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

Devin Kress[1,2], Melissa Fogarty[1], Heather Harris[2], Dr. Barry K. Logan[1,3]

[1]The Center for Forensic Science Research and Education, Willow Grove, PA [2]Arcadia University, Glenside, PA, [3]NMS Labs, Horsham, PA

INTRODUCTION

Serum separator tubes (SST) are a type of blood collection tube used primarily for clinical testing. These tubes contain a clot activator and a separator gel. The clot activator causes the blood to clot in the tube, and centrifugation allows the polymeric gel to separate the serum and red blood cells due to differences in densities. It is common practice with SSTs to store the remaining serum sample within the tube. A disadvantage to that is certain drugs are susceptible to being adsorbed into the gel polymer, resulting in a decreased concentration remaining in the serum, possibly to the point where the drug may be below detection limits. This is a concern when the sample may be submitted days, weeks, or even months after collection for analysis in a forensic toxicology laboratory. This phenomenon has been investigated with some traditional drugs, but there is little investigation for this effect with novel psychoactive substances (NPS), which are not always targeted in the initial investigation due to the ever-changing selection of NPS available.

STABILITY SAMPLE PREPARATION

1. Pooled citrated human whole blood (BioIVT) was spiked with analyte stock solutions to obtain a concentration of 100 ng/mL for each analyte
2. 5 mLs of spiked blood was pipetted into 5mL Becton Dickson Vacutainer SST
3. Each tube was recalcified with 37.6 µL of 2M calcium chloride solution, inverted six times, and left for 30 minutes to clot
4. The tubes were then centrifuged at 3000 RPM for 10 minutes
5. 1.5 mL of the serum from each SST was aliquoted into a borosilicate glass tube (GT) to be used as the control
6. Samples were stored in the refrigerator (approximately 4°C) until their analysis day: 0, 1, 2, 3, 14, 21, 60 and 90
7. Both SST and GT were extracted and analyzed in triplicate on their corresponding day

EXTRACTION PROCEDURE

1. Aliquoted 0.5 mL serum sample into test tube
2. Added internal standard (IS) working solution
   Stimulants: 100 uL of 1 ng/uL (Pentylone D3 and N-Ethyl Pentylone d5)
   Benzodiazepines: 50 uL of 1 ng/uL (Clonazolam D4, 8-Aminoclonazolam D4, Bromazolam D5, Flubromazolam D4, Flualprazolam D4, Flubromazepam D4)
3. Added buffer
   Stimulants: 1 mL of 0.1 M Borax buffer
   Benzodiazepines: 500 uL of Sodium Bicarbonate, pH=9
4. Added elution solvent
   Stimulants: 3 mL of n-butyl chloride: ethyl acetate (70:30)
   Benzodiazepines: 3 mL of MTBE: n-butyl chloride (60:40)
5. Caped tubes and rotated for 15 minutes
6. Centrifuged at 4600 rpm for 10 minutes
7. Aqueous layer was frozen in -80°C conditions for 15 minutes
8. Top organic layer was transferred to new test tube
9. *Stimulants only* - Add 100 uL of 10% HCl in MeOH to all samples
10. Evaporated organic layer to dryness at 35°C in TurboVap for 30 minutes
11. Reconstituted in 200 uL of initial conditions mobile phase
    Stimulants: 5mM Ammonium Formate:0.1% Formic Acid in ACN (90:10)
    Benzodiazepines: 0.1% Formic Acid in Water:0.1% Formic Acid in MeOH (50:50)

