Evaluation of Novel Psychoactive Substance Drug Loss from Storage in Serum Separator Tubes

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DISCLOSURE

- No conflicts of interest to disclose

SERUM SEPARATOR TUBES

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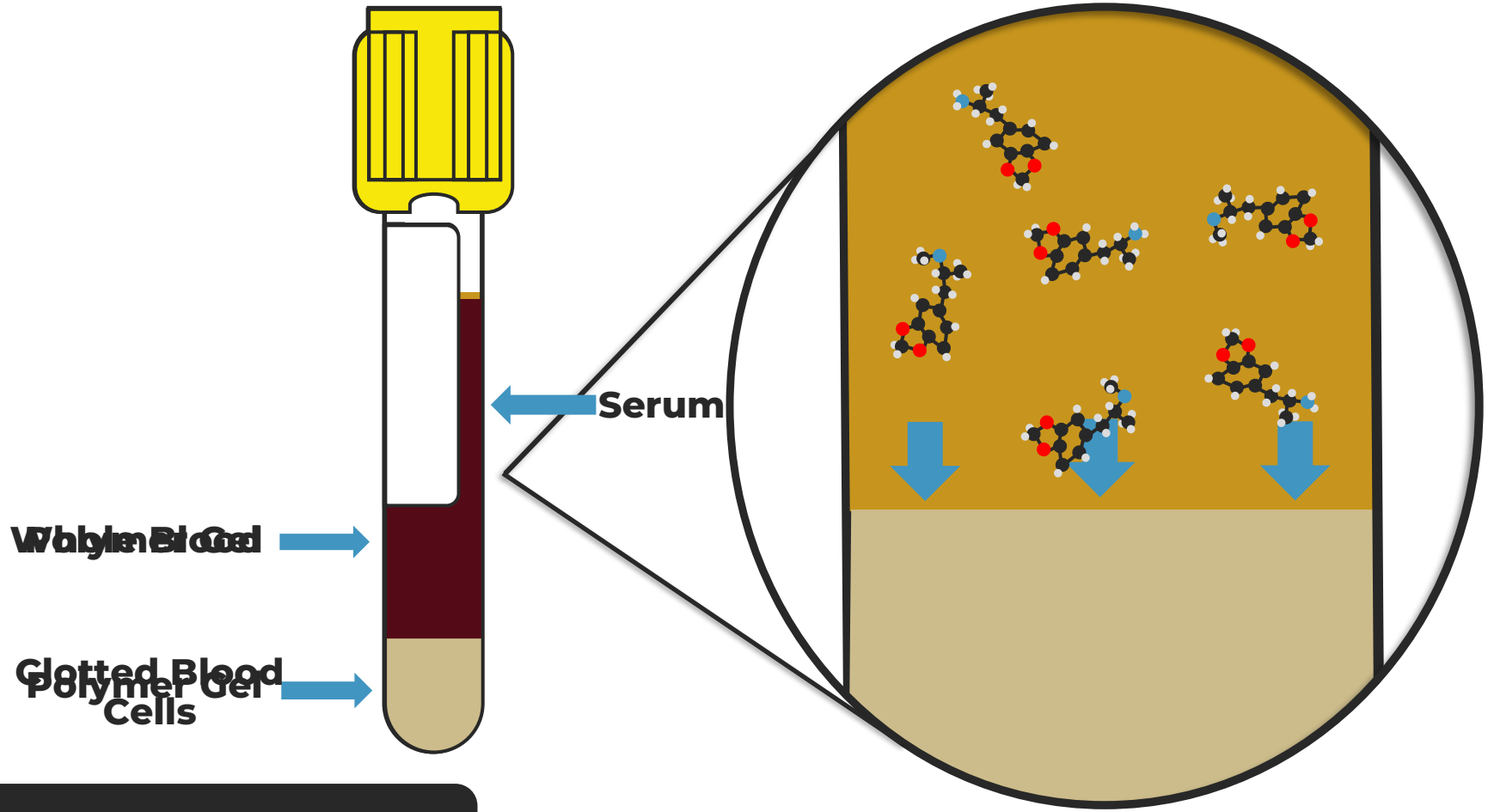
SERUM SEPARATOR TUBES (SST)

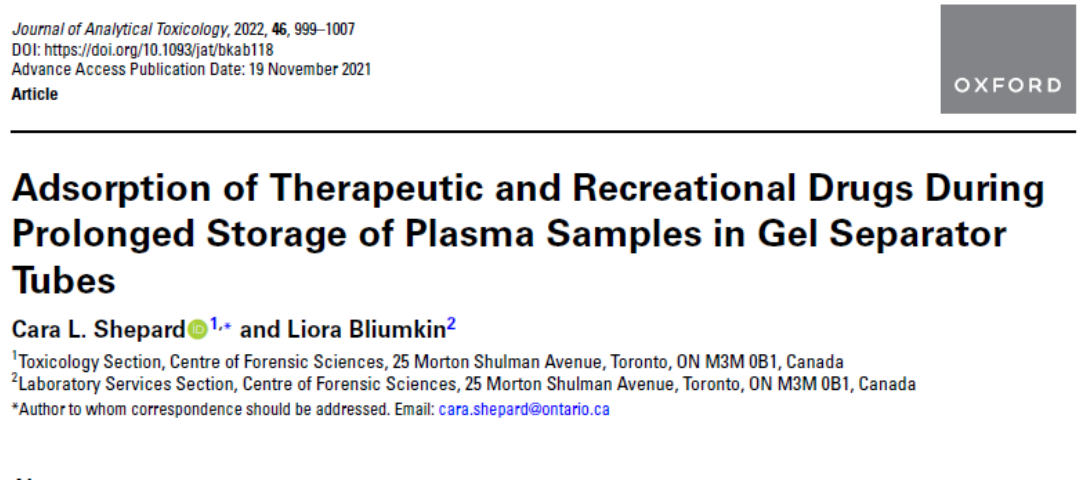
- “Marble Top”, “Tiger Top”, “Gold Top”
- Separates serum from the cellular constituents of blood
- Primarily used in hospital and clinical settings for chemistry and serology testing
    - Not recommended for toxicology/drug testing

SERUM SEPARATOR TUBES (SST)



PREVIOUS RESEARCH

- Research on drug susceptibility to gel adsorption is not typically centered around drugs which are forensically relevant
- Shepard and Bliumkin (2021)
  - Research focused on plasma separator tubes, not serum separator tubes
  - Evaluated traditional drugs, not novel psychoactive substances (NPS)
- No studies conducted focusing on NPS interacting with separator gel were found in the literature



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Article

OXFORD

Adsorption of Therapeutic and Recreational Drugs During Prolonged Storage of Plasma Samples in Gel Separator Tubes

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OBJECTIVE

This study was designed to determine if the following NPS were susceptible to loss in concentration due to adsorption by the gel in SST while stored in refrigerated conditions (4°C) for 0, 1, 2, 7, 14, 60, and 90 days.

