Stability of Synthetic Cathinones in Biological Evidence

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NIJ AWARD

NIJ Award # 2013-R2-CX-K006

Solicitation: Applied Research and Development in Forensic Science for Criminal Justice Purposes

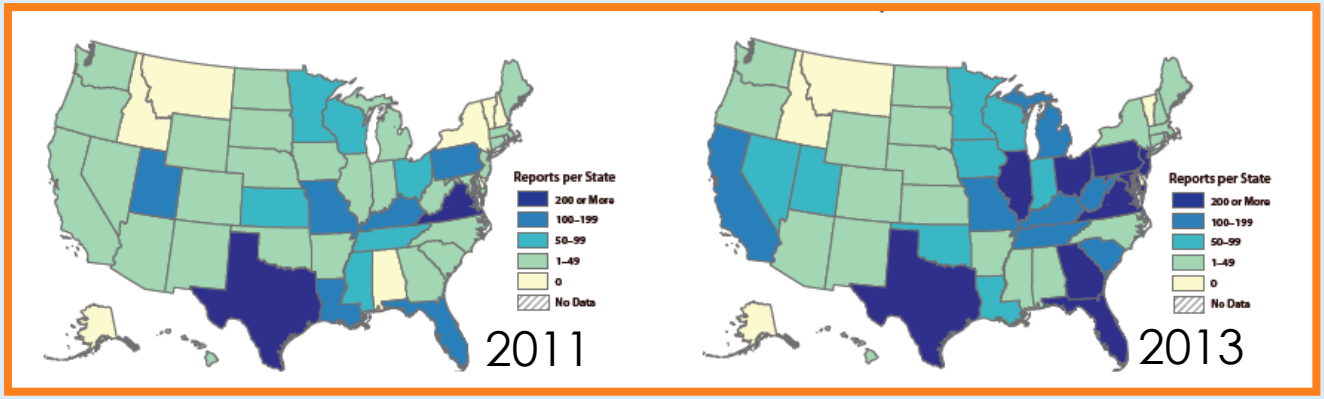
SYNTHETIC CATHINONE BACKGROUND

- 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”

PREVALENCE

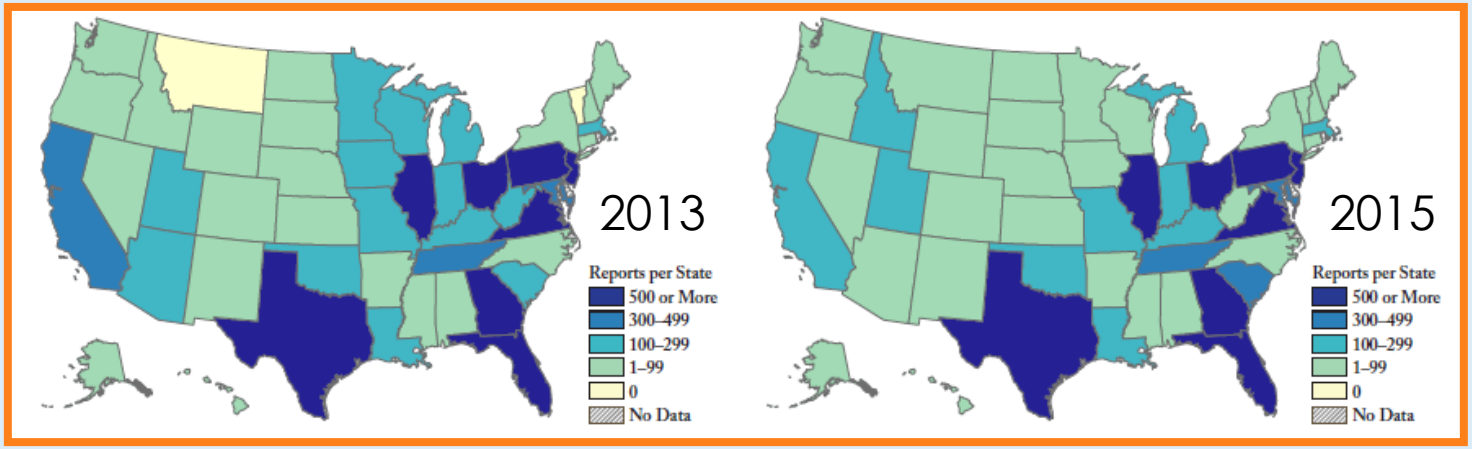
National Forensic Laboratory Information System (NFLIS)

- Special Report 2013-2015



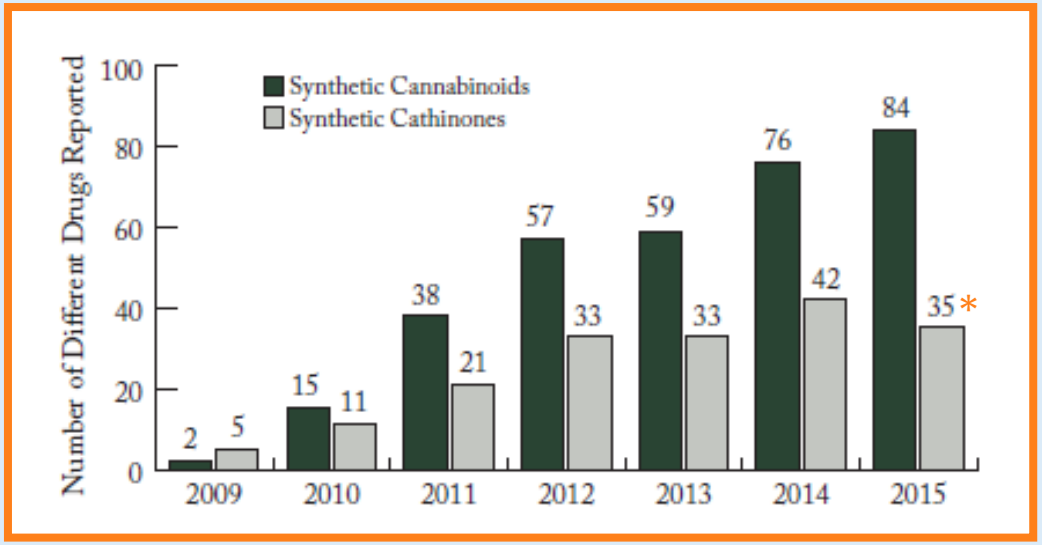
2010 – 2013:
29,648 reports

2013 – 2015:
51,824 reports



PREVALENCE

2010 – 2013	
Methylone	Naphyrone
MDPV	MDPBP
α-PVP	MPHP
4-MEC	Ethylcathinone
Pentedrone	3,4-DMMC
Mephedrone	Methedrone
Butylone	
FMC	
Pentylone	
4-MePPP	
α-PBP	
Ethylone	
Buphedrone	
Methcathinone	



2013 – 2015	
Methylone	**Butylone**
α-PVP	**Pentedrone**
Ethylone	α-PHP
MDPV	**α-PBP**
4-MEC	Dibutylone
Dimethylone	PV8
4-BMC	
4-CMC	
Pentylone	
FMC	
4-EMC	
Mephedrone	
Buphedrone	
4-MePPP	

SCHEDULING

Two Acts for Drug Scheduling

1. Controlled Substance Act of 1970
2. Controlled Substance Analogue Enforcement Act of 1986

1993: Schedule I: methcathinone

2011: Schedule I: methylone, mephedrone, and MDPV

2011: States (30) introduce legislation

2012: President Obama signs Food and Drug Administration Safety and Innovation Act of 2012

- contains Synthetic Drug Abuse Prevention Act of 2012

2013: 48 states + DC have legislation

2014: Schedule I (temporary): 4-MEC, alpha-PVP, butylone, pentedrone, pentylone, 4-FMC, 3-FMC, and naphyrone

2016: Temporary Schedule I from 2014 extended

PHARMACOLOGY

Demographics: males, ages 10-73 (mean of 26)

Routes of Administration: IN, PO, IV, or IM

Desired Effects: stimulant and euphoric symptoms

- Increased energy, mood enhancement, empathy, sociability, concentration, euphoria
- Onset: 10-20 minutes
- Peak: 45-90 minutes
- Duration: 6-8 hours

Adverse Effects: neurological, cardiovascular, and psychopathological symptoms

- Hallucinations, delusions, confusion, violence, homicidal tendencies, death
- Duration: hours to days

SYNTHETIC CATHINONES IN TOXICOLOGY CASEWORK

Antemortem Cases

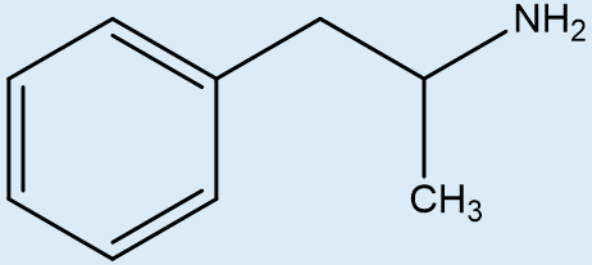
- Motor Vehicle accidents
- DUID
- Urine: 5-1,300 ng/mL
- Blood: 3-240 ng/mL

Postmortem Cases

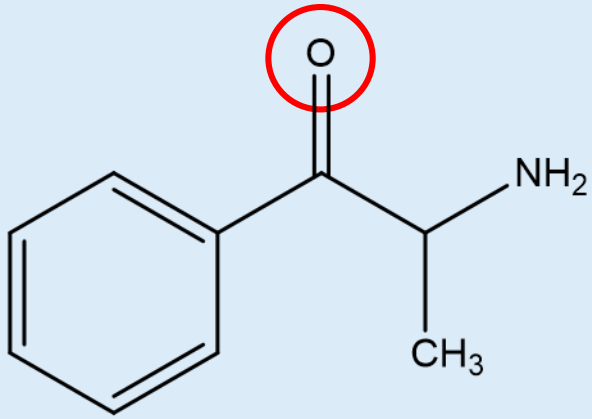
- Overdose
- Suicide
- Homicide
- Urine: 220-20,000 ng/mL
- Blood: 3-5,000 ng/mL

CHEMISTRY

- Phenethylamines
- Beta-ketone
- Substituents on the alkyl chain and benzyl ring



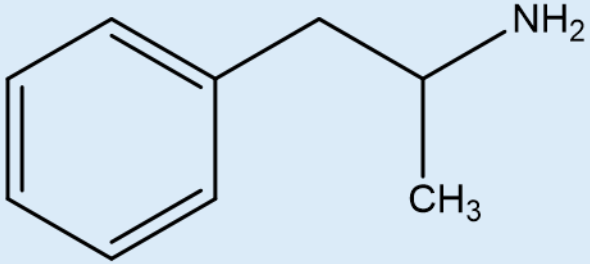
Methamphetamine



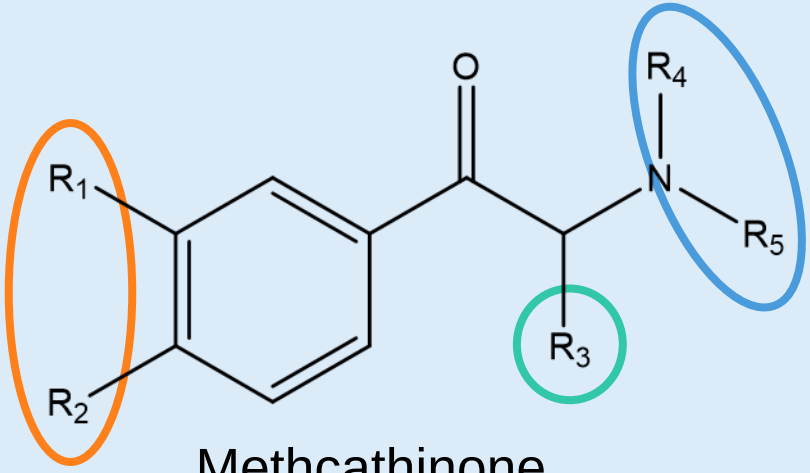
Methcathinone

CHEMISTRY

- Phenethylamines
- Beta-ketone
- Substituents on the alkyl chain and benzyl ring



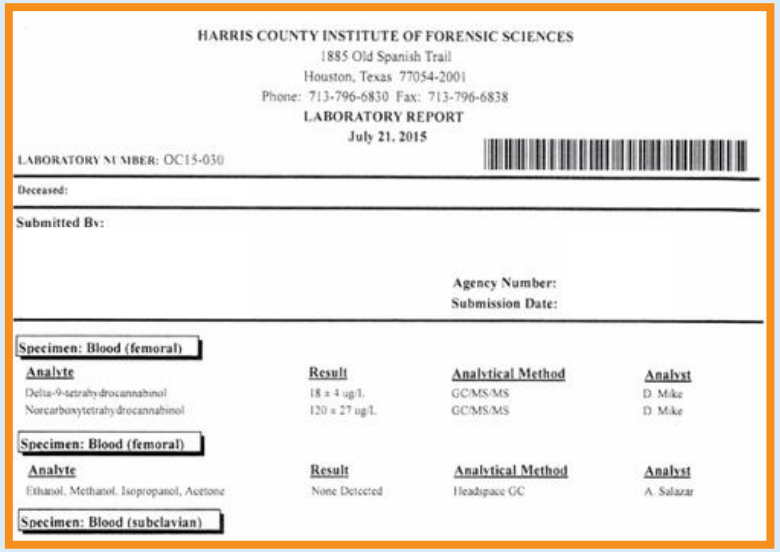
Methamphetamine



Methcathinone

TOXICOLOGICAL INTERPRETATION

- Relies on reliable quantitative and qualitative results
- Concentration at time of death or time of collection most relevant