RESULTS: NPS STIMULANTS

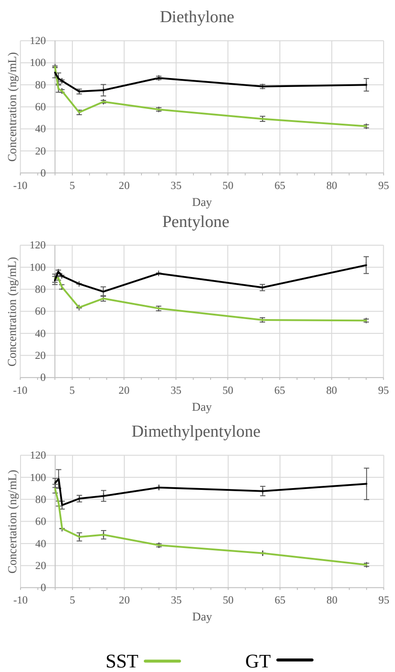

Table 1: Stimulant Data	Day 0 SST (ng/mL)	Day 90 SST (ng/mL)	% Diff SST	Day 0 GT (ng/mL)	Day 90 GT (ng/mL)	% Diff GT
Dimethylpentylone	89	20	-77%	94	94	0 %
Hexylone	86	28	-67%	85	79	-7.3%
Diethylone	96	42	-56%	91	79	-12%
N-Ethyl Pentylone	71	35	-50%	72	57	-21%
Pentylone	90	51	-42%	88	101	+15%
Tertylone	74	51	-29%	78	102	+31%
Eutylone	86	61	-28%	87	98	+12%

RESULTS: NPS BENZODIAZEPINES

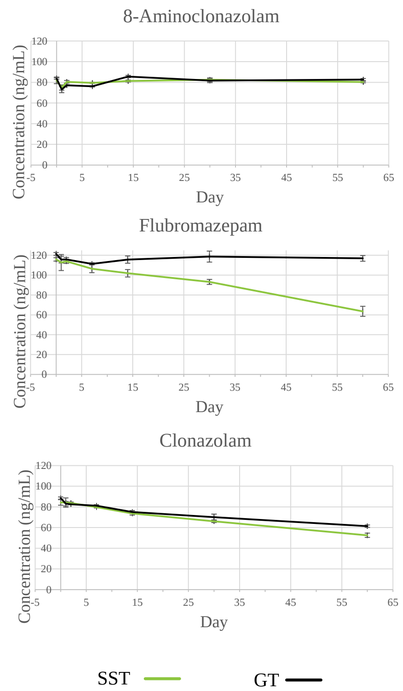

Table 2: Benzo Data	Day 0 SST (ng/mL)	Day 60 SST (ng/mL)	% Diff SST	Day 0 GT (ng/mL)	Day 60 GT (ng/mL)	% Diff GT
Flubromazepam	115	63	-45%	121	116	-3.6%
Clonazolam	84	52	-37%	88	61	-30%
Bromazolam	96	81	-14%	99	103	+3.7%
Flualprazolam	103	89	-13%	105	103	-2.4%
Etizolam	110	100	-8.8%	113	115	+2.5%
Flubromazolam	101	97	-4.1%	105	108	+2.2%
8-Aminoclonazolam	81	82	-1.9%	83	80	-1.3%

DISCUSSION: NPS STIMULANTS

- Loss in concentration was observed across all seven novel stimulants tested in this study when stored in SSTs
  - Over the 90-day timespan concentration loss ranged from 28% to 77% (Table 1)
- Dimethylpentylone concentration was affected the greatest with storage in the SST
  - Dimethylpentylone concentration decreased by 77% in 90 days, while the GT concentration decreased by only 1% in 90 days

DISCUSSION: NPS BENZODIAZEPINES

- Of the seven novel benzodiazepines tested in this study, only flubromazepam experienced loss due to storage in an SST
  - The concentration of flubromazepam decreased by 45% after being stored in an SST for 60 days (Table 2)
- Clonazolam had a similar decrease in concentration in the SST and GT of 37% and 30%, respectively
  - This indicates that the concentration loss is due to the inherent instability of clonazolam rather than adsorption into the gel

CONCLUSION

While some compounds in this study had a lower concentration for the SST then the GT for the final analysis day, this difference was minimal for the majority of the NPS benzodiazepines but much more evident with the NPS stimulants. One theory for this could be that the NPS stimulants are far less polar due to their carbon chains, and thus they get adsorbed by the nonpolar separator gel. On the other hand, the halogens of the NPS benzodiazepines provide an increase in polarity, thus a decrease in gel adsorption. This study showed that SST can produce additional interpretation issues for forensic toxicology cases in terms of quantitation. When submitting a sample for forensic testing that was stored in a SST, it would be advantageous to disclose the collection date/storage time of the specimen to assist with interpretation should the specimen now be negative.

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