NPS Benzodiazepines
8-Aminoclonazolam
Clonazolam
Bromazolam
Etizolam
Flualprazolam
Flubromazepam
Flubromazolam

NPS Stimulants
Diethylone
Eutylone
Tertylone
Pentylone
Dimethylpentylone
N-Ethyl Pentylone
Hexylone

NPS Opioids (Nitazenes)
Etodesnitazene
4'-hydroxy Nitazene
Metonitazene
N-desethyl Isotonitazene
Isotonitazene
Protonitazene

STUDY DESIGN

- A pooled control was created in citrated human whole blood
  - 100 ng/mL: NPS Stimulants & NPS Benzodiazepines
  - 10 ng/mL: NPS Opioids (Nitazenes)
- 5 mL of each pooled control was aliquoted into 5 mL gold-top Becton Dickinson Vacutainer SST
- Each tube was re-calcified via 37.6 µL of 2M calcium chloride, inverted 6 times, and left for 30 mins to allow the blood to clot
- The tubes were centrifuged at 3000 RPM for 10 minutes to separate the serum
- Approximately half (~1.5mL) was transferred to a 13x100 mm borosilicate glass tube (GT) and the remaining sample was stored in the SST
- In total there were three SST and GT for each test day (0, 1, 2, 7, 14, 30, 60, and 90) and all were stored via refrigeration (4°C) until analysis

STATISTICAL ANALYSIS

- Average SST and GT concentrations for each drug on each test day were calculated and plotted to illustrate loss over the 90-day analysis
  - Error bars were included to show the standard error for each average
    - Standard error = $\frac{Standard\ Deviation}{\sqrt{n}}$
- Percent difference was calculated between the final and initial test day to show concentration loss for both SST and GT
  - Percent difference = $\frac{\text{Day 90}-\text{Day 0}}{\text{Day 0}} \times 100$
- Two tailed, two-sample paired variance t-test ($p<0.05$) used to compare SST and GT concentrations on corresponding days and determine if a statistically significant difference exists

RESULTS/DISCUSSION: NPS BENZODIAZEPINES

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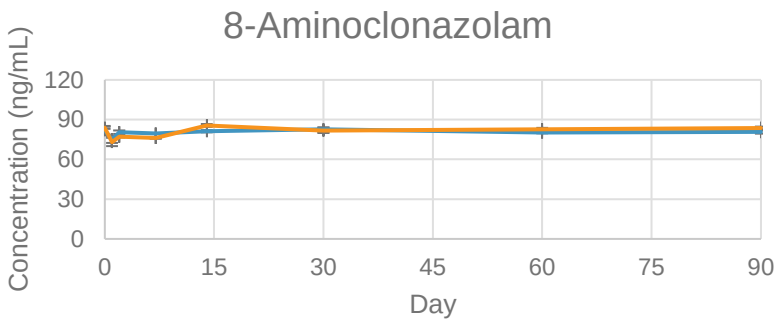
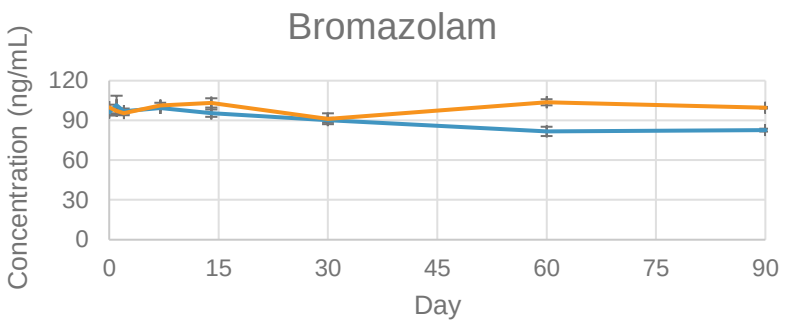
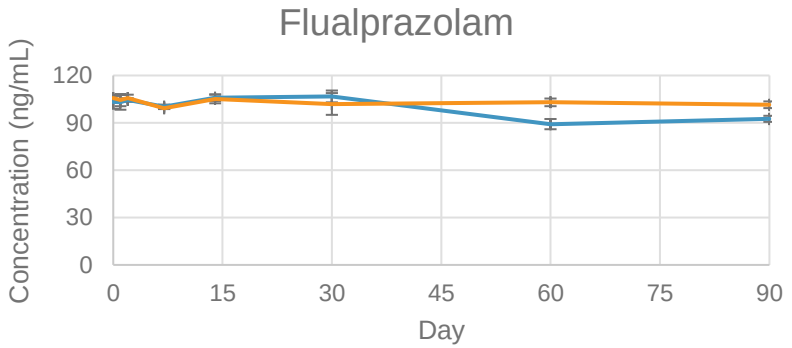
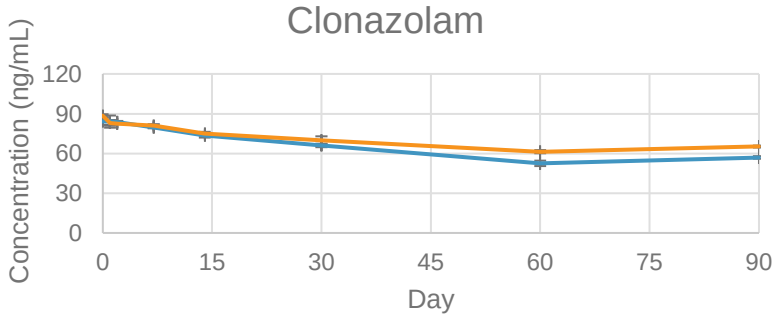
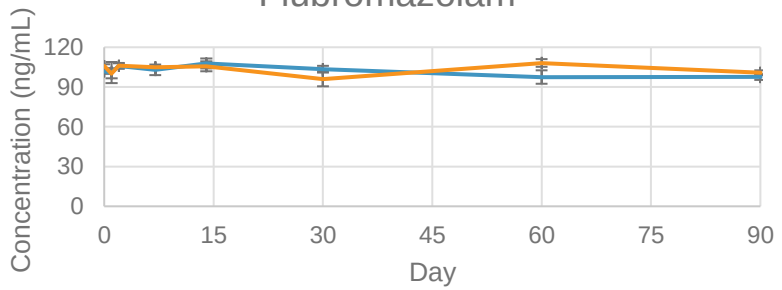
RESULTS: NPS BENZODIAZEPINES

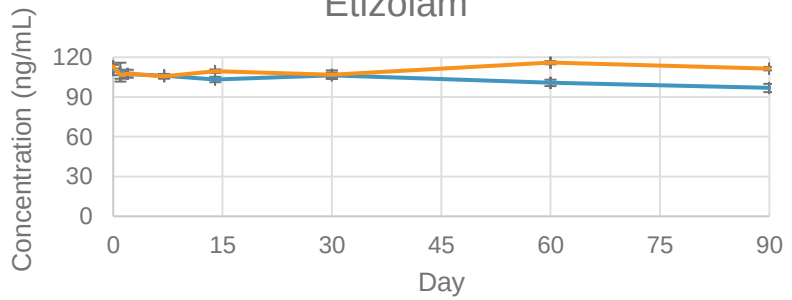












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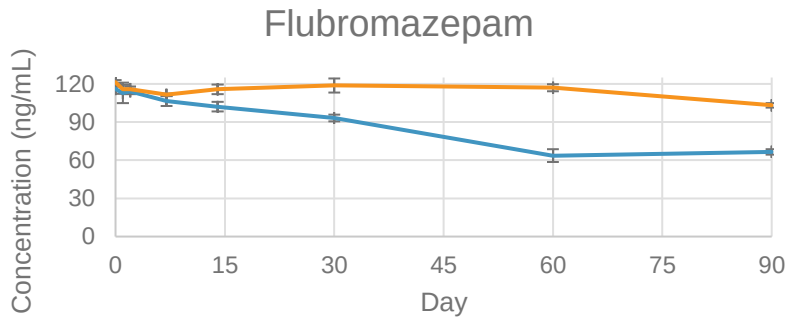