HARRIS COUNTY INSTITUTE OF FORENSIC SCIENCES
1885 Old Spanish Trail
Houston, Texas 77054-2001
Phone: 713-796-6830 Fax: 713-796-6838
LABORATORY REPORT
July 21, 2015

LABORATORY NUMBER: OC15-030

Deceased:

Submitted By:

Agency Number:
Submission Date:

Specimen: Blood (femoral)

Analyte	Result	Analytical Method	Analyst
Delta-9-tetrahydrocannabinol	18 ± 4 ug/L	GC/MS/MS	D. Mike
Norcarboxytetrahydrocannabinol	120 ± 27 ug/L	GC/MS/MS	D. Mike

Specimen: Blood (femoral)

Analyte	Result	Analytical Method	Analyst
Ethanol, Methanol, Isopropanol, Acetone	None Detected	Headspace GC	A. Salazar

Specimen: Blood (subclavian)

https://www.nytimes.com/

IMPORTANCE OF STABILITY

- 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

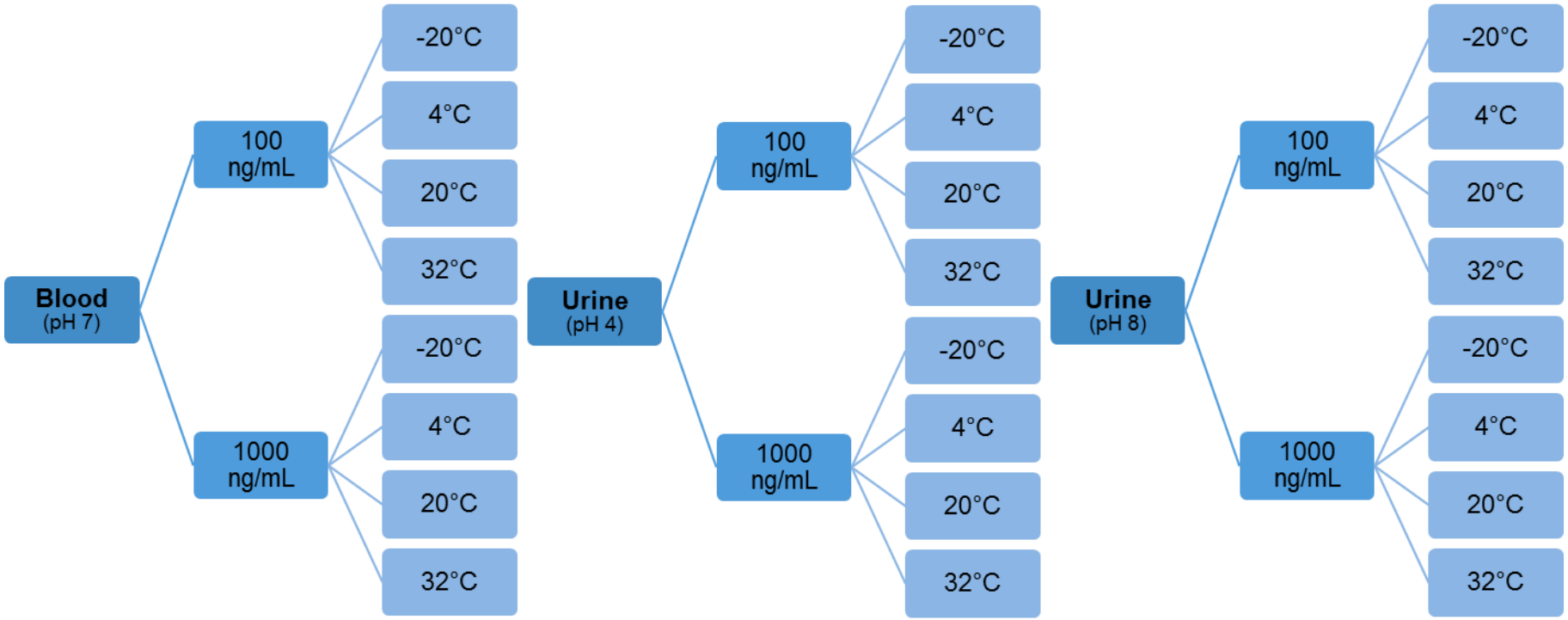
STABILITY STUDIES

Reference	# Synthetic Cathinones	# Biological Matrices	# Temperatures	Time Length
Paul and Cole	2	1	2	3 months
Sorensen	7	2	2	7 days
Tsujikawa	5	N/A	1	48 hours
Johnson and Botch-Jones	2	3	3	14 days
Soh and Elliot	1	2	2	7 days
Proposed Research	22	2	4	6 months

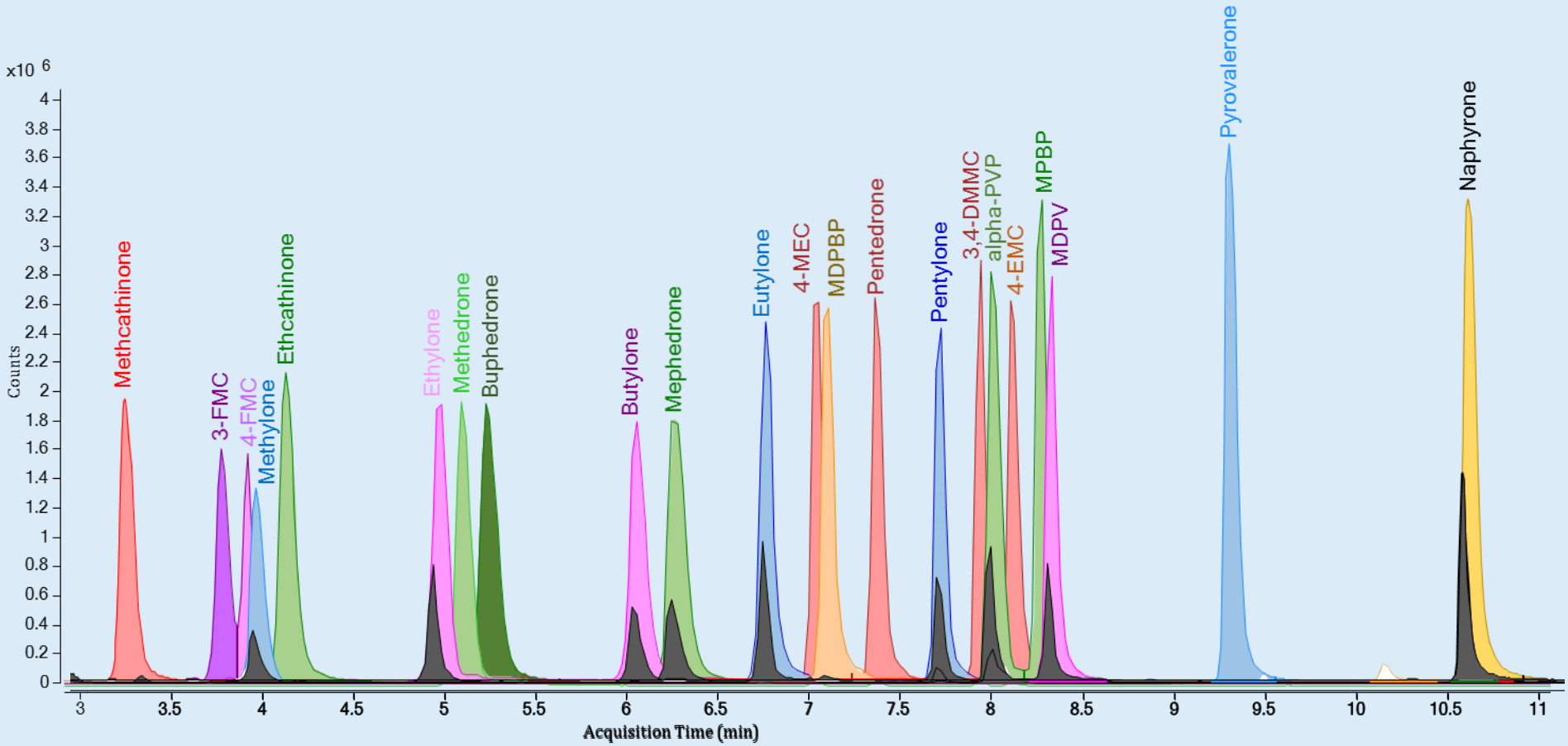
Comprehensive study assessing stability as it relates:

1. pH
2. Concentration
3. Temperature
4. Biological Matrix
5. Storage Time
6. Chemical Structure

RESEARCH DESIGN



CHROMATOGRAPHIC SEPARATION



VALIDATION SUMMARY

SWGTOX Standard Practices for Method Validation

Validation Parameter	Urine	Blood
LOD	0.25 – 5 ng/mL	1 – 5 ng/mL
LOQ	0.25 – 5 ng/mL	1 – 5 ng/mL
Precision	± 15%	± 15%
Bias	± 15%	± 15%
Analytical Recovery	84 – 104%	81 – 91%
Matrix Effects	± 20%	± 20%
Interferences	No interferences	No interferences

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

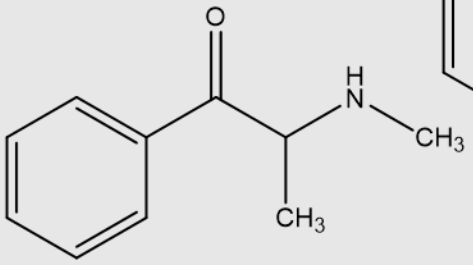
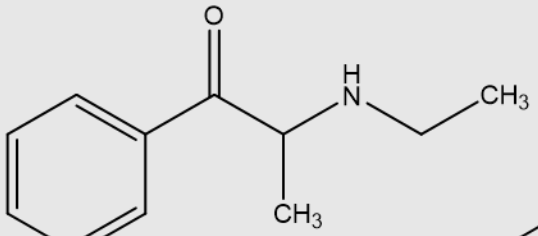
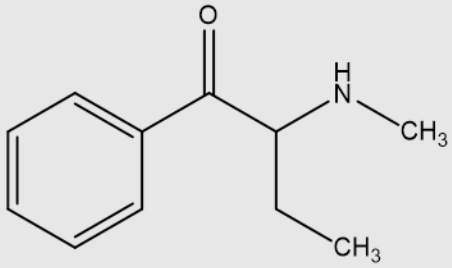
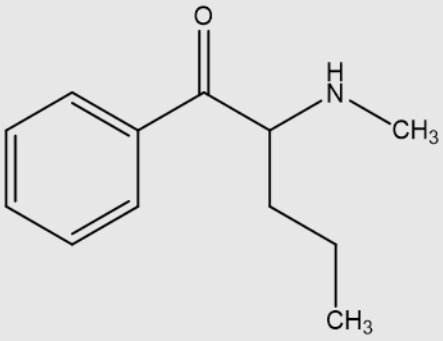
- Samples in duplicate (n=2)
  - 1000 ng/mL samples 1:4 dilution
- Calibrators extracted with every run
  - 10, 25, 100, 250, 350, and 500 ng/mL
- Negative and Positive (100 ng/mL) Controls

Analysis

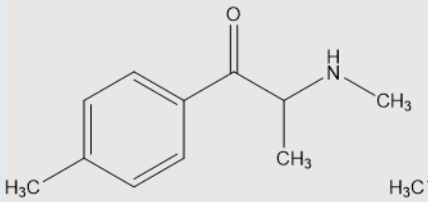
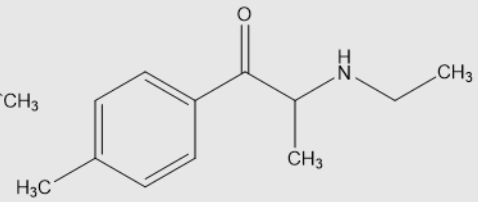
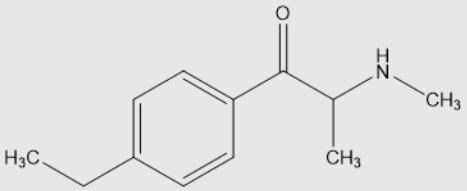
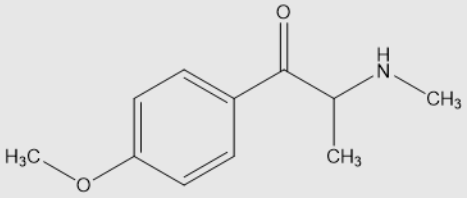
- Concentration Mean (n=2)
- Error bars omitted for clarity
- Significant >20% loss

