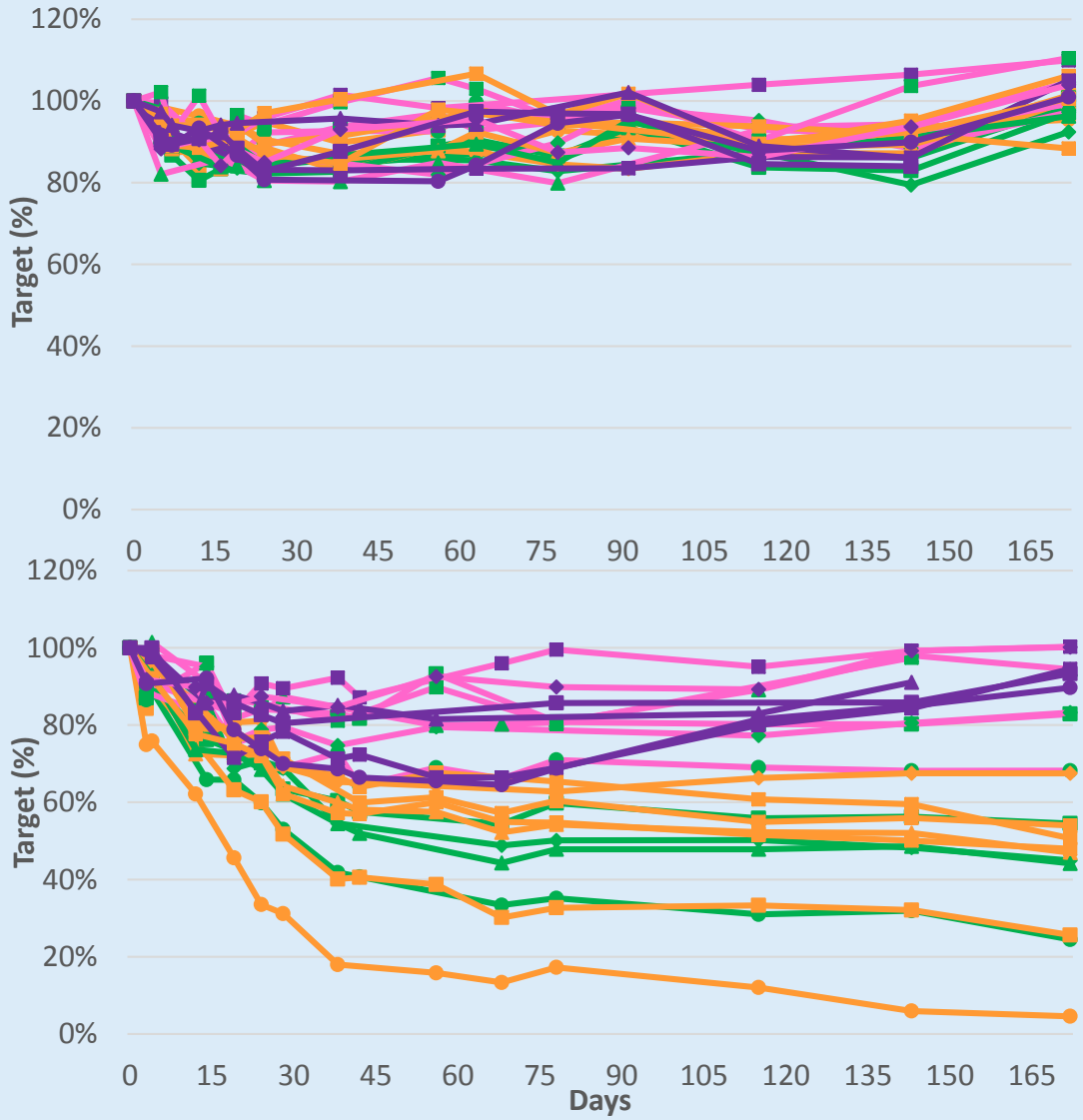
pH 8

PH DEPENDENCE (100 NG/ML)