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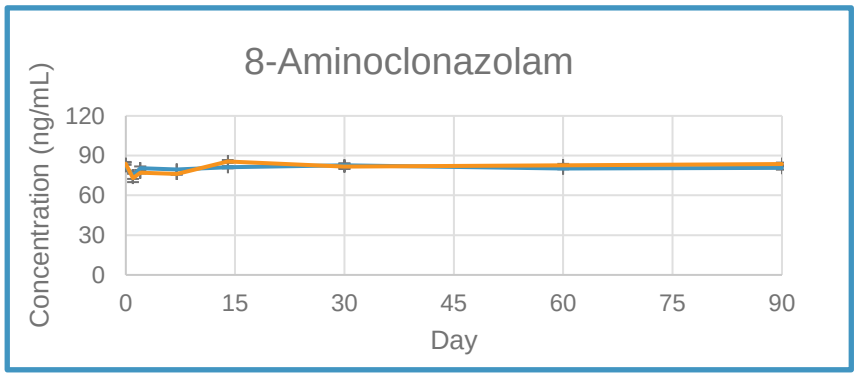
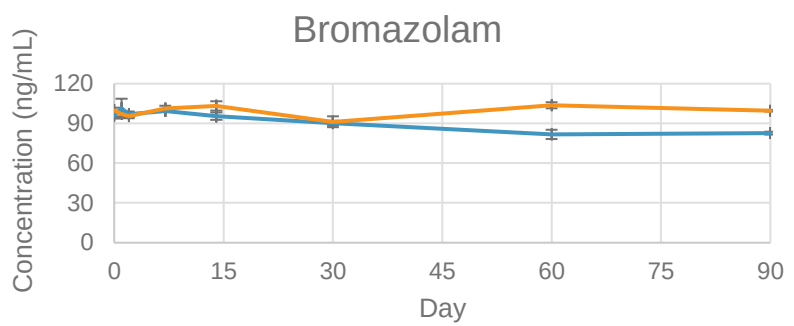
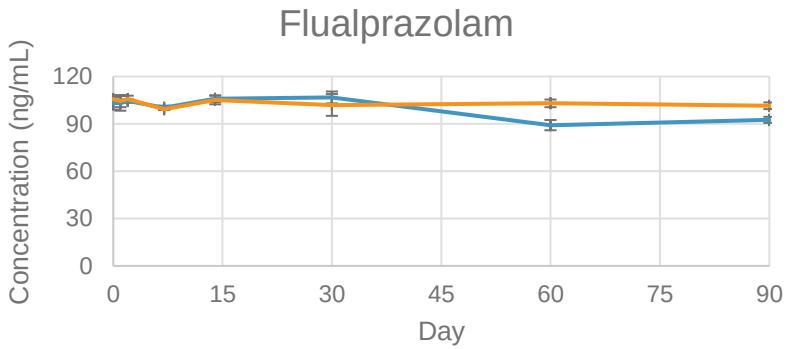
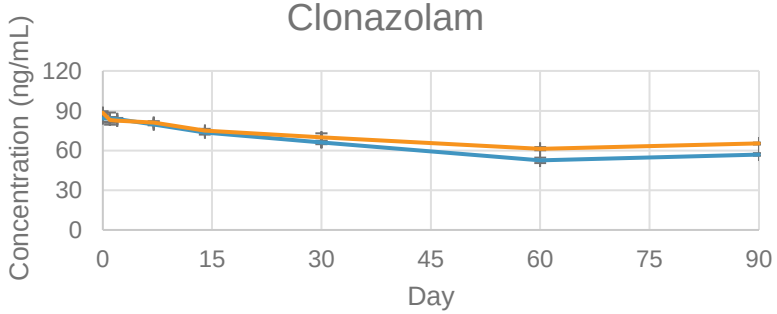
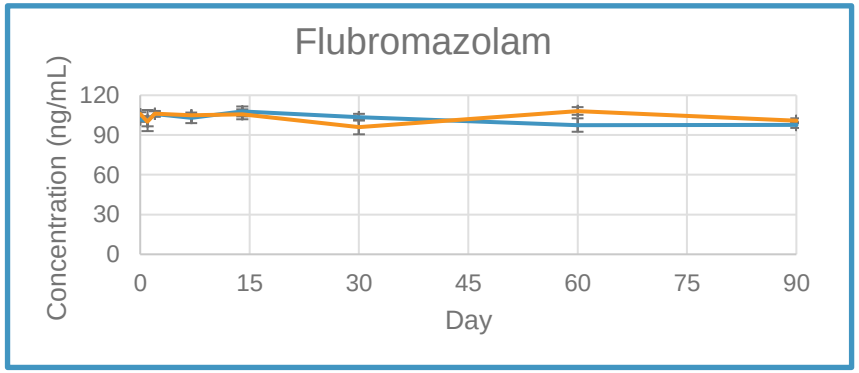
RESULTS: NPS BENZODIAZEPINES

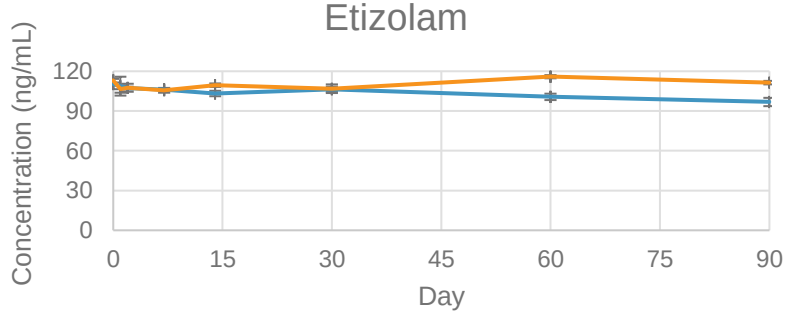












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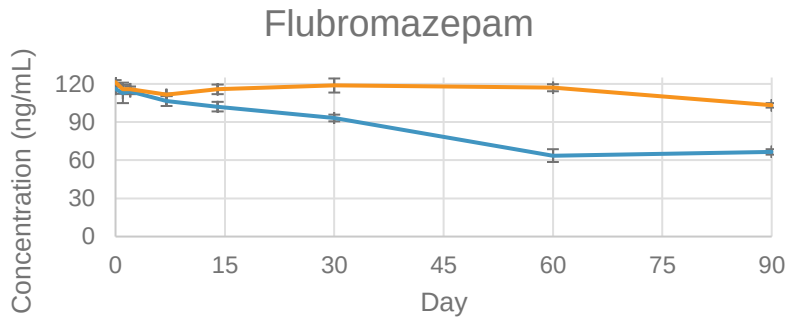

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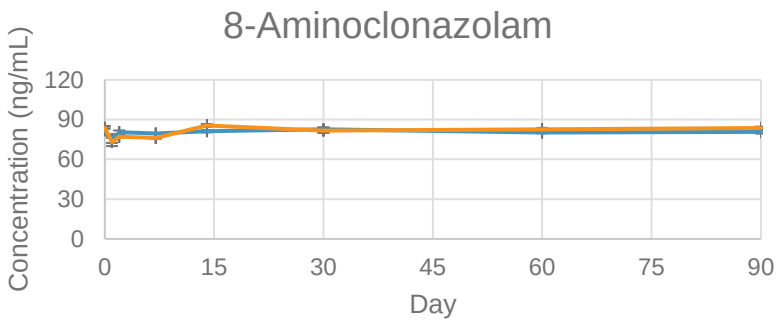
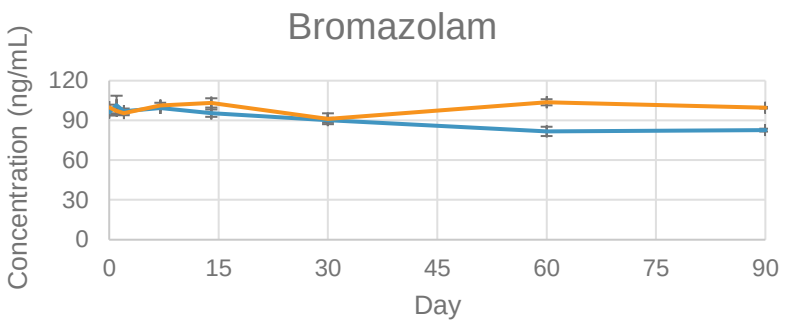
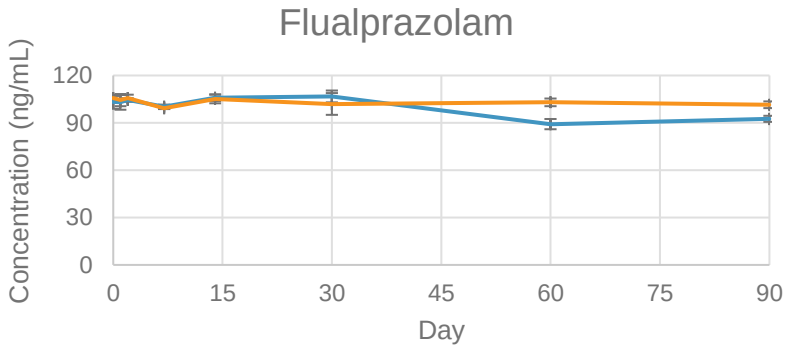
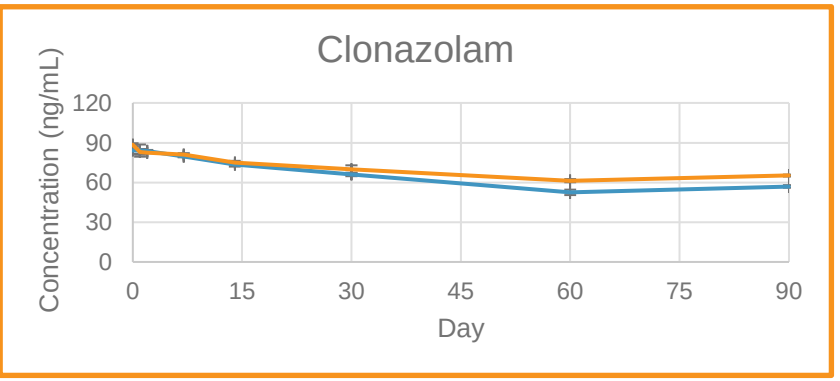
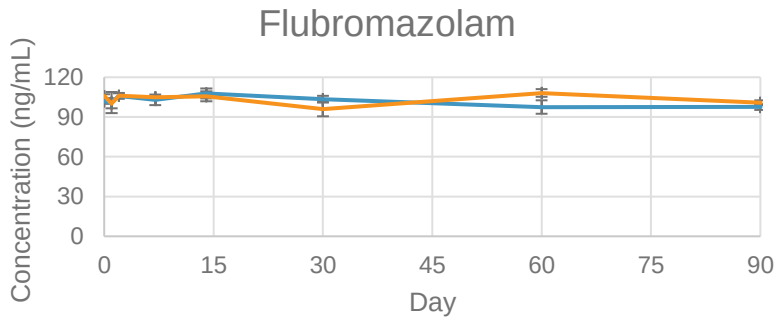
RESULTS: NPS BENZODIAZEPINES