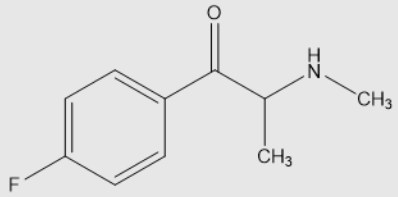
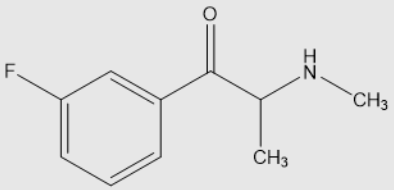
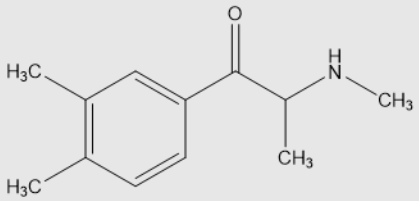
Month	Samplings/Week
1	4
2-3	2-3*
4-6	1
6-12	1/month

SECONDARY AMINES, NO RING SUBSTITUENTS

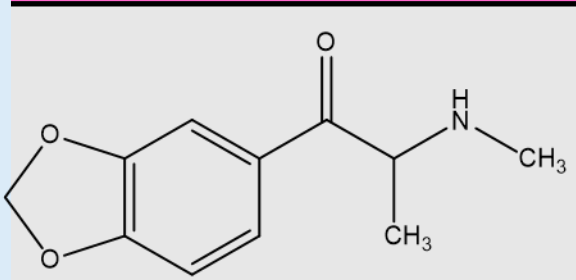
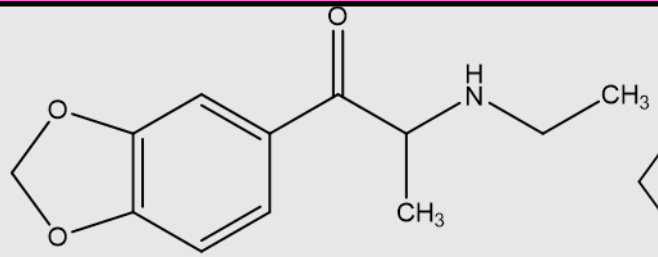
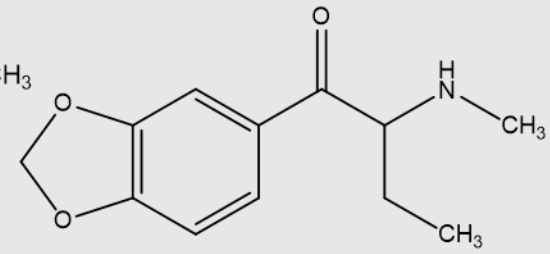
Methcathinone	Ethcathinone	Buphedrone	Pentedrone
			

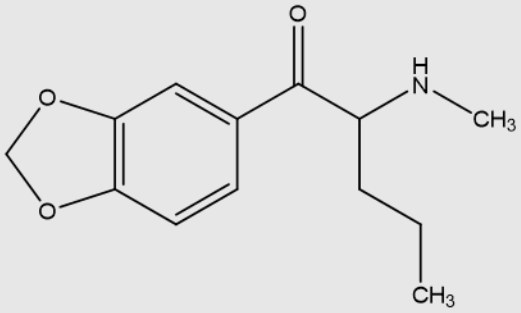
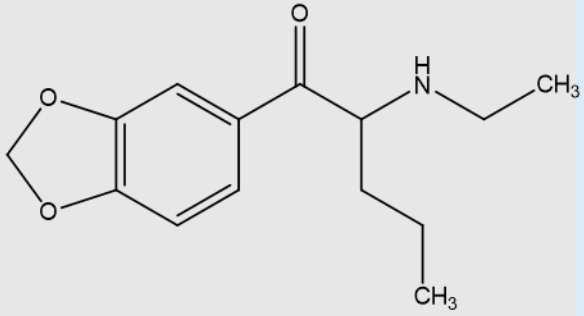
SECONDARY AMINES, RING SUBSTITUTED

Mephedrone*	4-MEC	4-EMC	Methedrone
			

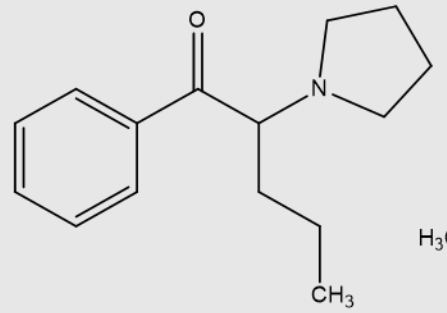
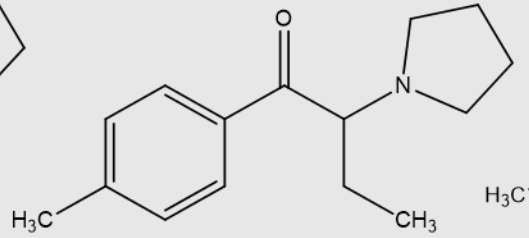
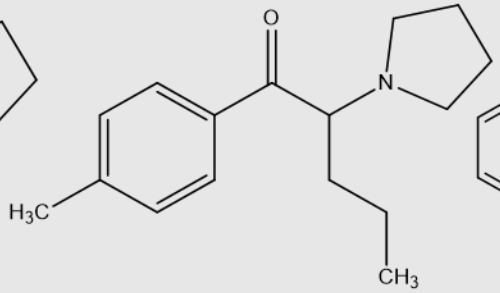
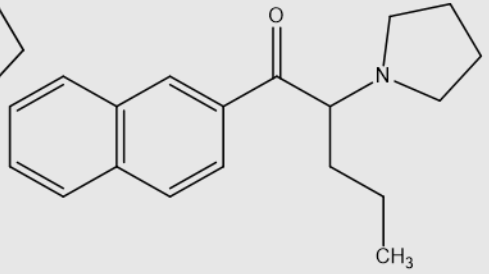
Flephedrone	3-FMC	3,4-DMMC
		

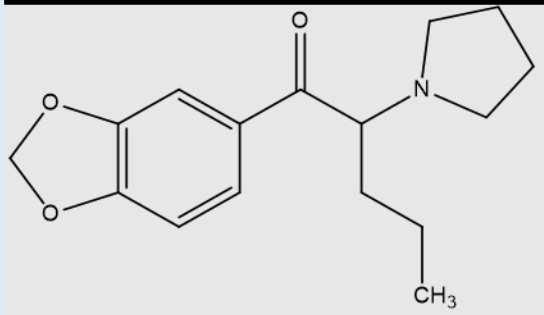
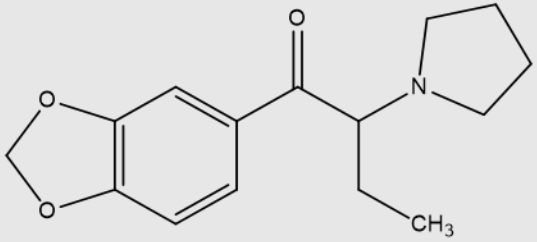
SECONDARY AMINES, METHYLENEDIOXY TYPE

