Introduction: Serum separator tubes (SST) are a type of blood collection tube used primarily for clinical testing. After testing is completed, it is common practice to store remaining serum in a SST. A concern that arises with this practice is certain drugs are susceptible to adsorption into the polymer gel that separates the serum and red blood cells, which can result in a decreased concentration of drug in the serum. This is problematic for forensic toxicology as the lower concentration of the drug can affect interpretation. This phenomenon has been investigated with some drugs, but information on the impact of storage in SST on novel psychoactive substances (NPS) is limited.

Objectives: The aim of this study is to determine if statistically significant loss of NPS opioids (nitazenes), NPS stimulants, and NPS benzodiazepines occurs during storage in SST for 14, 60, and 90 days in refrigerated conditions, respectively.

Methods: A control was prepared in citrated human whole blood. 5 mL of the control was then aliquoted into a 5 mL gold-top Becton Dickinson Vacutainer SST. The blood was re-calcified with 37.6 µL of 2M calcium chloride, inverted six times, left undisturbed for 30 minutes to clot, and centrifuged at 3000 RPM for 10 minutes. 1.5 mL of serum was transferred to a 13x100 mm borosilicate glass tube (GT) and the remaining serum was left in the SST. These aliquots were stored refrigerated (4°C) and analyzed on days 0, 1, 2, 7, 14, 30, 60, and 90.

Results: Relative percent difference in concentration over the time period and two tailed, two-sample paired variance t-test ($p<0.05$) were calculated from the resulting mean of each test day for the SST and GT controls.

NPS Benzodiazepines:

The percent change in concentration from day 0 to day 90 in the SST and GT ranged from -42% to -1.5% and -26% to 0%, respectively. By day 90, five of the seven NPS benzodiazepines had a statistically significant (t-test, $p<0.05$) decrease in concentration by day 90. Flubromazolam and 8-aminoclonazolam were unaffected by storage in the SST. Clonazolam showed a 32% decrease in concentration when stored in the SST, however, it showed a similar decrease in the GT (26%) suggesting a stability issue of the compound rather than loss of the drug into the gel polymer in the SST.

NPS Stimulants:

All seven NPS stimulants demonstrated a statistically significant decrease in concentration when the sample was stored in the SST. The percent change in concentration from day 0 to day 60 in the SST ranged from -65% to -23%, respectively, while the percent change in the GT from day 0 to day 60 ranged from -13% to +1.3%, respectively. Dimethylpentylone suffered the largest loss in concentration while being stored in the SST while showing almost no difference in concentration between day 0 and 60 when stored in GT.

NPS Opioids (Nitazenes):

All six NPS opioids displayed a statistically significant decrease by day 2. The percent change in concentration from day 0 to day 14 in the SST and GT ranged from -88% to -51% and -3.5% to +29%, respectively. Testing will continue to day 90 to determine if this trend continues.

Discussion: There was significant loss of basic drugs into the gel polymer, and in some cases this could cause the concentration to fall below the limit of detection. In cases where blood is collected and stored in a SST, the best practice is to decant the serum as soon as possible into another tube. In the event that quantitative testing is performed on serum from a SST, the possibility of drug loss should be considered in any interpretation.