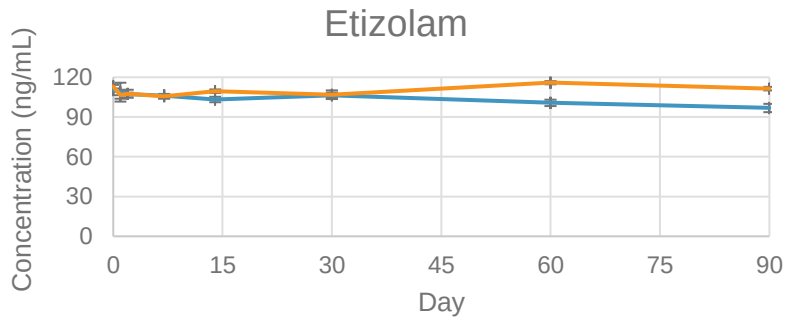




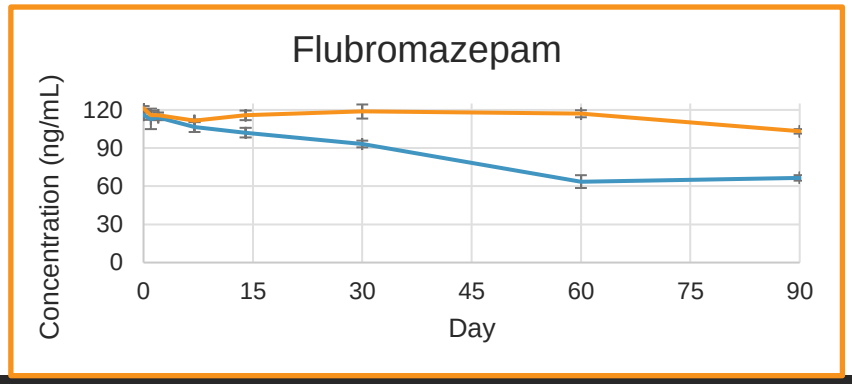




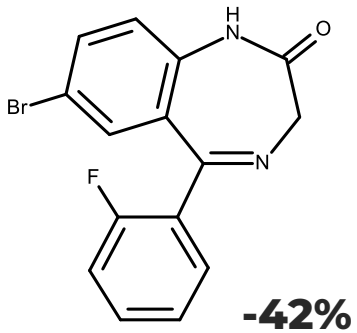
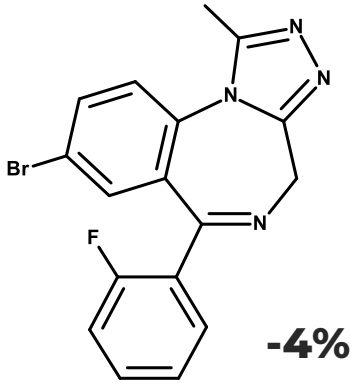
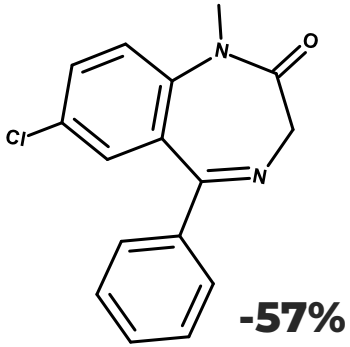
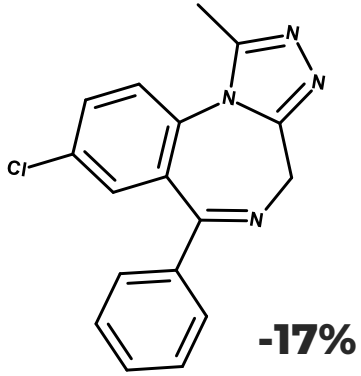




SST GT



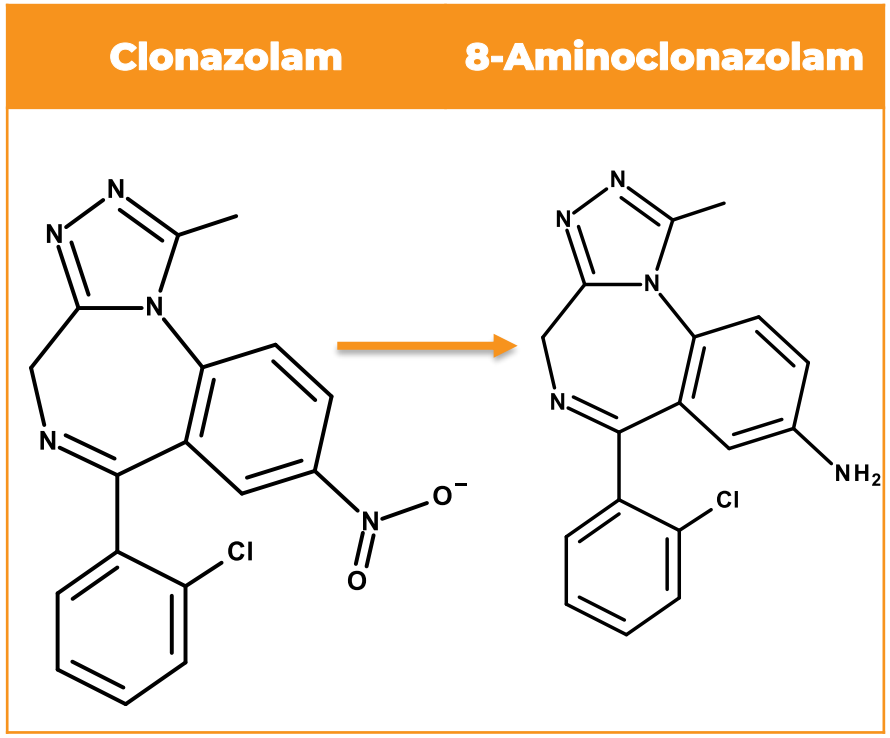
NPS BENZODIAZEPINES

Flubromazepam	Flubromazolam
 -42%	 -4%
Diazepam	**Alprazolam**
 -57%	 -17%

- Flubromazepam showed the largest decrease (-42%) from day 0 (115 ng/mL) to day 90 (66 ng/mL)
  - Only 2-keto-benzodiazepine tested
    - All others were triazolo-benzodiazepines
- Aligns with previous research of traditional benzodiazepines
  - Shepard and Bliumkin reported 2-keto-benzodiazepines being more susceptible to the gel than triazolo-benzodiazepines
    - Polarity of triazolo