Methylone*	Ethylone*	Butylone*
		

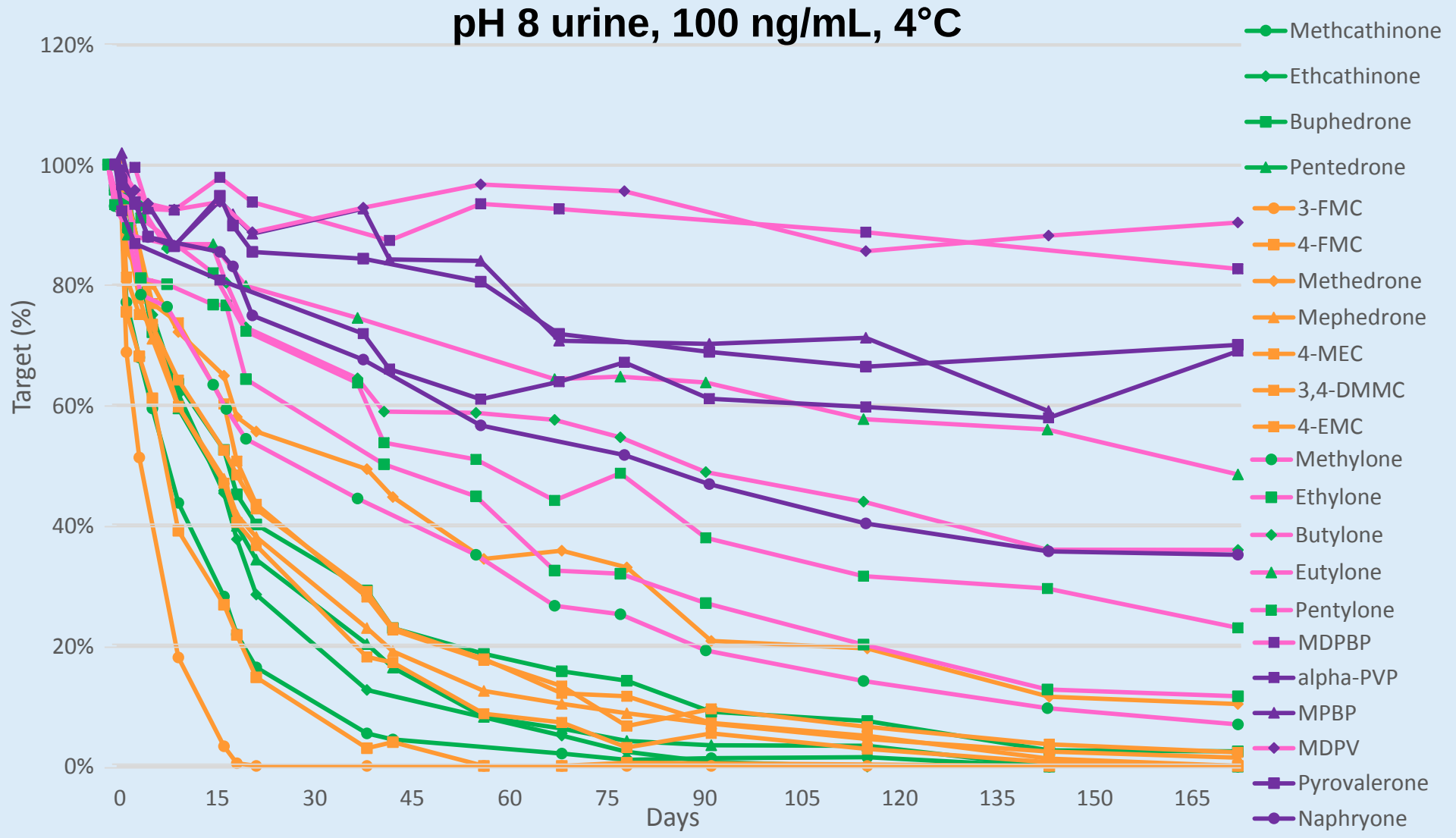
Pentylone*	Eutylone*
	

TERTIARY AMINES, PYRROLIDINE TYPE

Alpha-PVP*	MPBP	Pyrovalerone	Naphyrone*
			

MDPV*	MDPBP
	

CHEMICAL STRUCTURE DEPENDENCE



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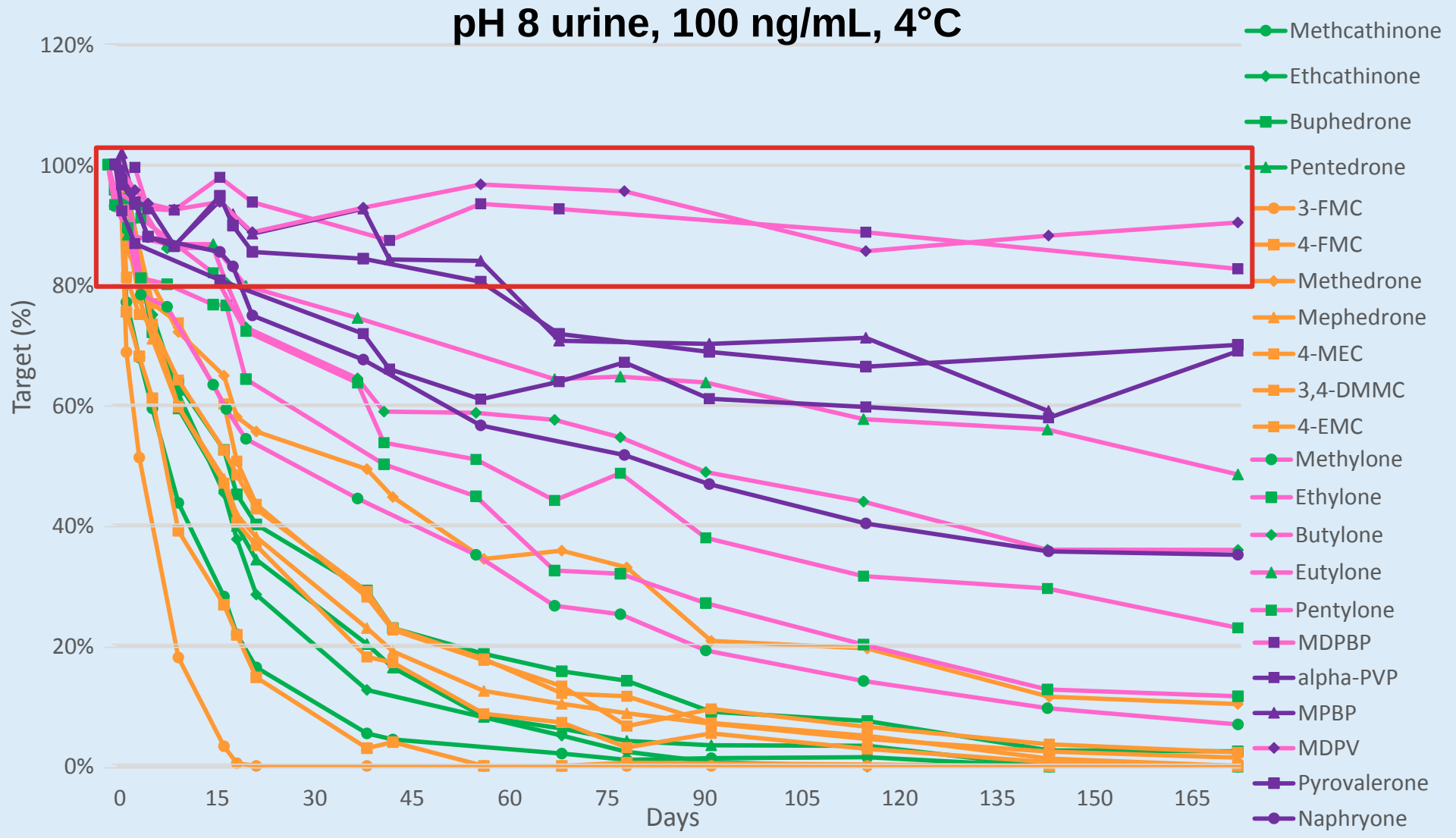
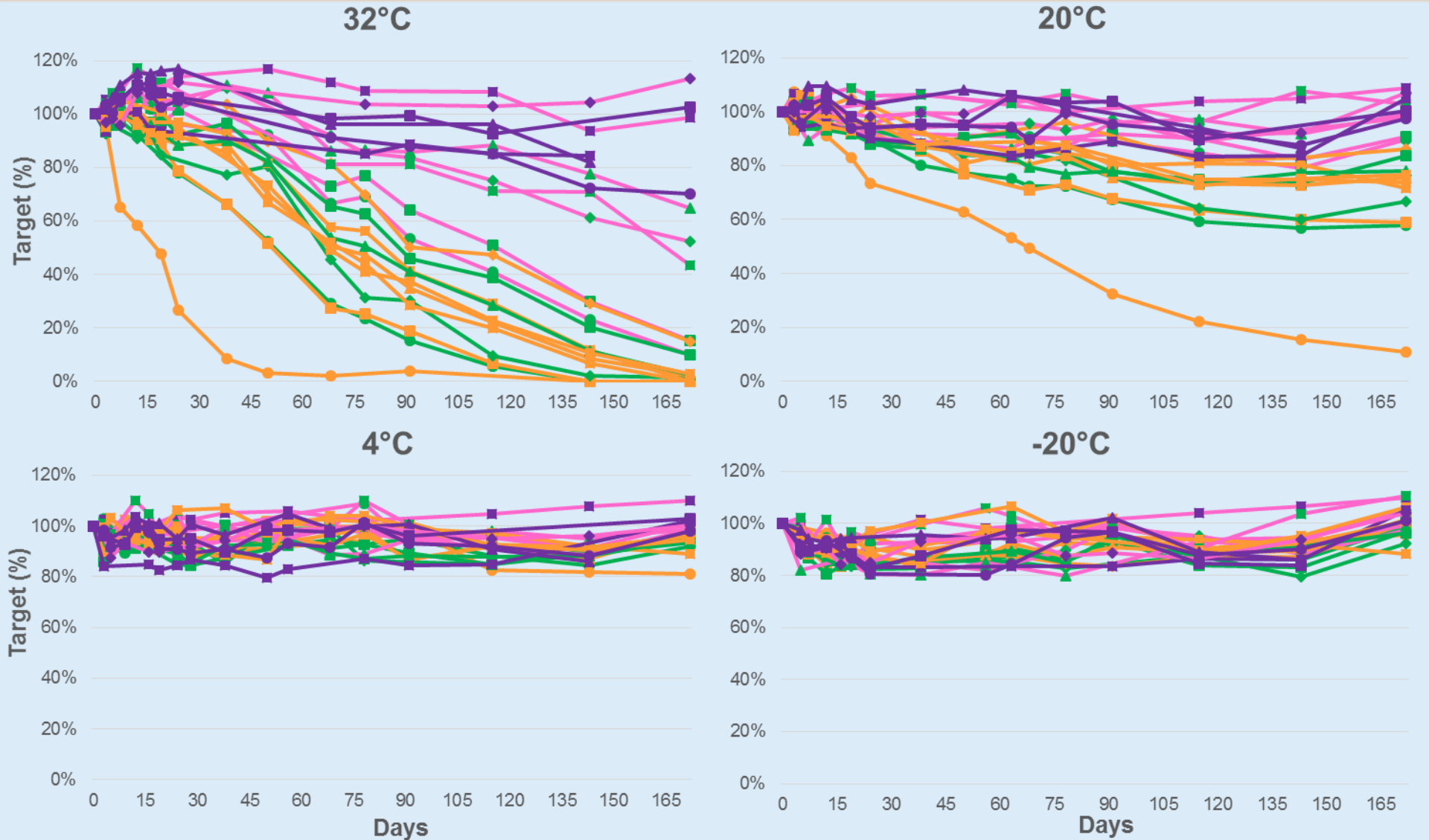
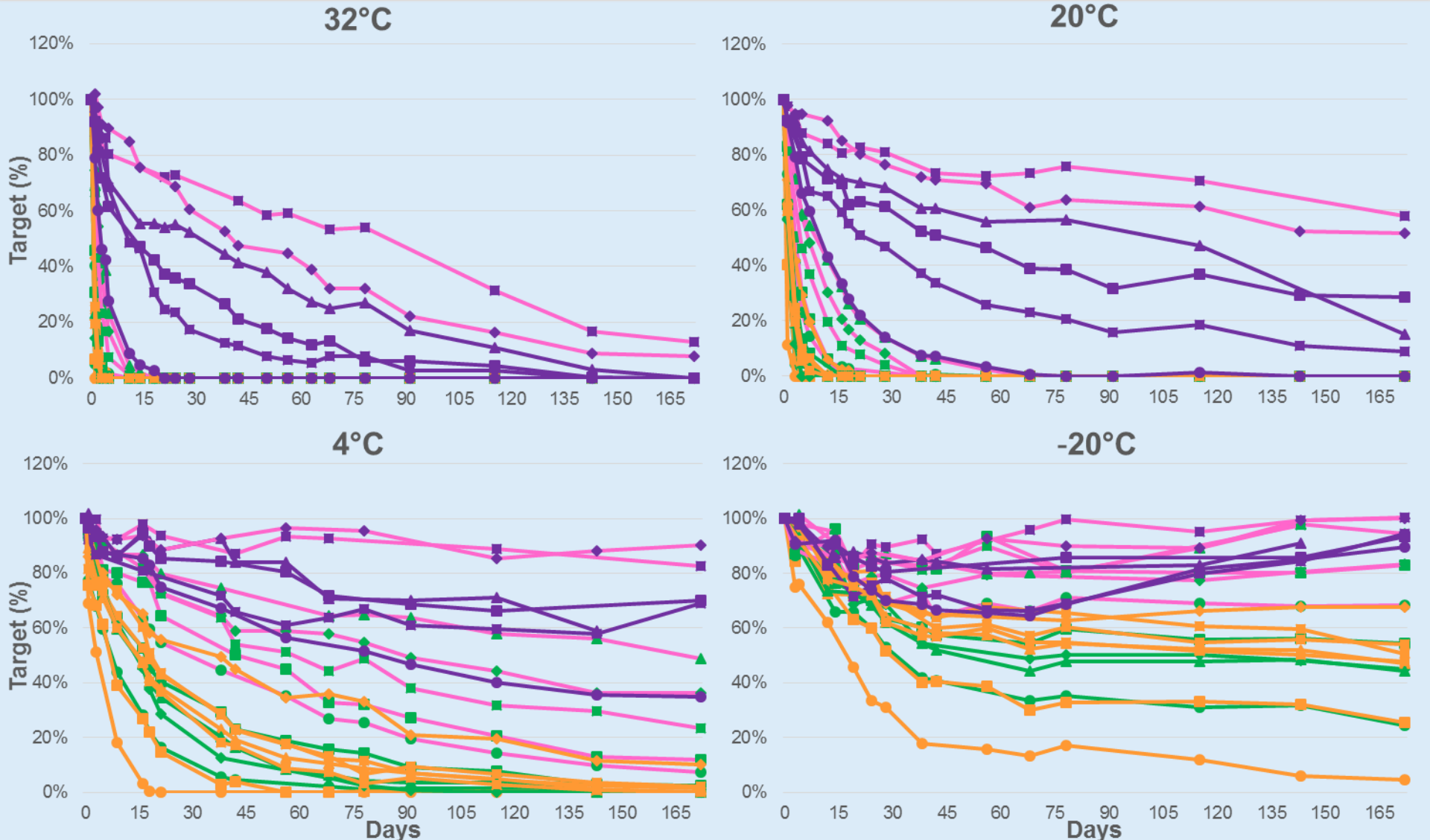


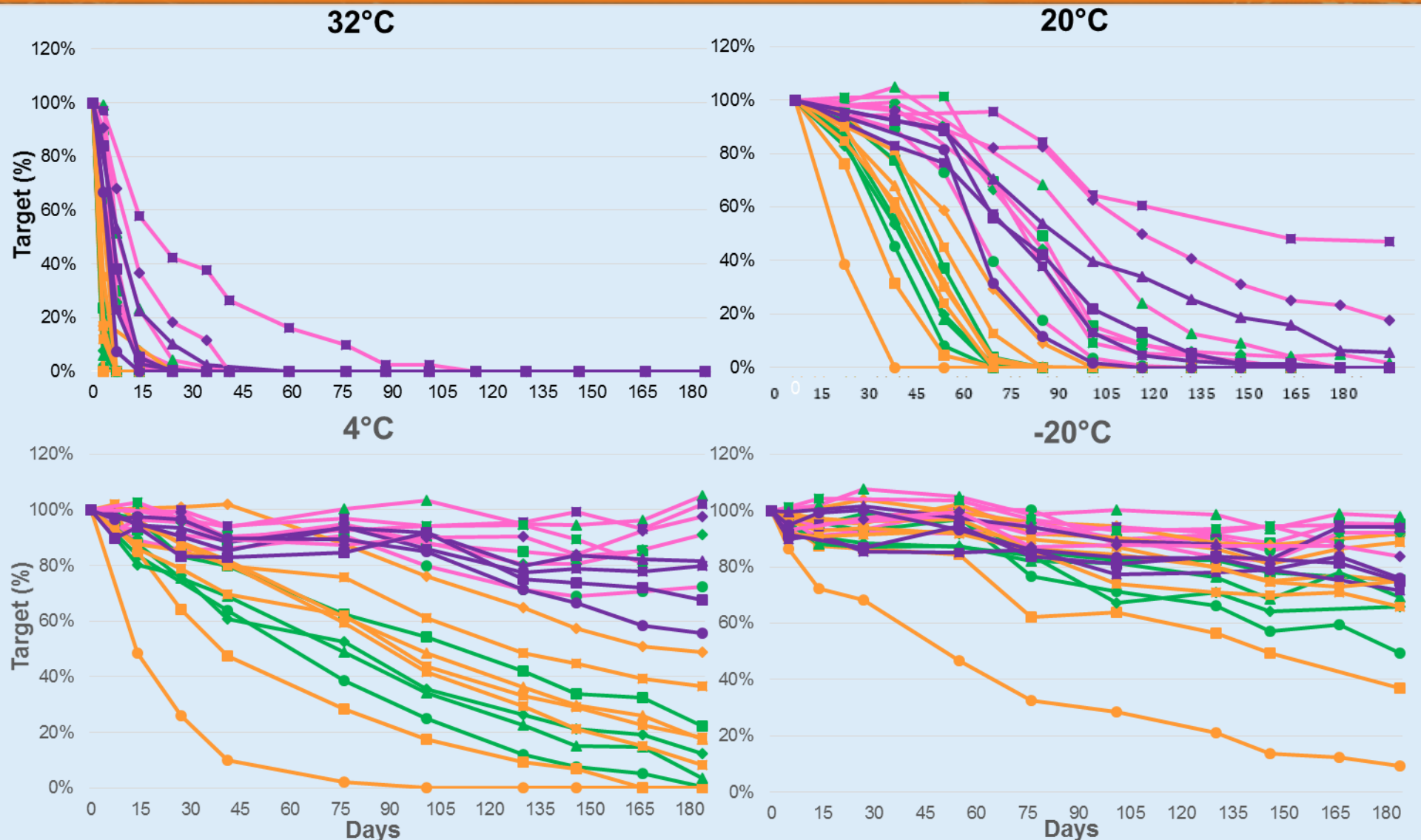
TEMPERATURE DEPENDENCE (URINE, PH 4, 100 NG/ML)