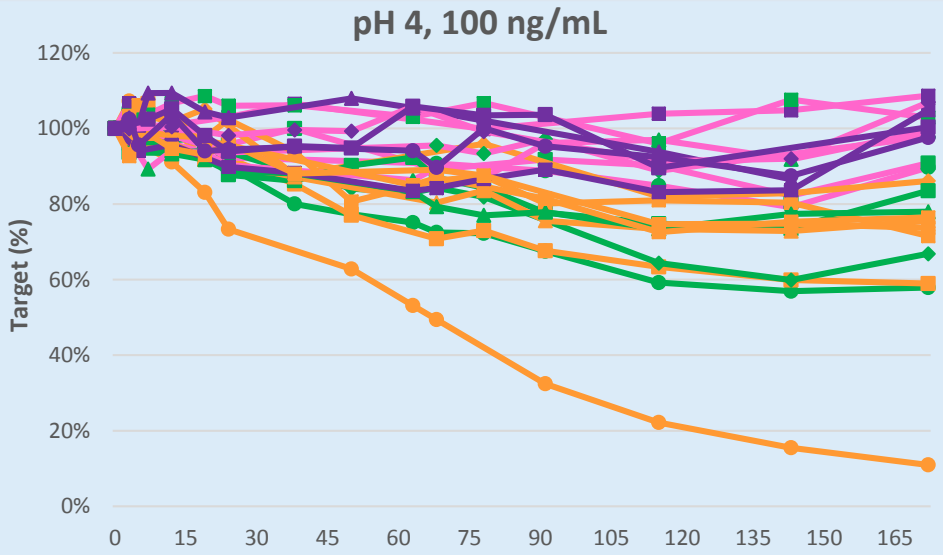
-20°C

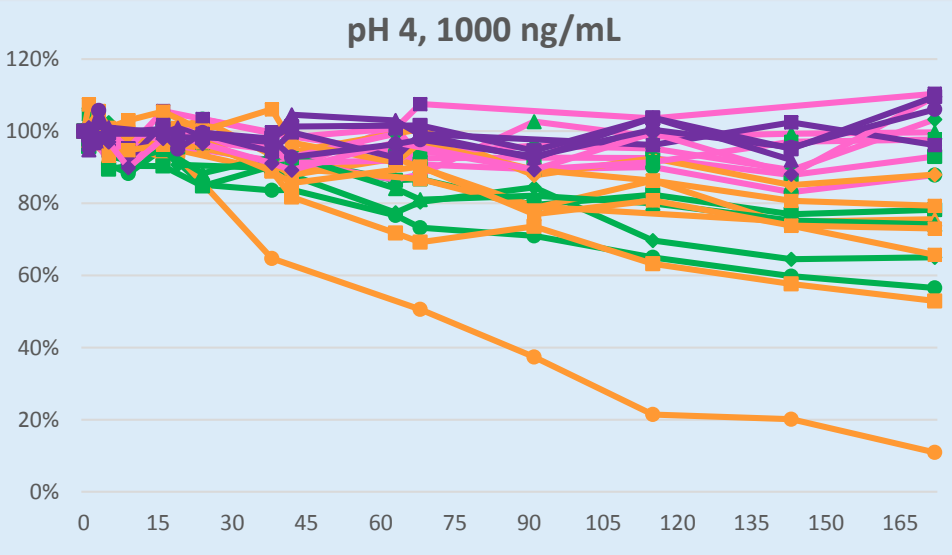
pH 4

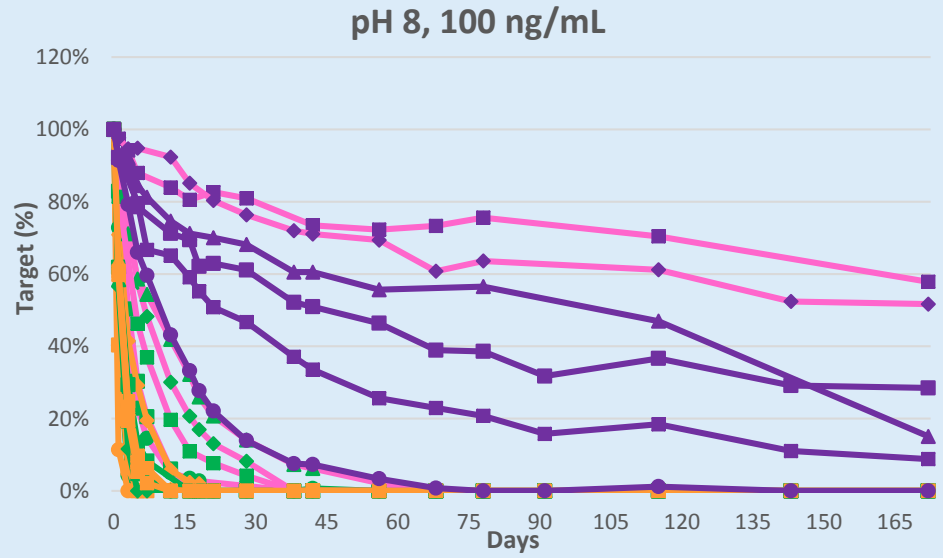
pH 8

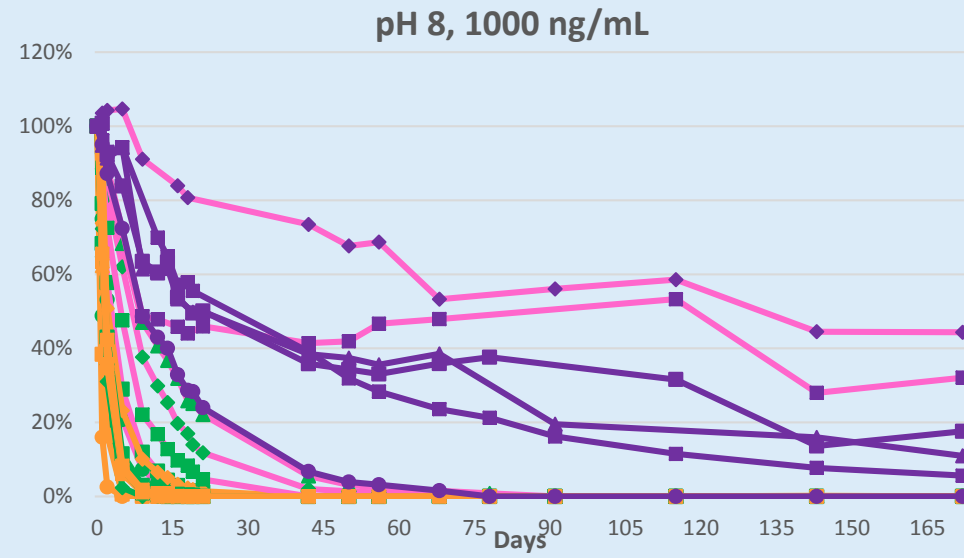