Benzodiazepines	Day 0 SST (ng/mL)	Day 90 SST (ng/mL)	% Diff SST	Day 0 GT (ng/mL)	Day 90 GT (ng/mL)	% Diff GT
Flubromazepam	**115**	**66**	**-42%**	**121**	**103**	**-15%**
Clonazolam	84	57	-32%	88	65	-26%
Bromazolam	96	82	-13%	99	99	0%
Flualprazolam	103	92	-10%	105	101	-3%
Etizolam	110	96	-12%	113	111	-1.4%
Flubromazolam	**101**	**97**	**-4%**	**105**	**100**	**-4%**
8-Aminoclonazolam	81	80	-1.5%	83	83	0%

NPS BENZODIAZEPINES



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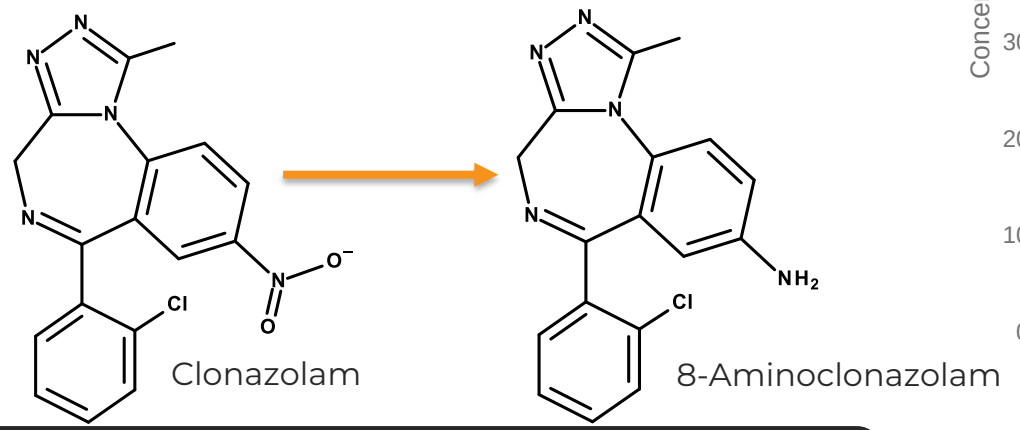
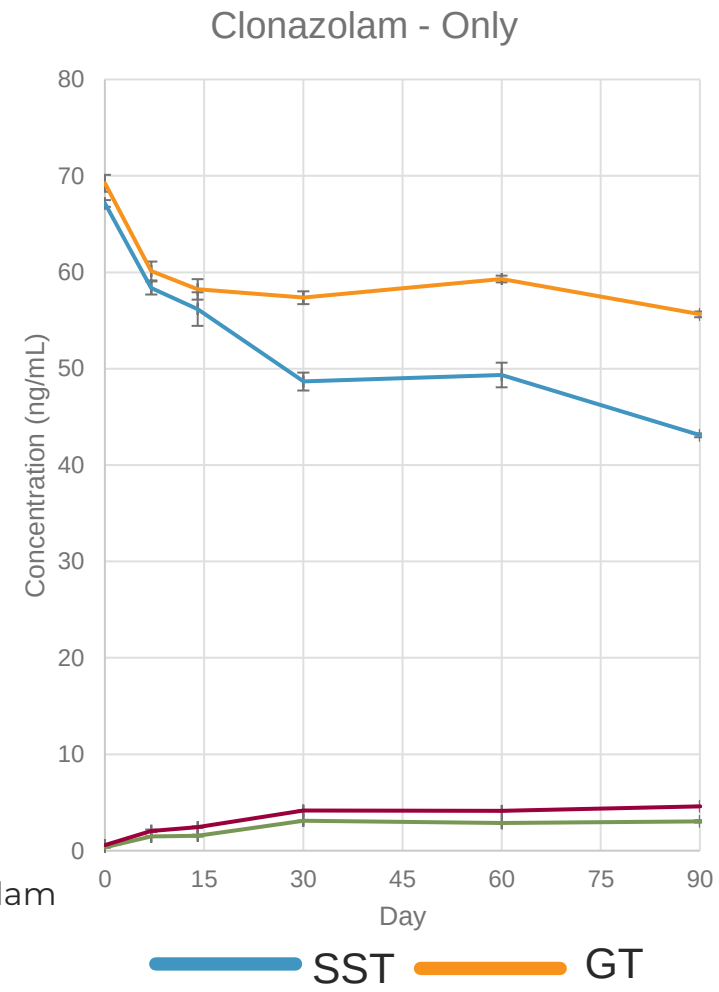
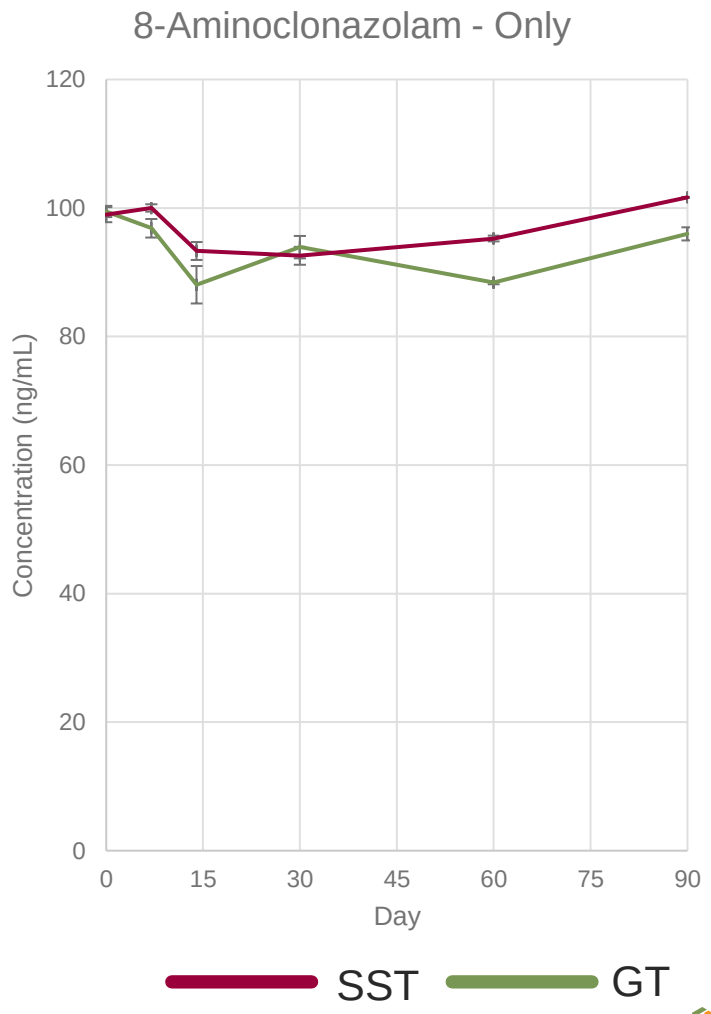
8-Aminoclonazolam	**81**	**80**	**-1.5%**	**83**	**83**	**0%**

CLONAZOLAM AND 8-AMINOCLONAZOLAM

- When testing clonazolam only, 8-aminoclonazolam was detected by day 7
  - Further indicating loss is due to instability rather than adsorption into separator gel
- When testing 8-aminoclonazolam only, the contribution from the clonazolam breakdown was not significant to affect the prior results