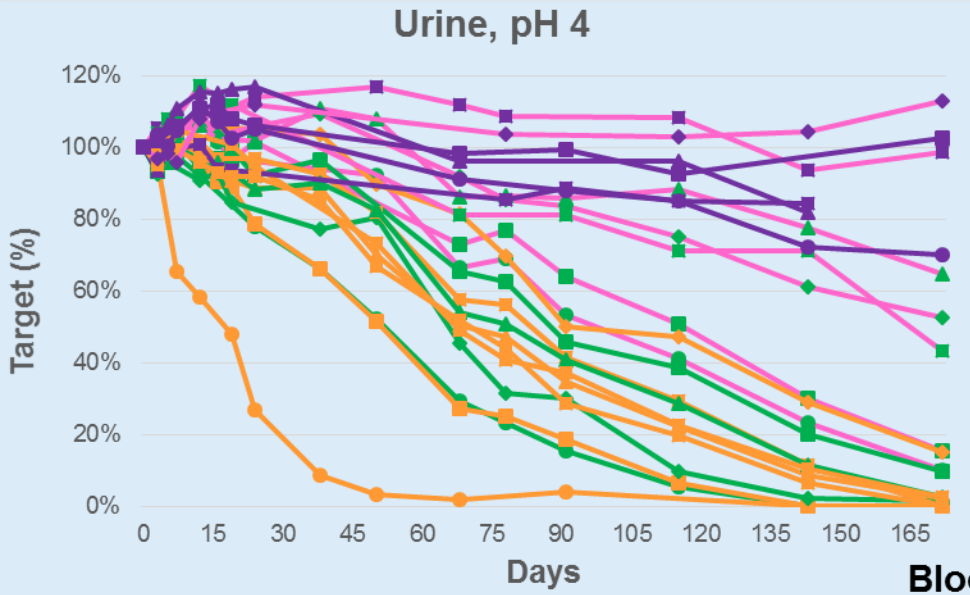
TEMPERATURE DEPENDENCE (URINE, PH 8, 100 NG/ML)

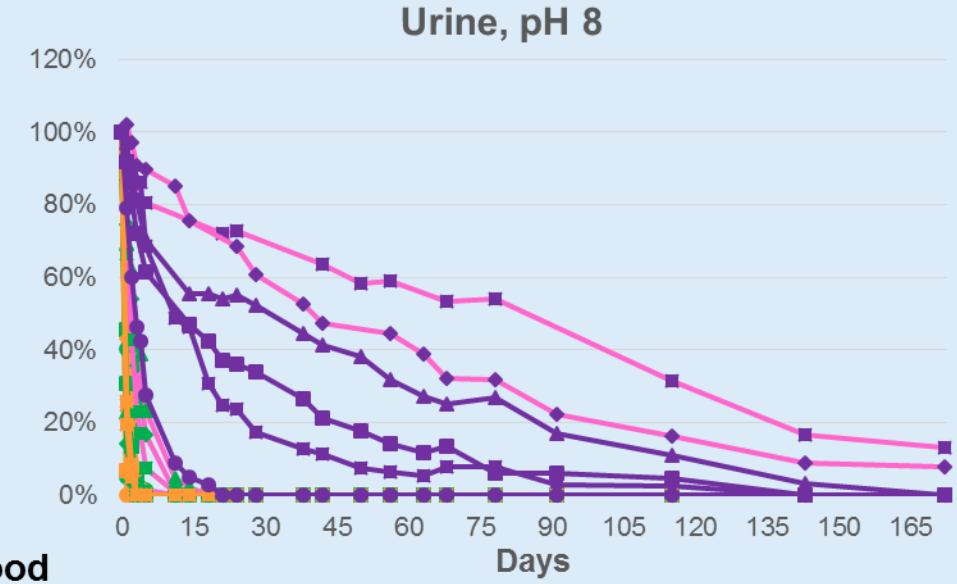


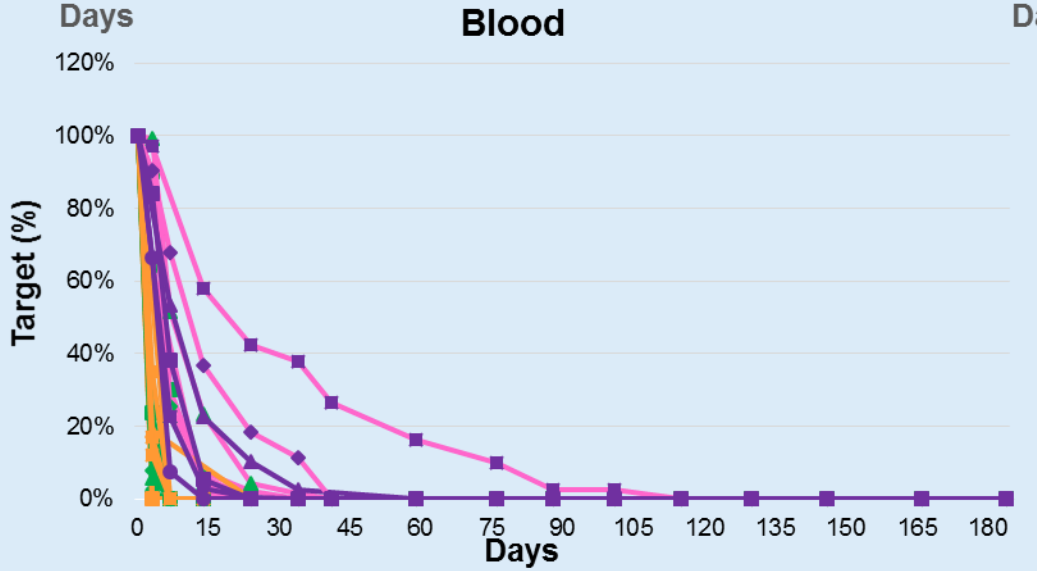
TEMPERATURE DEPENDENCE (BLOOD, 100 NG/ML)



pH DEPENDENCE (100 NG/ML)

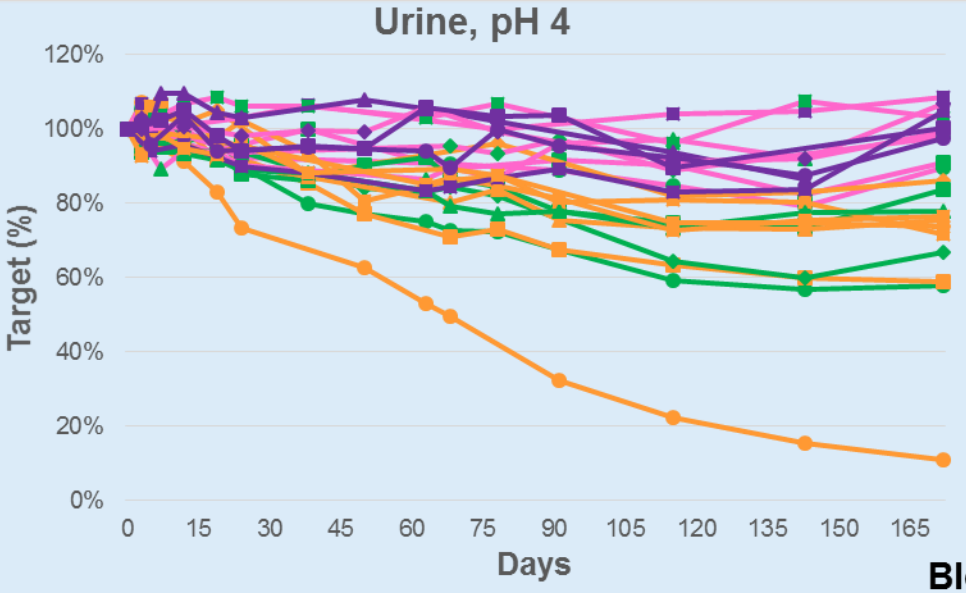


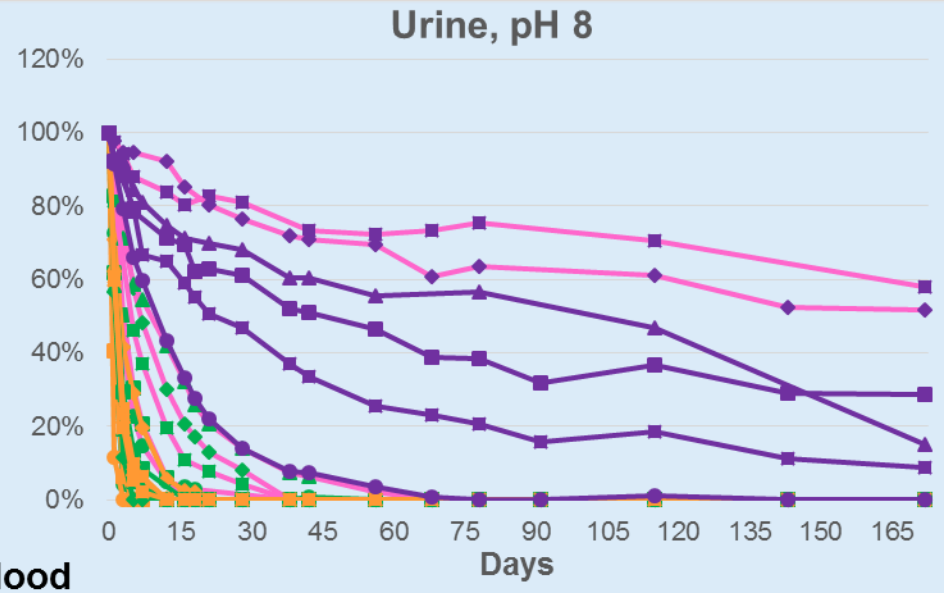


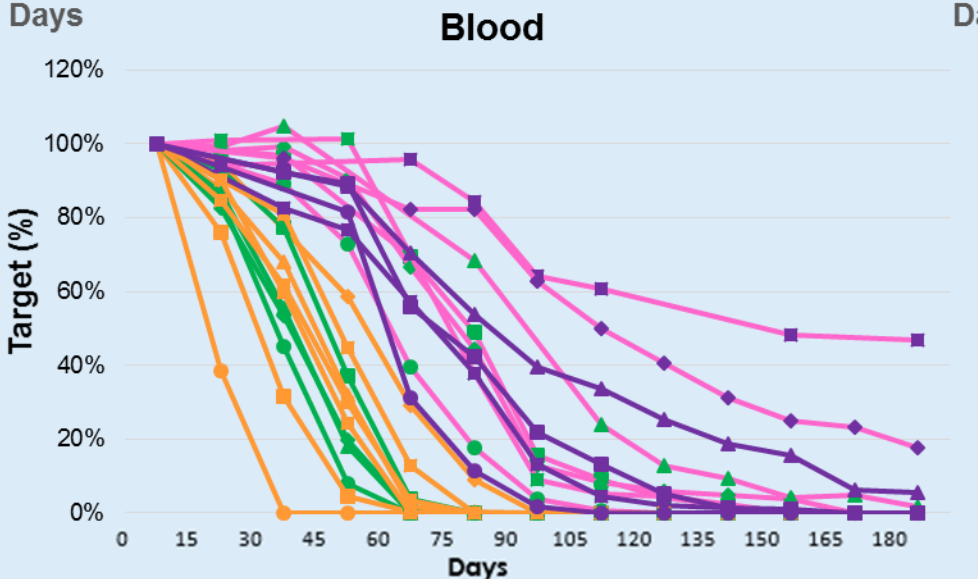


32°C

pH DEPENDENCE (100 NG/ML)

