WSLH Verification of American Toxicology Products Grey Topped Glass Tubes for Blood Drug Testing

A study of tubes supplied by American Toxicology Products was completed to assess if the tubes are fit for purpose by monitoring physical integrity and maintaining analyte stability over the course of one month for a variety of drugs. Completed by Cole Pajunen and Allen Mello, and Utilizing methods and operating procedures approved by the Wisconsin State Laboratory of Hygiene (WSLH), six different drug categories were assessed: THC, Methamphetamine/Amphetamine (MAMP/AMP), Benzodiazepines, Opiates, Cocaine, and Fentanyl. The analyte breakdown of each drug method is listed in Table 1.

Table 1: Breakdown of Analytes Tested Per Method

THC	MAMP:AMP	Benzodiazepines	Opiates	Cocaine	Fentanyl
Delta-9-THC	Methamphetamine	7-amino clonazepam	Codeine	Cocaine	Fentanyl
11-Hydroxy-THC	Amphetamine	Zolpidem	Morphine	Benzoylecgonine	
Carboxy THC		Chlordiazepoxide (CDX)	Hydrocodone	Cocaethylene	
		NorCDX	6-Acetylmorphine		
		Midazolam	Hydromorphone		
		a-OH-Alprazolam	Oxycodone		
		Nordiazepam	Oxymorphone		
		Oxazepam			
		Alprazolam			
		Lorazepam			
		Clonazepam			
		Triazolam			
		Diazepam			
		Zaleplon			
		Temazepam			

To accurately assess the stability of analytes in the American Toxicology Products tubes over the course of one month, samples were prepared by adding 8 mL of blank blood to each of 6 - 10mL tubes per method, for a total 36 samples. Five tubes were fortified with a known concentration of each analyte corresponding to five concentration levels. The sixth tube was used as a blank control and analyzed alongside the fortified samples. The six American Toxicology Products prepared samples were typically analyzed as part of valid production runs containing method specific quality controls including but not limited to: externally prepared quality control material, internally prepared quality control material/inhouse controls, blank specimens, and spiked recovery samples consisting of 0.5ml of the case sample and 0.5ml of fortified blank matrix.

The evaluation took place over a period of 3 months with analysis intervals of one day, one week, and one month for each method. Tests completed at the Day 1 interval were analyzed within one day of preparation to achieve a baseline concentration for each analyte. Tests completed at the Week 1 interval were tested a minimum of 7 days after the initial prep date of the tube. Tests completed at the Month 1 interval were tested a minimum of 28 days after the initial prep date of the tubes. Each set of tubes were analyzed with validated instrumentation and sample preparation optimized for each approved quantitative method using Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry.

Each analyte was evaluated for acceptability in accordance with WSLH standard operating procedures. Each analyte result was considered valid if it was within ±20% of the target value.

Cocaine Stability

Cocaine and cocaethylene were assessed at concentrations of 50, 100, 200, 400, and 800 ng/mL. Benzoylecgonine was assessed at concentrations of 100, 200, 400, 800, and 1000 ng/mL. Both cocaine and cocaethylene showed degradation over the course of one month while benzoylecgonine remained stable. Between the Day 1 and Week 1 timepoints, cocaine and cocaethylene were within the acceptability criteria of ±20% of the target concentration. At the Month 1 timepoint, both analytes at all concentrations quantitated below 80% of the target values.

Benzoylecgonine remained stable over the course of one month, with its measured values within acceptability at all concentrations.

Changes in concentration of cocaine and its metabolites, cocaethylene and benzoylecgonine, are expected after one month of storage due to potential hydrolyzing of cocaine and cocaethylene in the blood, forming benzoylecgonine.

Fentanyl Stability

Fentanyl was assessed at concentrations of 1.0, 2.0, 5.0, 10, and 20 ng/mL. Overall, fentanyl remained stable over the course of one month.

One sample changed from its target concentration by more than 20%. The 10 ng/mL sample during the Month 1 analysis was quantitated at 14.13 ng/mL, showing an increase in the concentration. The Day 1 and Week 1 analyses by comparison, had calculated concentrations of 10.33 ng/mL and 10.35 ng/mL, respectively. This is the only sample to show this pattern. In addition, when looking at the data for this sample, the response for fentanyl is similar to the 10 ng/mL calibrator but the internal standard showed a noticeable decrease which likely caused the elevated result. All other samples calculated within 20% of their target concentration.