RESULTS/DISCUSSION: NPS STIMULANTS

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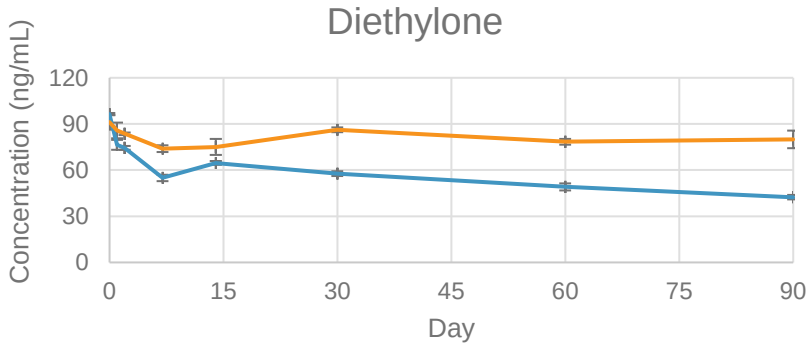
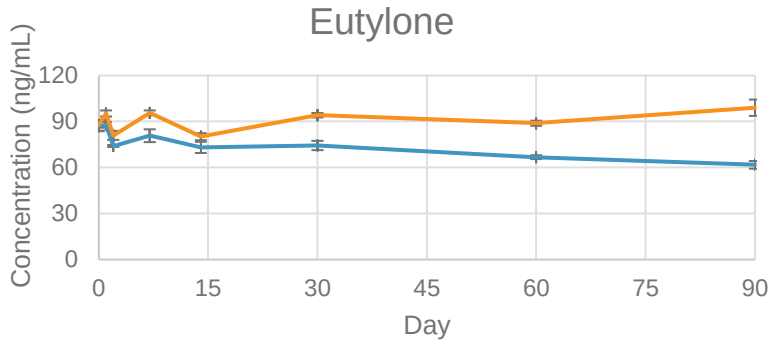
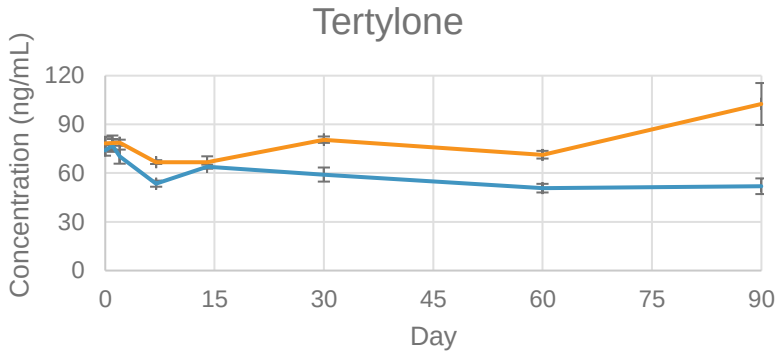
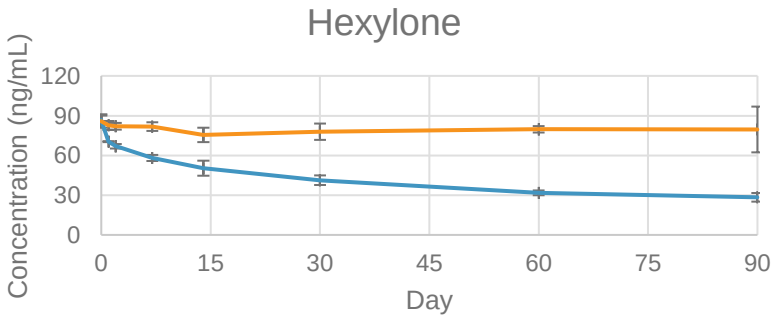
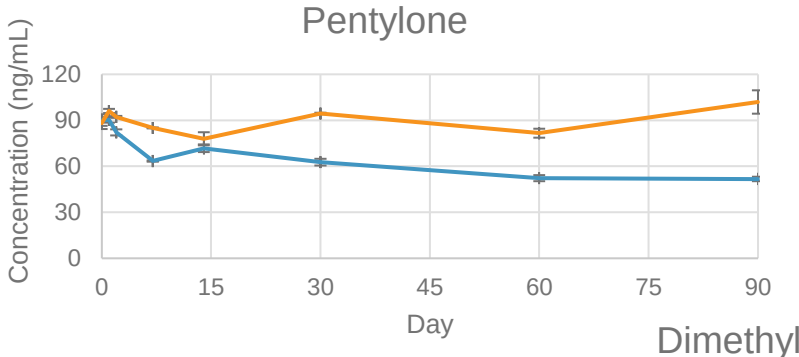
RESULTS: NPS STIMULANTS

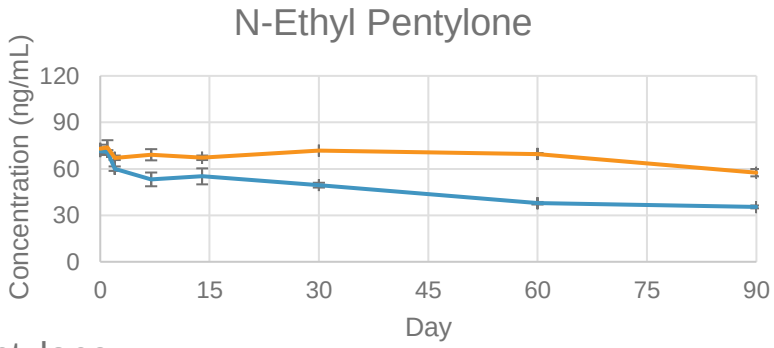


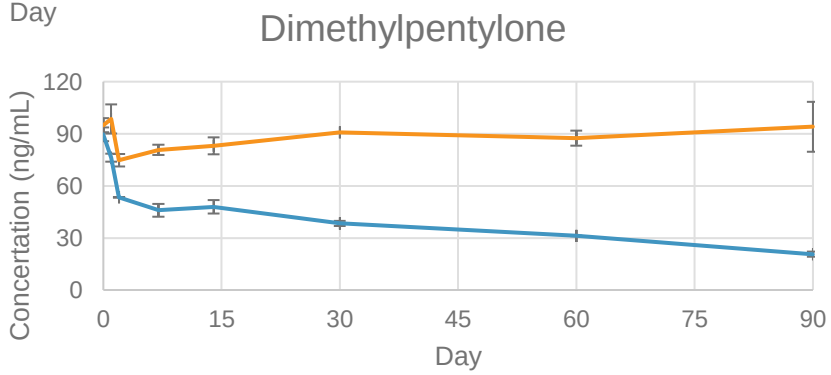




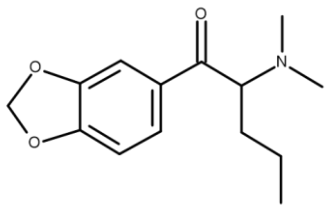




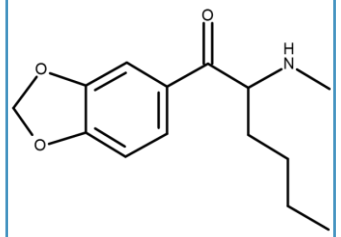




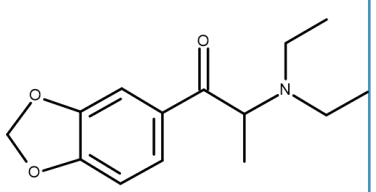
NPS STIMULANTS



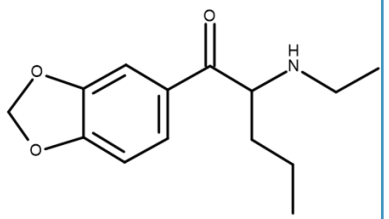
Dimethylpentylone



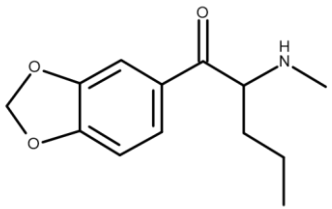
Hexylone



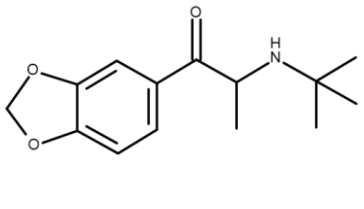
Diethylone



N-Ethyl Pentylone



Pentylone



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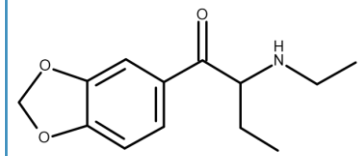

Eutylone

Most Affected → Least Affected

- Considerations
  - Substitutions are long carbon chains
    - ↓ polarity, ↑ affinity
  - Free hydrogen increased hydrogen bonding