CONCENTRATION DEPENDENCE





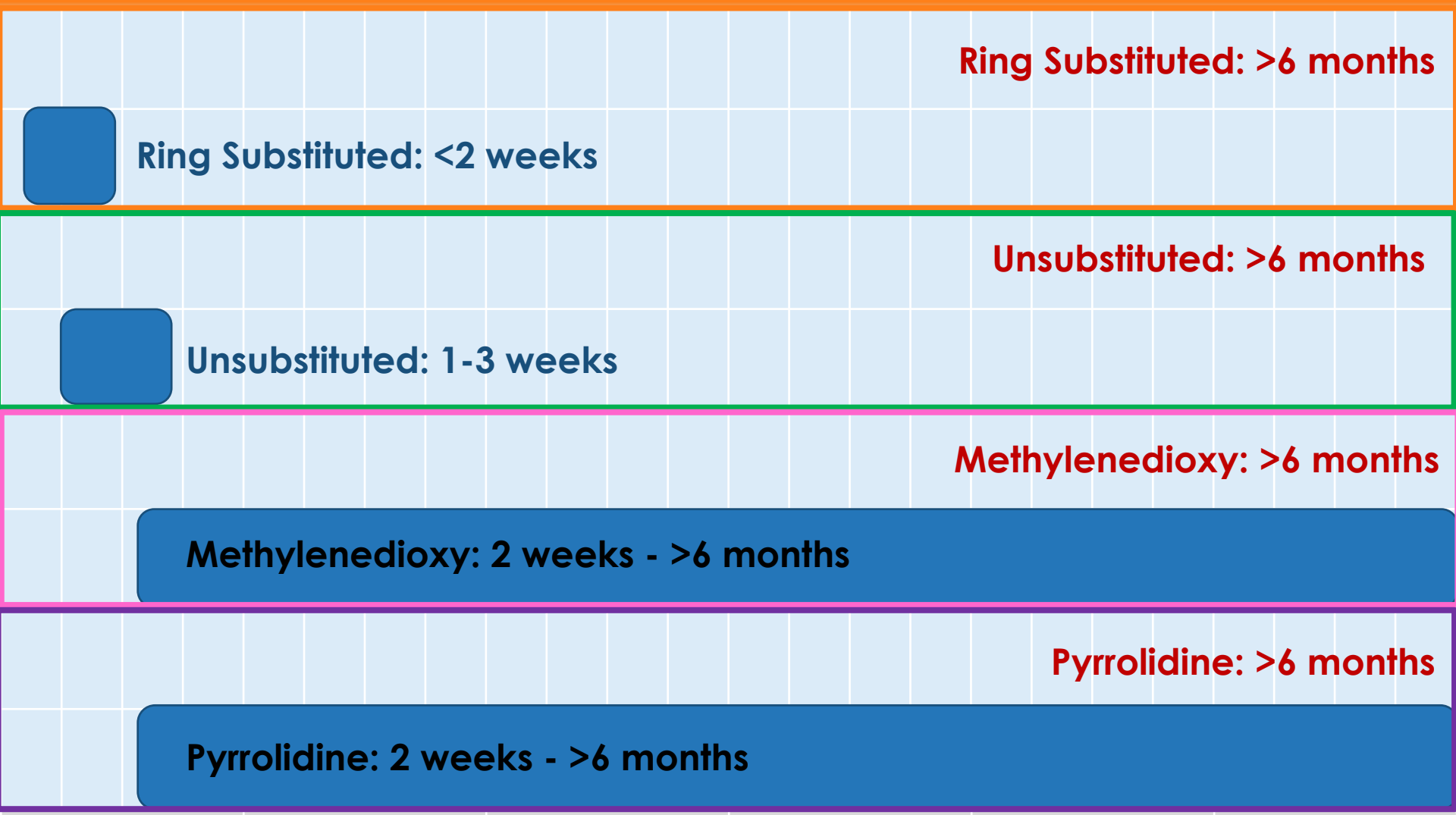




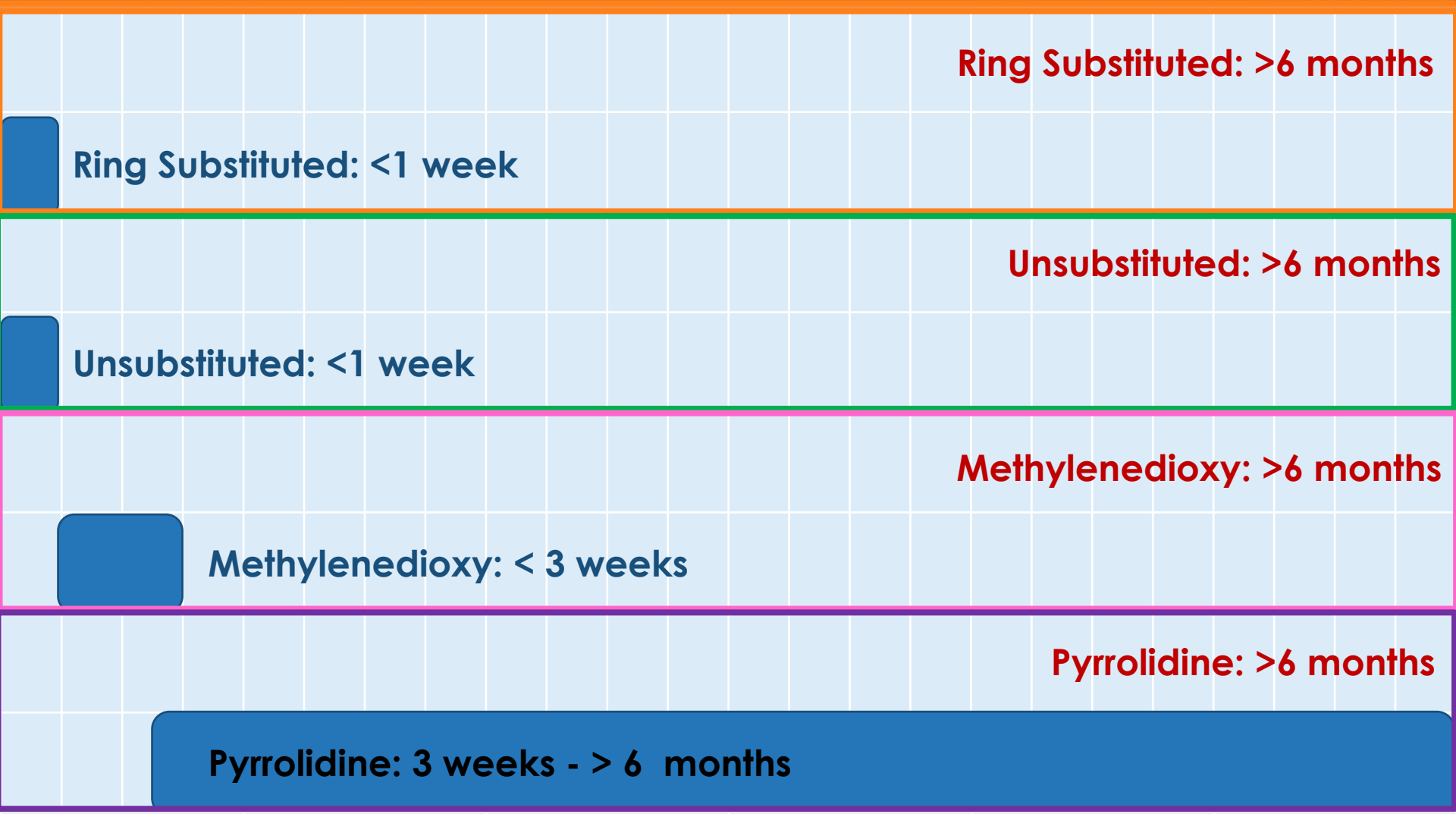
DAYS TO >20% LOSS

Cathinone Structural Group	32°C		20°C		4°C		-20°C	
	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8
Ring Substituted	7-78	<1	24 - >172	≤1	>172	1-5	>172	3-12
Unsubstituted	21-68	<1	42-115	≤1	>172	1-5	>172	7-19
Methylenedioxy	68-143	≤1	>172	1-3	>172	5-21	>172	16 - >172
Pyrrolidine	>143	1-14	>172	3-42	>172	19 - >172	>172	16 - >172

