Evaluation of Novel Psychoactive Substance Drug Loss from Storage in Serum Separator Tubes AAFS YFSF Abstract (Draft) Devin Kress

Introduction/ Background: 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. These tubes are used to collect blood due to their many advantages, including the convenience of simply storing any remaining sample in the vacutainer tube. A disadvantage that comes with storage in SST is that 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 is submitted days, weeks, or even months after collection for analysis in a forensic toxicology laboratory. Furthermore, there is little investigation for this effect with novel psychoactive substances (NPS), which are not always targeted in the initial investigation due to the everchanging selection of which are currently being used on the streets.

<u>Aim/Objective</u>: The aim of this study is to determine if a subset of trending NPS stimulants and benzodiazepines will be susceptible to adsorption into the SST polymeric gel during storage and the resulting decrease in concentration.

<u>Methods</u>: Samples were prepared by spiking citrated whole blood with known concentration of the following novel stimulant standards: diethylone, tertylone, *N*-ethyl pentylone, dimethylpentylone, eutylone, pentylone and hexylone. This drug fortified blood was aliquoted into twenty-one 5 mL Becton Dickson Vacutainer SST, recalcified with calcium chloride, and centrifuged at 3000 RPM for 10 mins to separate the serum. 1.5 mLs of the serum from each SST was aliquoted into a correspondingly labeled borosilicate glass tube to be used for the control where no adsorption would occur, the remaining serum remained in the SST for storage.

Serum aliquots were extracted and quantitatively analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS). These aliquots were stored in the refrigerator (approximately 4°C) and were analyzed on days 0, 1, 2, 7, 14, 30, 60, and 90. The resulting concentration from the analysis was then plotted against time for both the SST and borosilicate glass tube storage.

<u>Results</u>: All of the NPS stimulant compounds had a larger loss of drug in the SST versus the borosilicate glass tubes, and dimethylpentylone had the largest decrease over the 90-day time span. For dimethylpentylone, the concentration decreased in the SST by 77% while in the borosilicate glass tube, the concentration overall decreased by 0%. All NPS benzodiazepines, except for Flubromazepam experienced minimal loss over the 60 day storage in the SST. Flubromazepam concentration in SST decreased by 45% while the glass tube concentration only showed a 3.6% decrease.

<u>Discussion</u>: This study shows that while SST are useful for extended storage of serum samples in the clinical setting, it can produce additional interpretation issues for forensic toxicology cases in terms of quantitation. Without further testing of all NPS, it will be unknown if the concentration is or is not affected by adsorption into the separator gel. Until this testing can be completed, it would be advantageous that if hospitals suspect a novel drug was ingested, to store that serum in a secondary container or to send the SST to the laboratory as soon as possible.