Stimulants	Day 0 SST (ng/mL)	Day 90 SST (ng/mL)	% Diff SST	Day 0 GT (ng/mL)	Day 90 GT (ng/mL)	% Diff GT
Dimethylpentylone	89	20	-77%	94	94	0 %
Hexylone	86	28	-67%	85	79	-7.3%
Diethylone	96	42	-56%	91	79	-12%
N-Ethyl Pentylone	71	35	-50%	72	57	-21%
Pentylone	90	51	-42%	88	101	+15%
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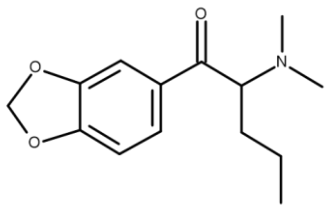
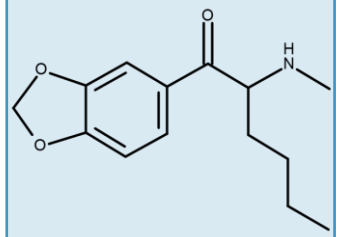
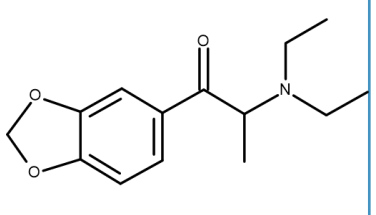
NPS STIMULANTS



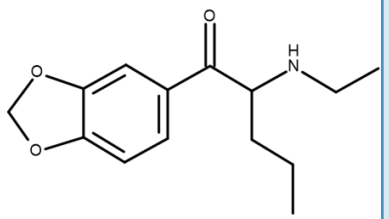
Dimethylpentylone



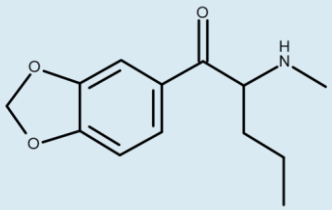
Hexylone



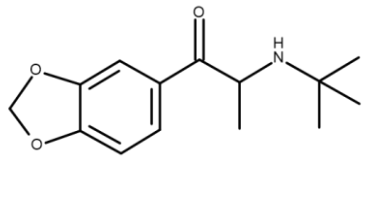
Diethylone



N-Ethyl Pentylone



Pentylone



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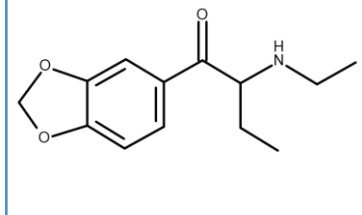

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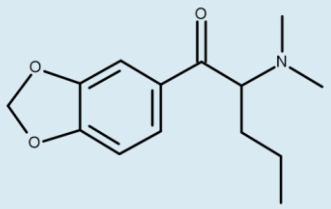

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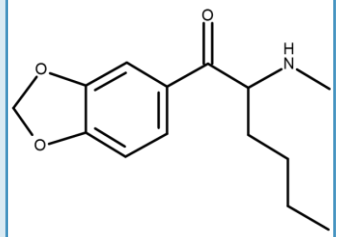
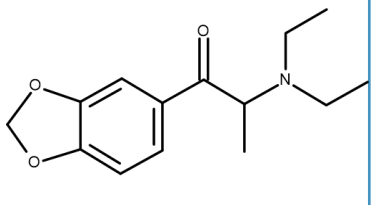
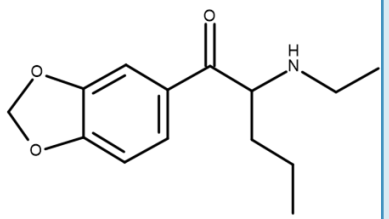


Hexylone



Diethylone



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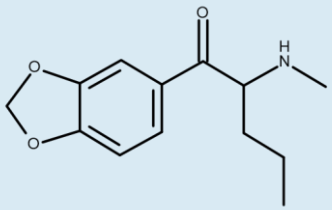

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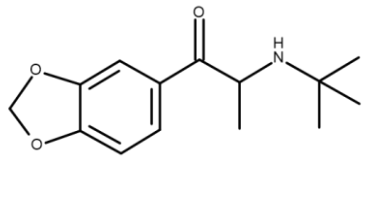
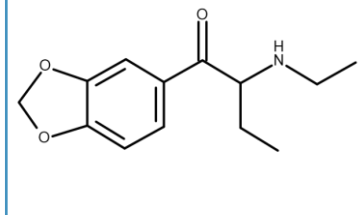


Tertylone



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RESULTS/DISCUSSION: NPS OPIOIDS (NITAZENES)

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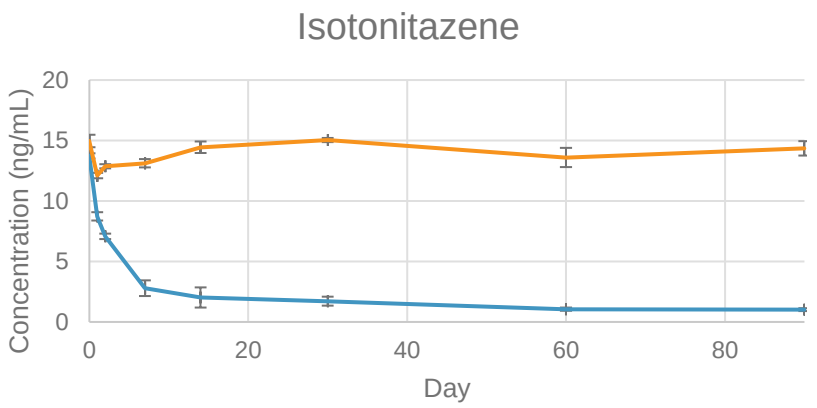
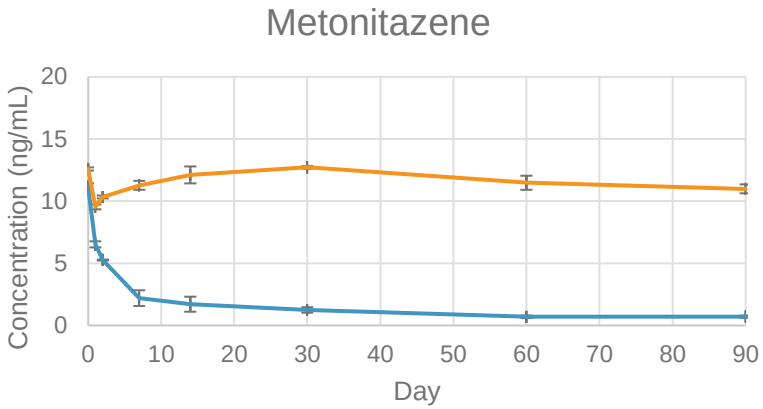
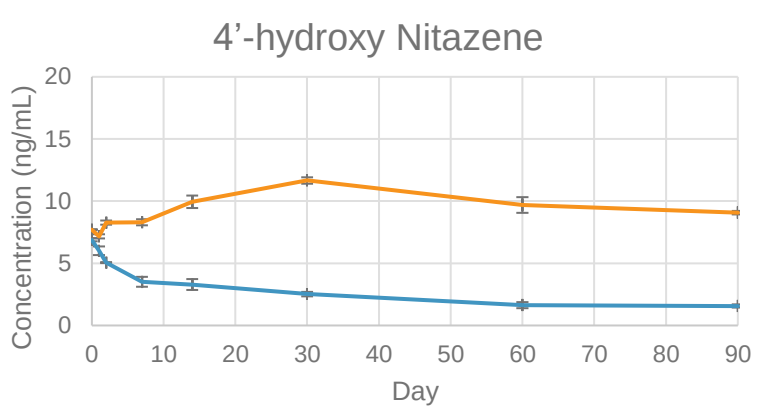
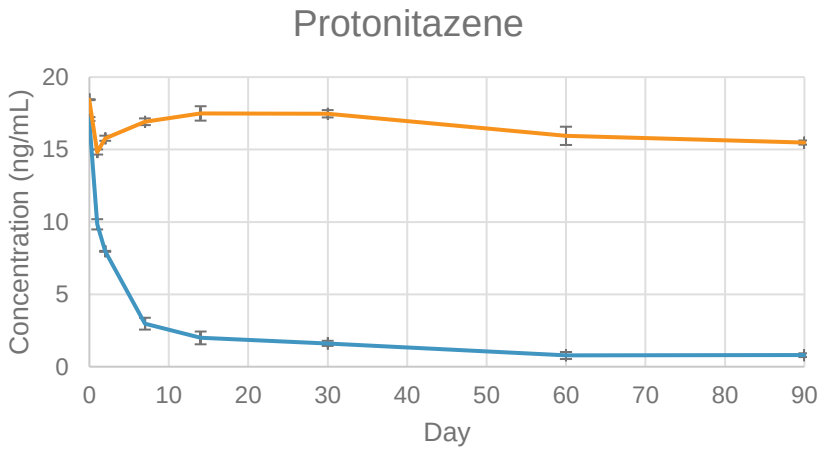
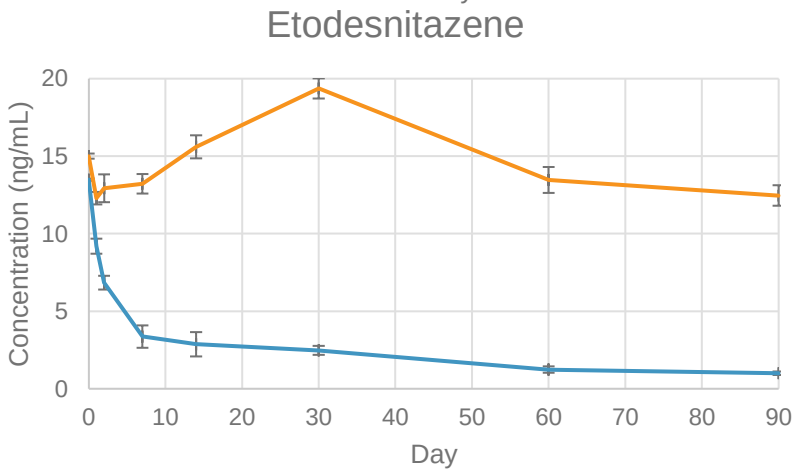
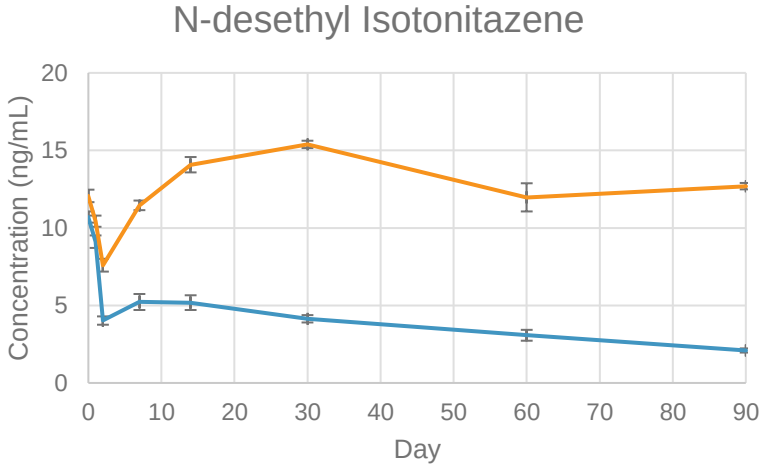