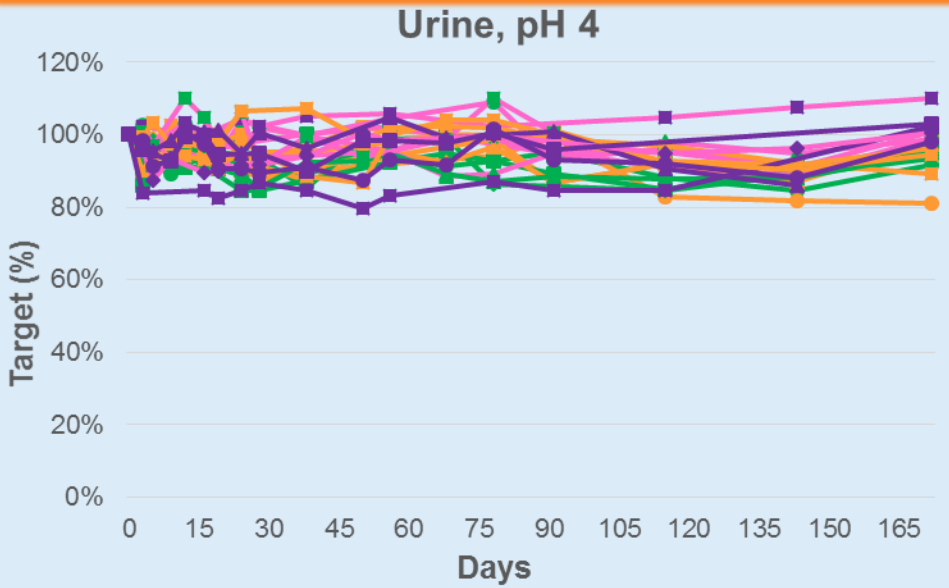


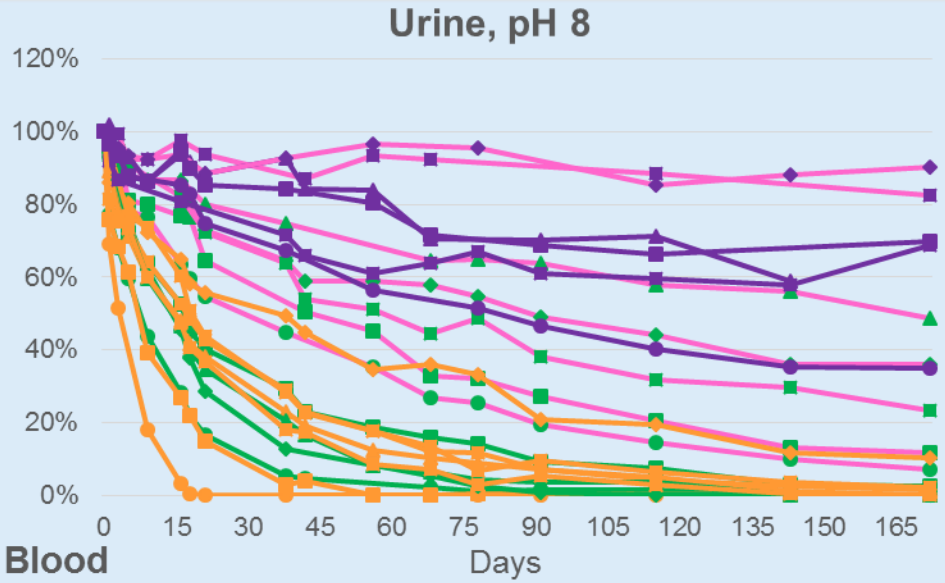


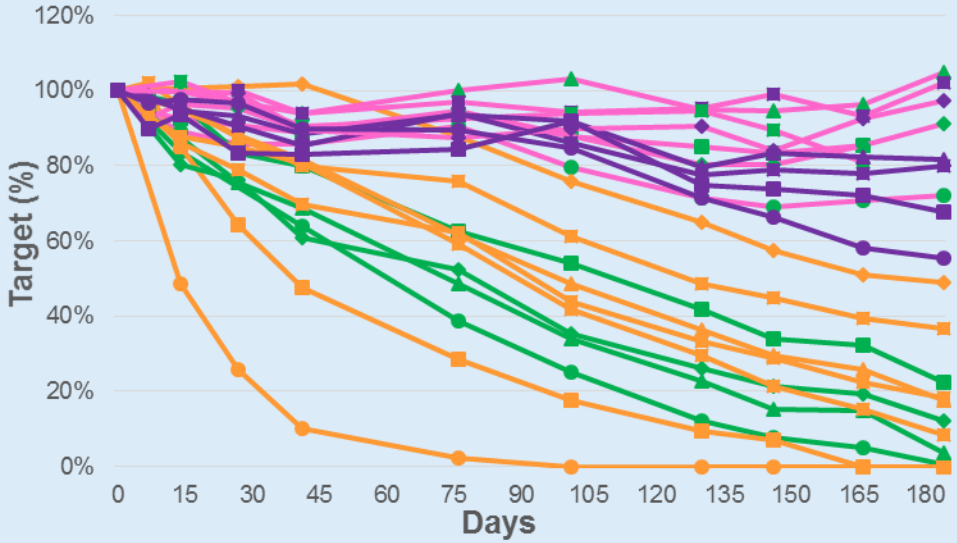


20°C

pH DEPENDENCE (100 NG/ML)

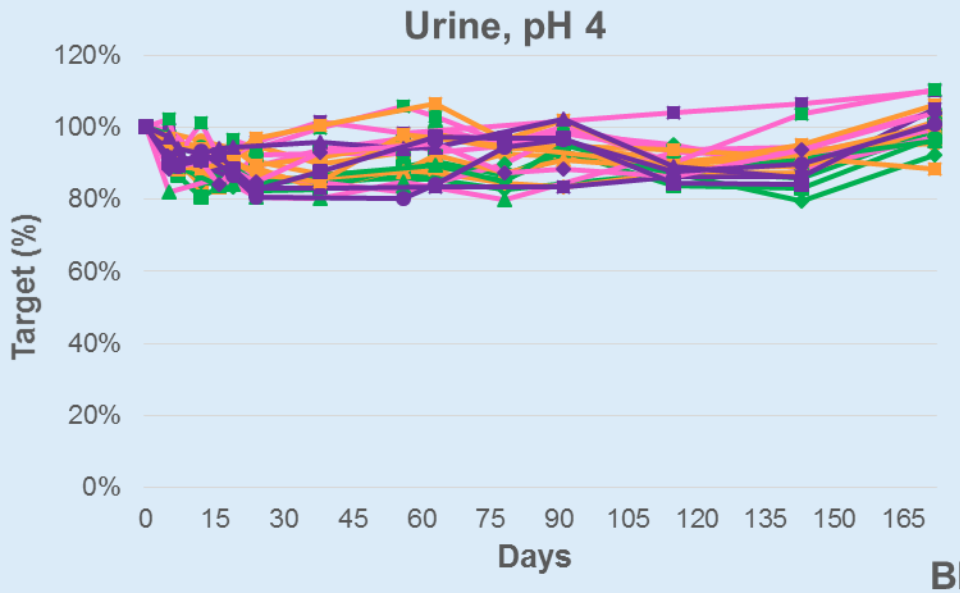


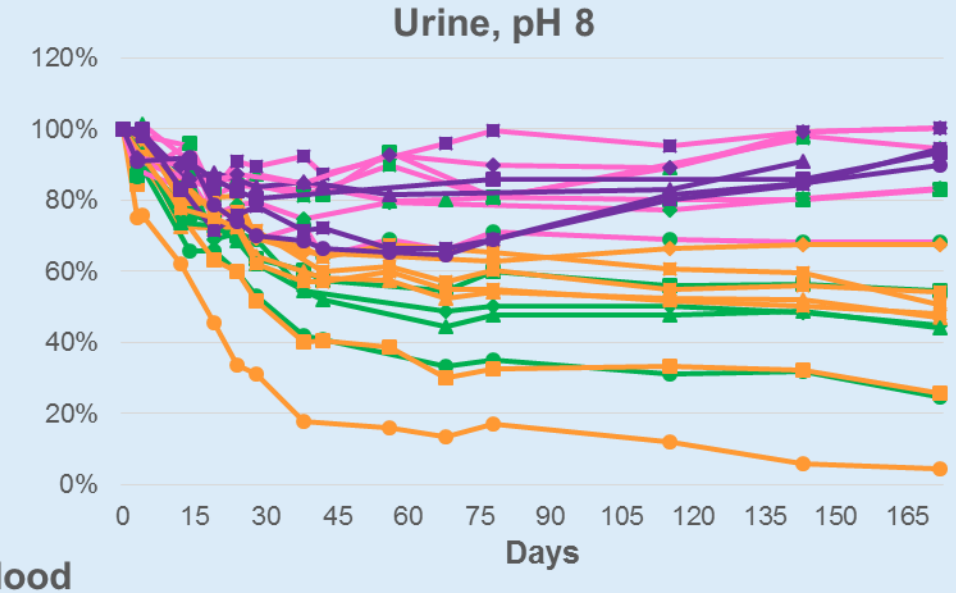


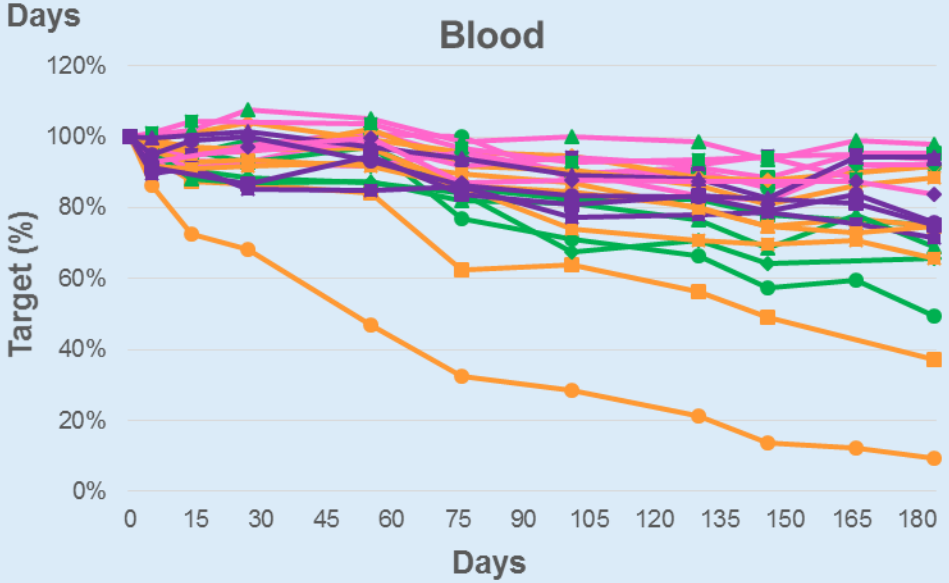


4°C

pH DEPENDENCE (100 NG/ML)