Benzodiazepine Stability

Each benzodiazepine listed in Table 1 was assessed at concentrations of 20, 50, 100, 200, and 500 ng/mL. Most analytes remained stable over the course of a month.

Certain analytes in the Week 1 analysis (including 7-amino clonazepam, midazolam, nordiazepam, oxazepam, clonazepam, and diazepam) and the Month 1 analysis (including zaleplon, nordiazepam, oxazepam, triazolam, and diazepam) had invalid calibration curves during testing. However, analyte concentrations remained consistent (within 20%) with target values at all levels. There was no evidence of any significant degradation for those analytes.

The only time any analyte resulted in a ±20% difference from the expected concentration was during the Week 1 analysis. Temazepam, at concentrations of 20 ng/mL and 50 ng/mL, was measured at 43.5% and 75% of its expected concentrations, respectively. In addition, both chlordiazepoxide and its metabolite, Norchlordiazepoxide, at 200ng/mL were measured at 77.5% and 77.7% of their expected values, respectively. Despite this, all analytes at the Month 1 analysis had acceptable values.

Overall, the analytes showed acceptable stability when tested over the course of a month. While some analytes had recovery issues during the Week 1 analysis, there is no evidence of analyte degradation since all analytes showed acceptable recovery during the Month 1 analysis.

THC Stability

Delta-9 THC and 11-OH-THC were assessed at concentrations of 1, 2, 5, 10 and 25 ng/ml. COOHTHC was assessed at concentrations of 5, 10, 25, 50, and 125 ng/mL.

For each timepoint, every analyte quantitated within +/- 20% of the target value. There was no significant increase or decrease in measured concentration across all analytes. Overall, stability of analytes in the tubes was acceptable.

Methamphetamine/Amphetamine Stability

Methamphetamine (MAMP) and Amphetamine (AMP) were assessed at concentrations of 20, 50, 100, 200, and 500 ng/mL. All measured concentrations for MAMP, were within the acceptability criteria (+/- 20%) for each target concentration.

Measured concentrations AMP at 100, 200, and 500 ng/mL, were consistently within 20% of the expected values. Measured concentrations for AMP at 20 ng/mL and 50 ng/mL, however, were quantitated at 55% and 68% of expected target values, respectively. The Day 1 reported value for the 20 ng/mL sample was acceptable at 19.75 ng/mL. In the 50 ng/mL sample only the Week 1 sample reported result for AMP was below 80% acceptability.

MAMP showed acceptable integrity at all levels, and AMP showed acceptable integrity greater than and equal to 100 ng/mL. Below 100 ng/mL, there was a decrease of AMP outside of 20% acceptability. Internal standard responses and quality control samples were consistent and acceptable, therefore, there is no analytical explanation for the observed decrease. AMP is also known to be a stable compound. Further study would be necessary to evaluate the decreased concentration in AMP over the month-long study.

Opiates Stability

A panel of Opiates were assessed for stability at varying concentrations. Codeine, Morphine, Hydrocodone, Hydromorphone, Oxycodone, and Oxymorphone were assessed at target concentrations of 10, 30, 50, 100, and 200 ng/mL. 6-Acetylmorphine (6-MAM) was assessed at 4, 12, 20, 40, and 80 ng/mL.

For most analytes (Codeine, Hydrocodone, Hydromorphone, 6-Acetylmorphine, Oxycodone and Oxymorphone), all values were within +/- 20% of target values. Morphine at 10ng/mL was consistently above 20% of the target concentration. The Morphine concentrations, while higher than the target value, were consistent across all timepoints. Overall, the concentrations remained stable throughout the onemonth evaluation for all opiate analytes.

Conclusion

Overall, the test tubes containing sodium fluoride and potassium oxalate from American Toxicology Products were proven to be fit for purpose as shown by stability of the majority of analytes over a one-

month period. While cocaine and cocaethylene showed degradation, the issue of stability is well known and is likely not caused by the tubes studied. Both analytes have been known to degrade over time in blood, even with a preservative present. Amphetamine below 100 ng/ml decreased in concentration after the Day 1 observation. It is unknown if this decrease in concentration of amphetamine was due to instrument error, extraction error, or analyte stability. It is unlikely that this issue was related to the test tubes as amphetamine is not known to adhere to test tube walls. All other studied analytes showed acceptable stability and no other consistent degradation was observed. The physical integrity of the test tubes maintained over the three-month testing period with no observed damage or wear to the tubes.