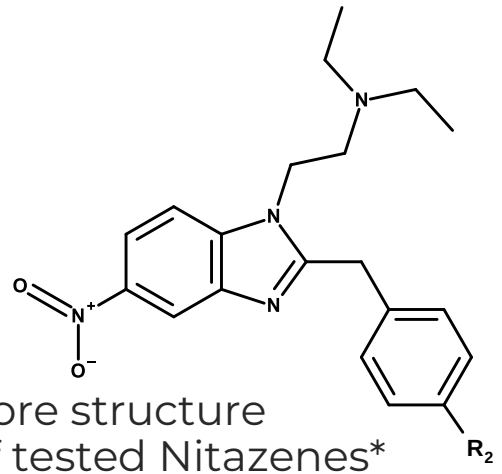




SST GT

NPS OPIOIDS

Compound	R_2 Functional Group
Protonitazene	$OCH_2CH_2CH_3$
Metonitazene	OCH_3
Isotonitazene	$OCH(CH_3)_2$
Etodesnitazene*	OCH_2CH_3
N-desethyl Isotonitazene*	$OCH(CH_3)_2$
4'-hydroxy Nitazene	OH



Core structure of tested Nitazenes*

- Nitazenes are lipophilic so they are highly adsorbed by the nonpolar gel in the tube
  - By day 7, all compounds experienced a lost of 50% or greater
- By day 60 concentrations may fall below analytical detection limits
  - May need to analyze for more polar metabolites

Nitazenes	Day 0 SST (ng/mL)	Day 90 SST (ng/mL)	% Diff SST	Day 0 GT (ng/mL)	Day 90 GT (ng/mL)	% Diff GT
Protonitazene	17	0.8	-95%	18	15	-16%
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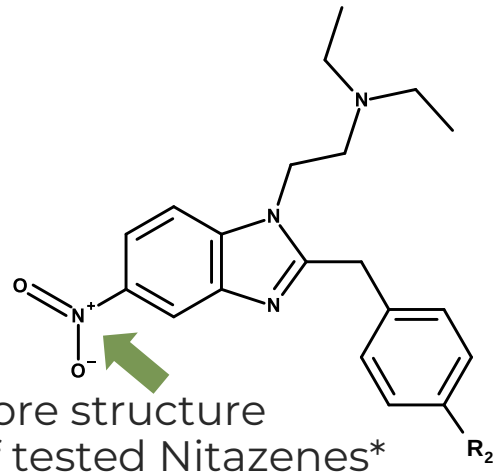

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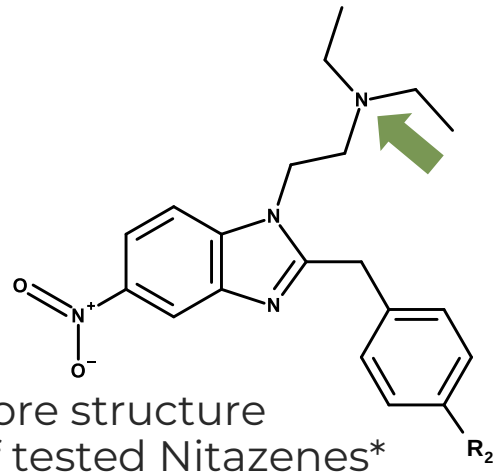

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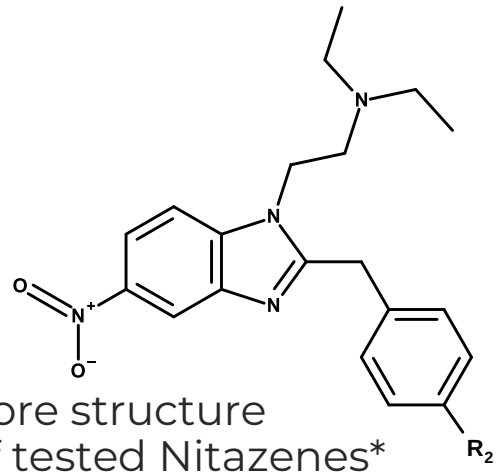

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OVERALL DISCUSSION/CONCLUSION

- Ideal to test serum immediately or transfer serum to a new container if NPS suspected
  - Most adsorption occurred over the first seven days
- Any interpretation made from SST results should consider drug loss due to gel adsorption
  - 2-keto benzodiazepines, beta-keto methylenedioxymethamphetamines and nitazenes most affected
  - Triazolo-benzodiazepines not as susceptible
- The adsorption may cause concentrations to fall below detection limits
  - Analysis of metabolites may still help in detection
- Future work
  - Eventual equilibrium theory
  - Group vs individual adsorption rate for nonpolar drugs
  - Combined lipophilic drug classes

ACKNOWLEDGMENTS

- I'd like the sincerely thank my fellow authors
  - Melissa Fogarty
  - Dr. Barry Logan
  - Heather Harris
- The Center for Forensic Science Research and Education and Staff
- Arcadia University

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THANK YOU! QUESTIONS?

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