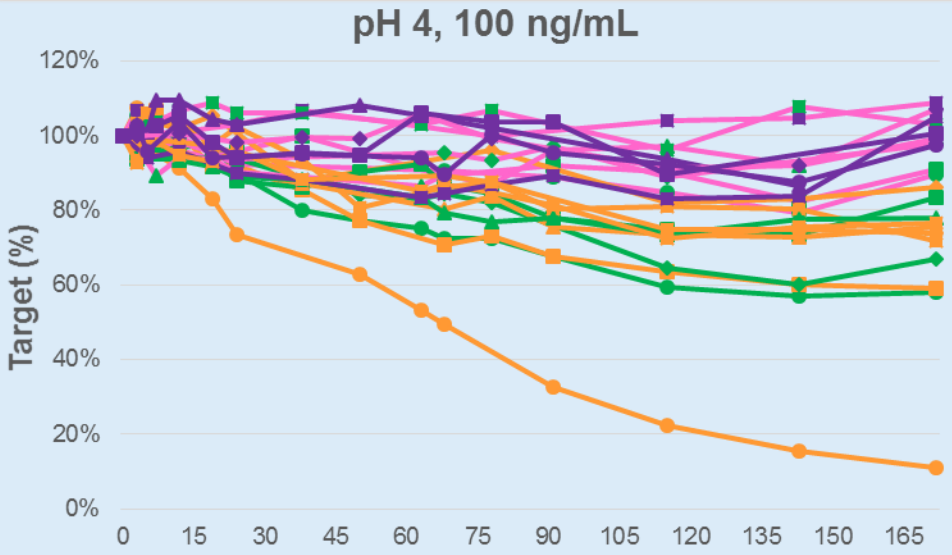


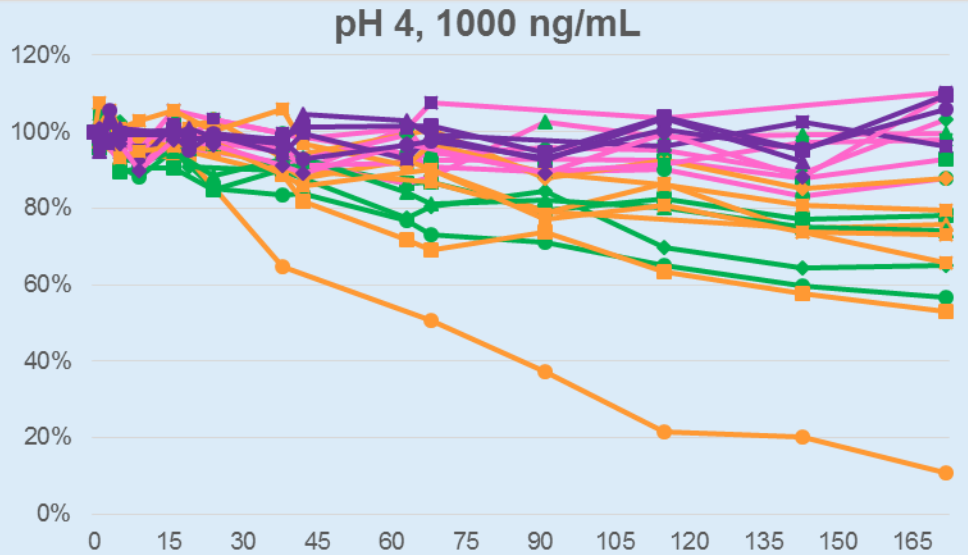


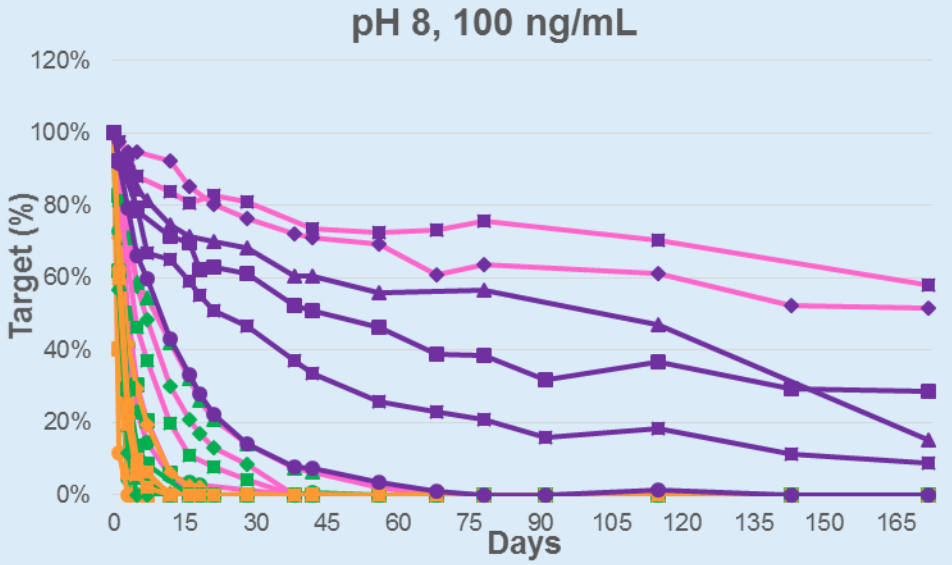


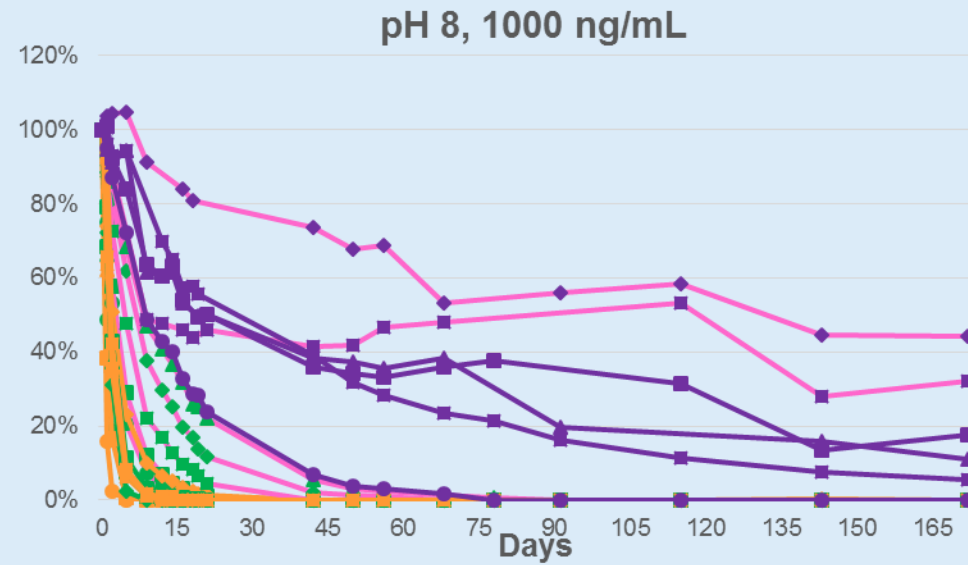
-20°C

CONCENTRATION DEPENDENCE: URINE

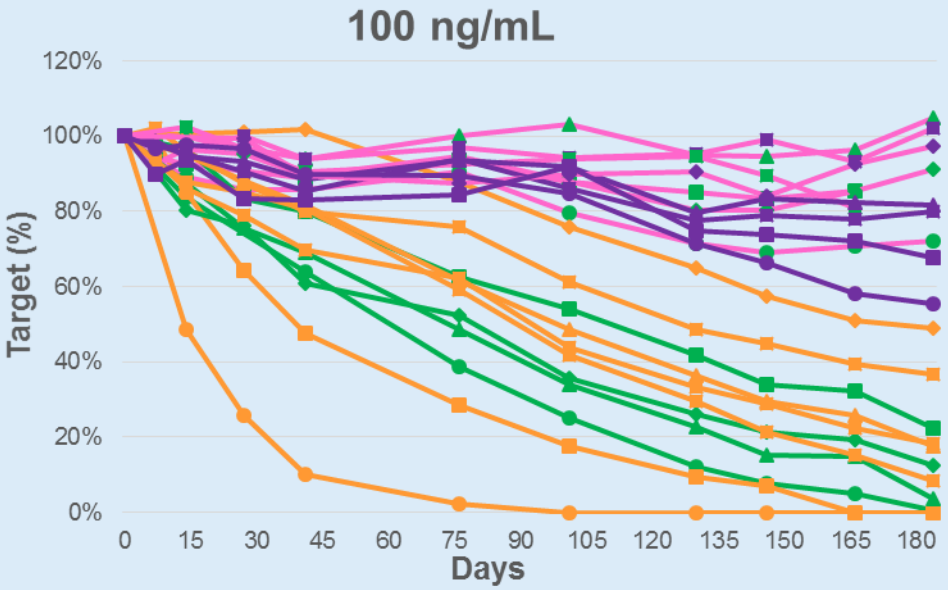


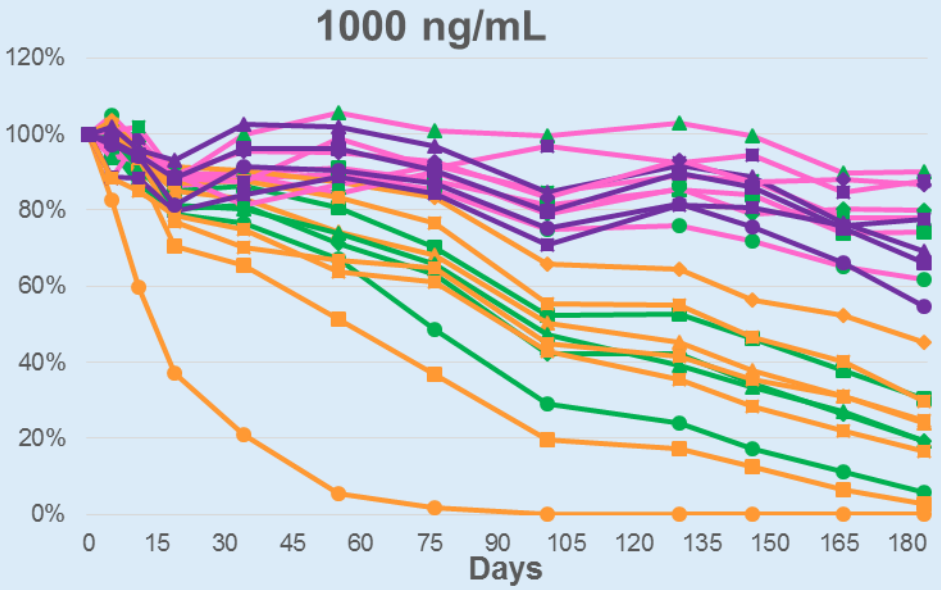






CONCENTRATION DEPENDENCE: BLOOD





CATHINONE STABILITY (IN DAYS)