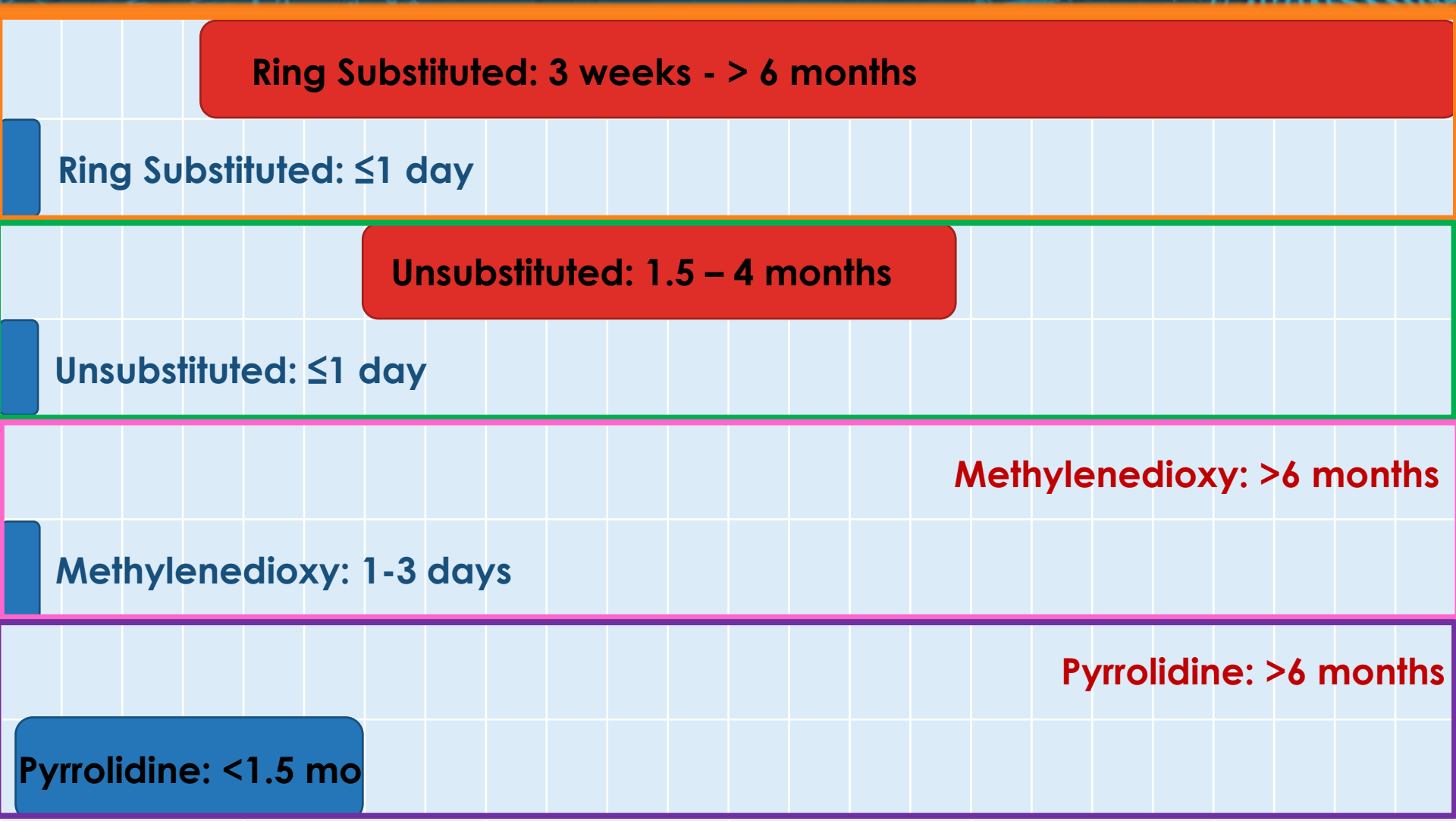
-20°C INSTABILITY



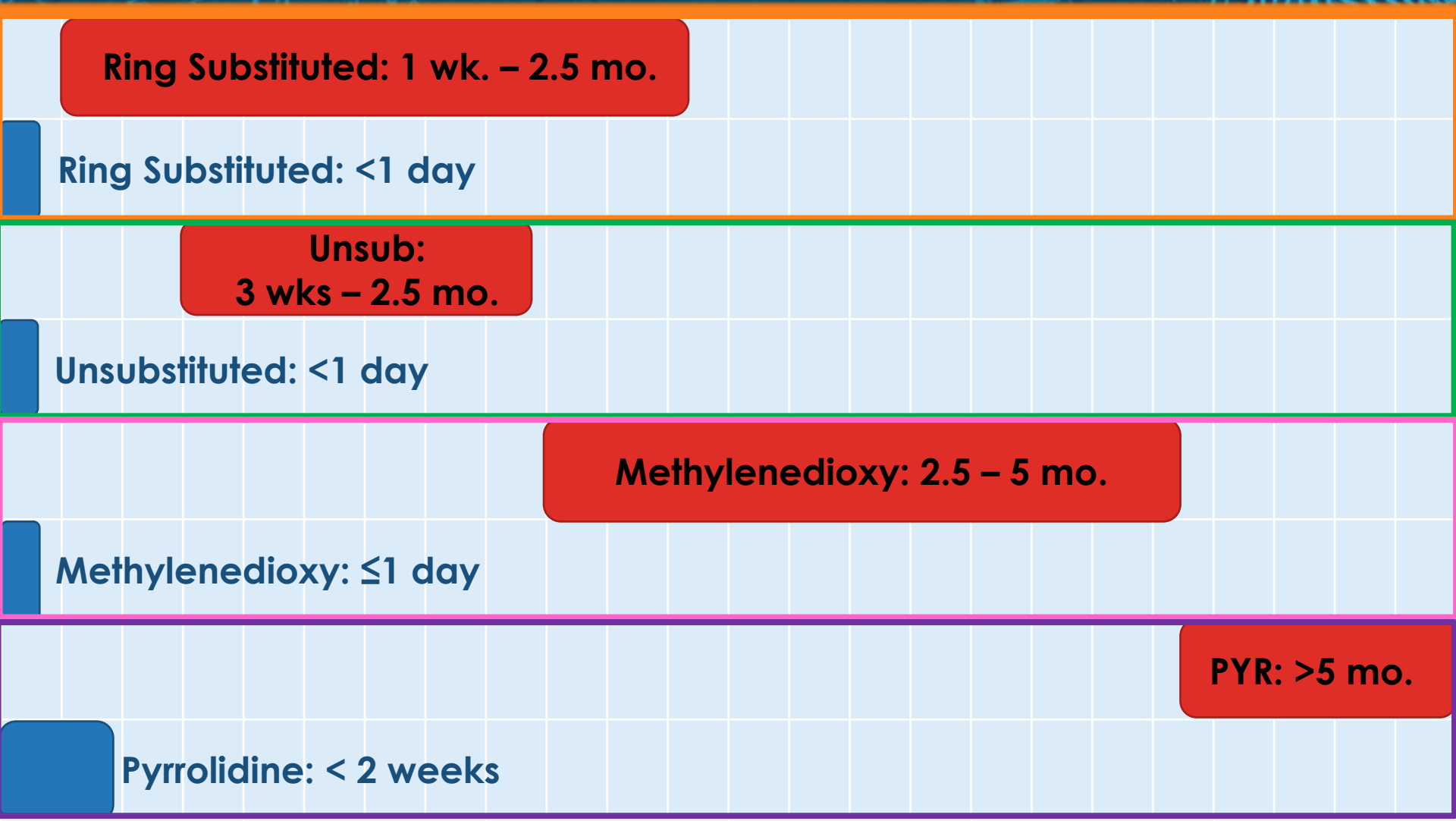
4°C INSTABILITY



20°C INSTABILITY



32°C INSTABILITY

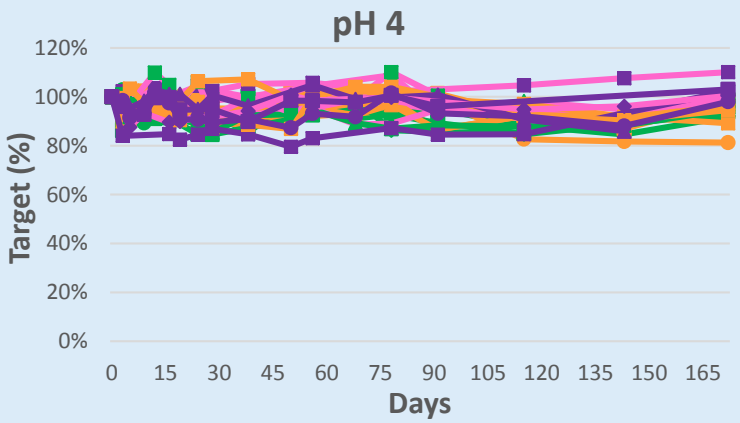


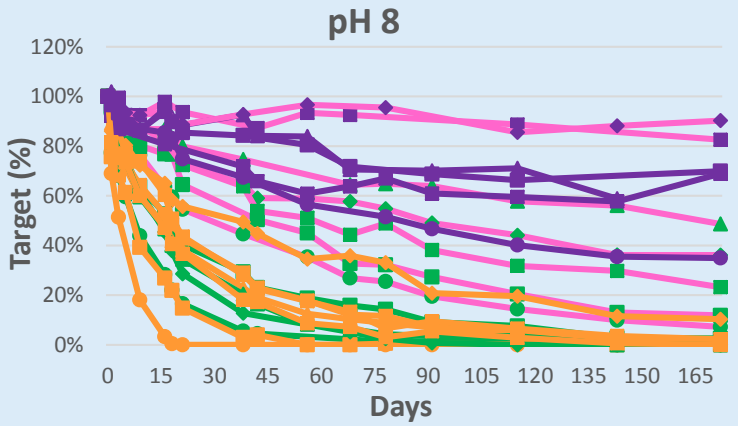
CONCLUSIONS

- pH Dependence
  Acidic > Alkaline
- 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

- **Significant loss on the order of hours (alkaline urine, 32°C and 20°C)**
  - 3-FMC: 32°C—undetectable after 6 hours
  - Substituted and Unsubstituted
  - 32°C: 20-88% loss after 6 hours
  - 20°C: 21-89% loss after 22 hours
- **Instability at common storage conditions (4°C)**





- **Significant Structural Influence**

ACKNOWLEDGEMENTS

This project was supported by Award No. 2013-R2-CX-K006 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Questions

Lindsay Glicksberg
lcg012@shsu.edu

Sam Houston State University