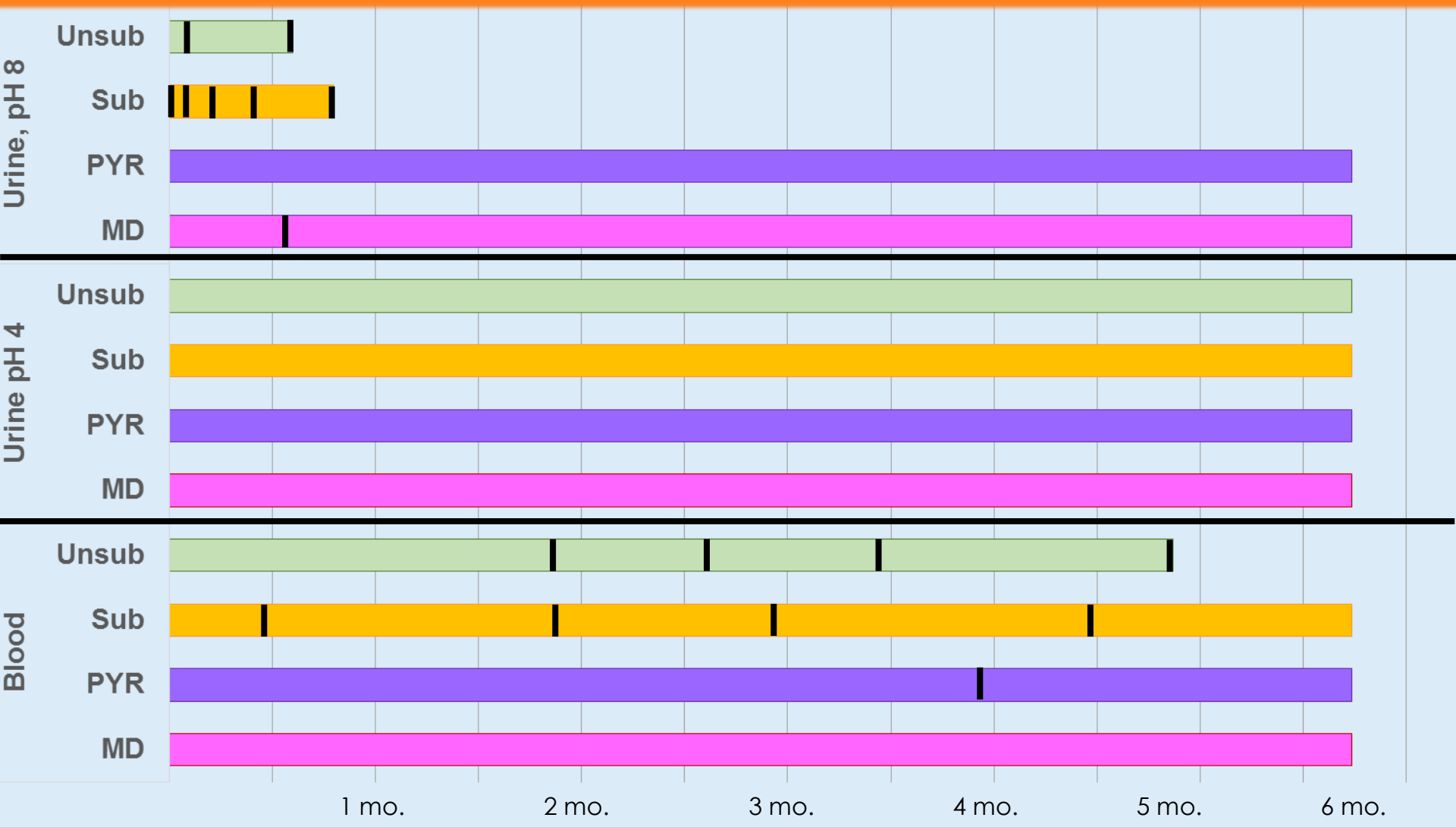
Urine

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

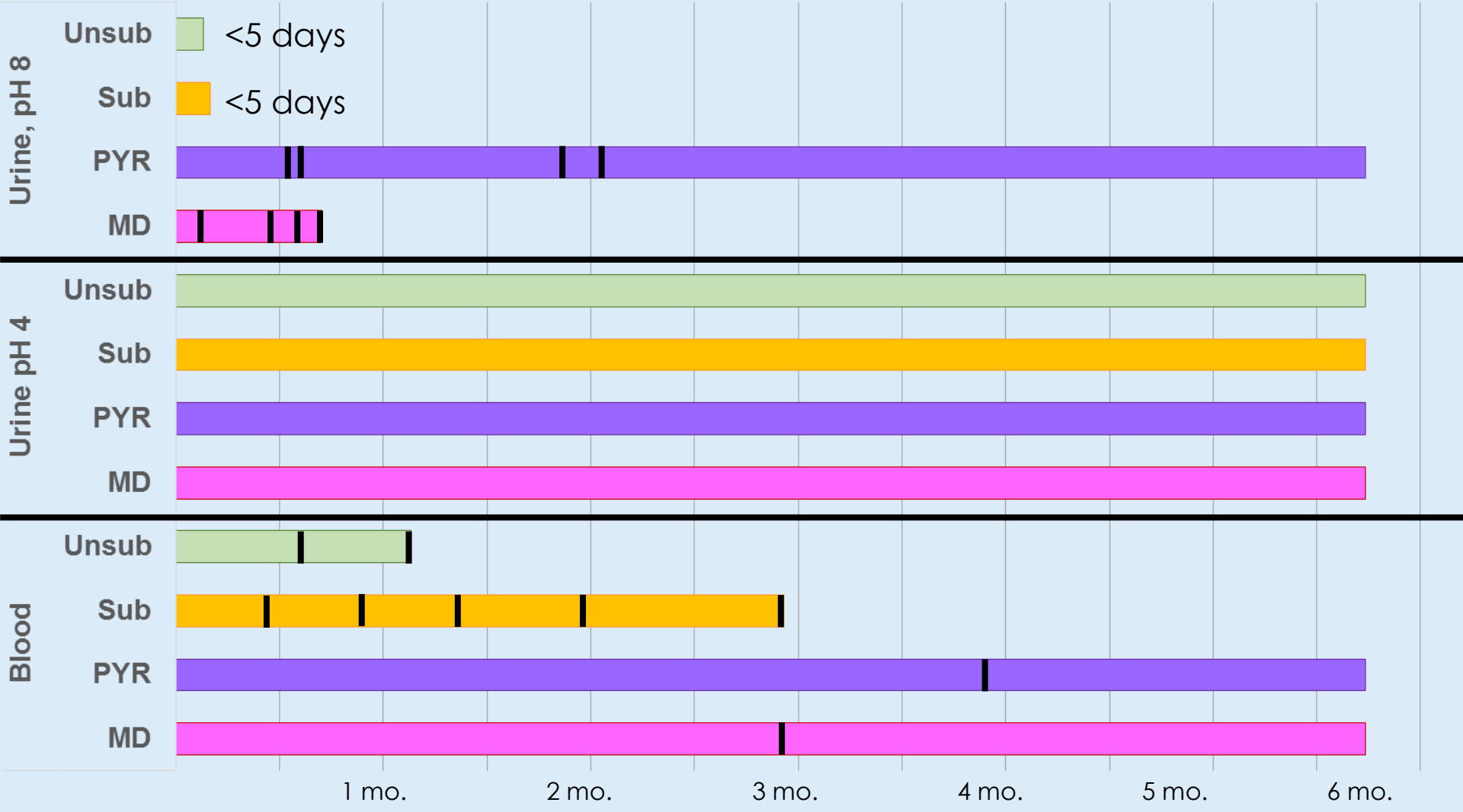
Blood

Cathinone Structural Group	32°C	20°C	4°C	-20°C
Unsubstituted	<2	3 – 7	19 – 41	76 – 146
Ring Substituted	<1 – 2	1 – 8	4 – 101	14 - >184
Methylenedioxy	2 – 4	11 – 27	101 - >184	>184
Pyrrolidine	3 – 9	14 - 55	103 - >184	>184

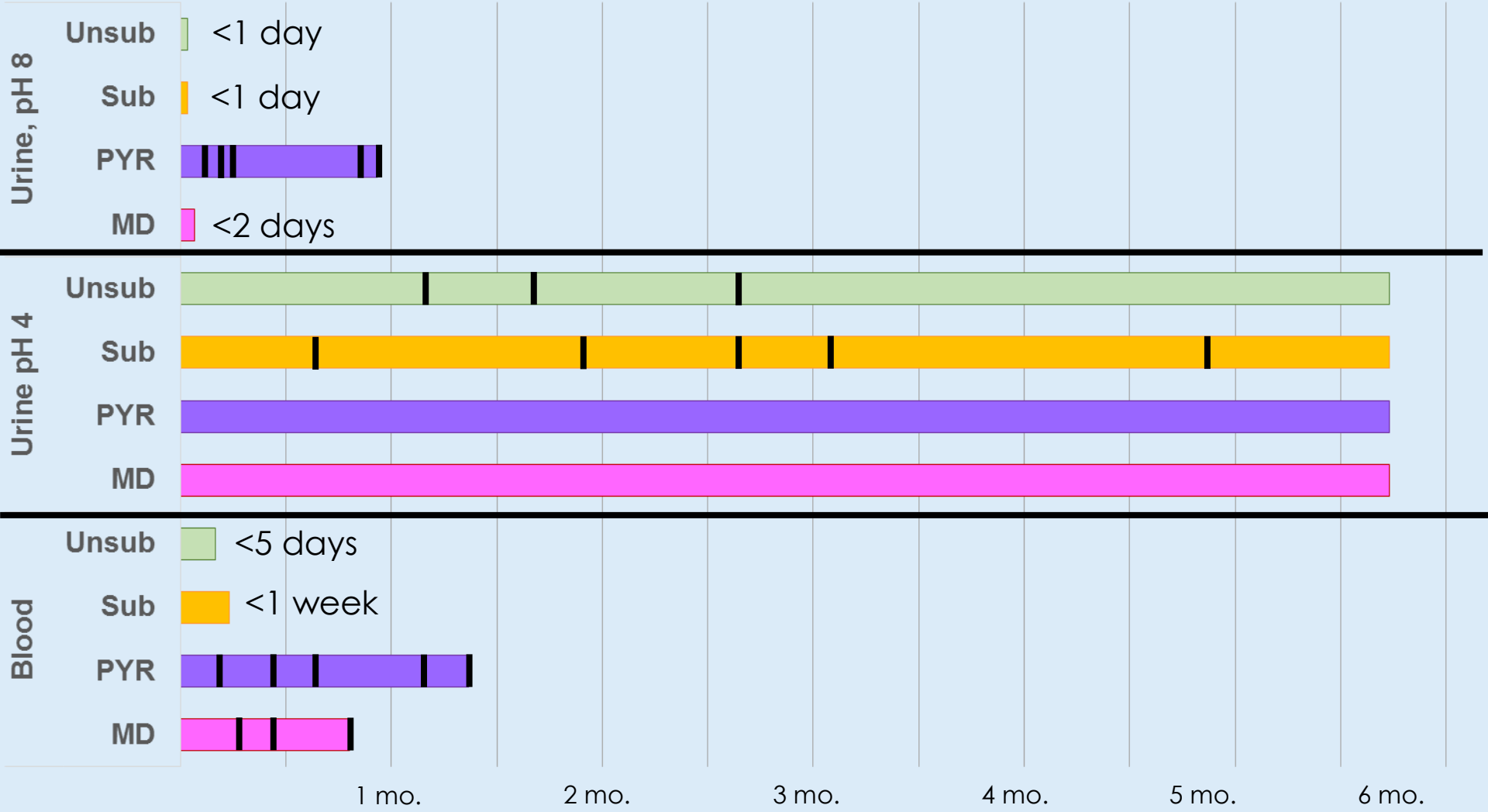
CATHINONE STABILITY -20°C



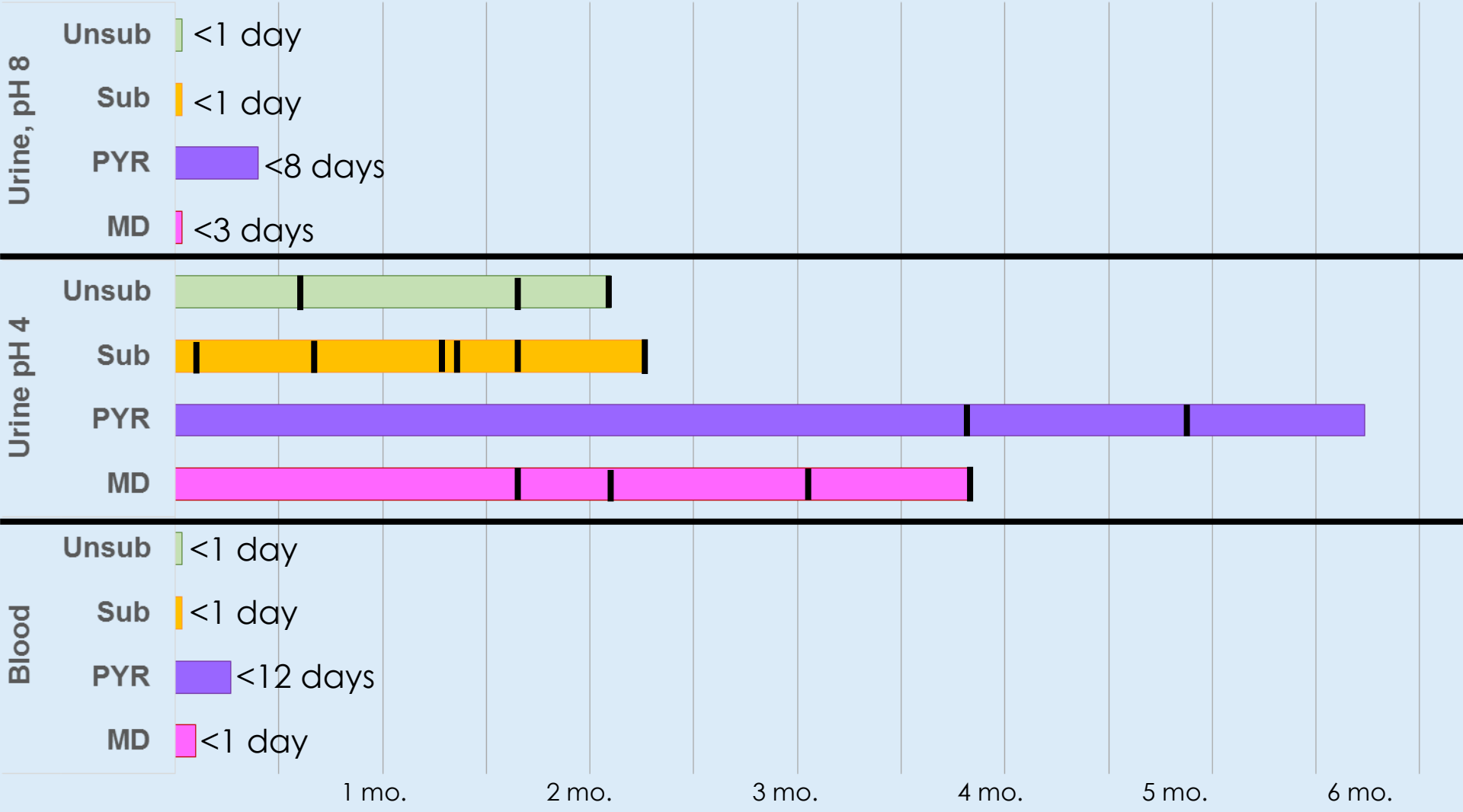
CATHINONE STABILITY 4°C



CATHINONE STABILITY 20°C



CATHINONE STABILITY 32°C



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 CONT.

- **Significant loss on the order of hours**

  alkaline urine and blood, 32°C and 20°C

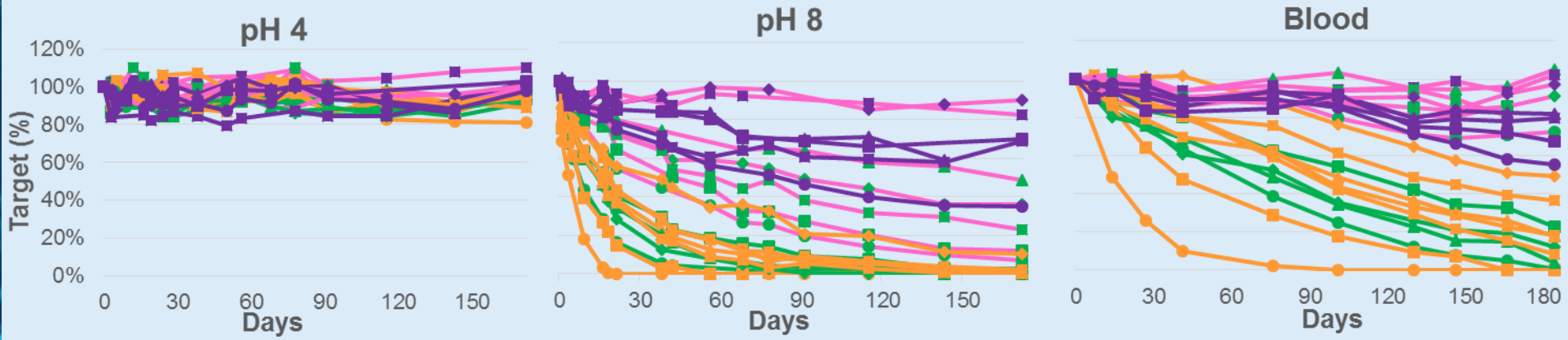
  3-FMC: 32°C—undetectable after 6 hours

  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**

SYNTHETIC CATHINONE INSTABILITY

Least Stable

- 3-FMC
- 4-FMC
- Methcathinone
- Ethcathinone
- 4-EMC
- Pentedrone
- Mephedrone
- 4-MEC
- 3,4-DMMC
- Buphedrone
- Methylone
- Methedrone
- Ethylone
- Pentylone
- Eutylone
- Butylone
- Naphyrone
- α-PVP
- Pyrovalerone
- MPBP
- MDPBP
- MDPV

Most Stable

POTENTIAL IMPACT

Increase understanding of synthetic cathinone stability

Emphasizes the importance of timely analysis and proper storage conditions

Ability to predict the stability of new cathinones based on chemical structure

ACKNOWLEDGEMENTS

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