VALIDATION SUMMARY	
Procedure Name	Alkaline Drug Quantitation / Confirmation
Validation Summary	Five Calibration curves of the following drugs/metabolites were analyzed as follows: Ketamine & tramadol: 50-1000ng/mL, PCP, zolpidem & norfentanyl: 10-200ng/mL, methadone: 20-1000ng/mL and fentanyl: 1-100ng/mL. Fentanyl – did not meet quantitative validation criteria and will be validated separately. Calibration Model: Ketamine (linear curve, 1/x2 weighting), Norfentanyl (linear curve, 1/x2 weighting) Tramadol (linear curve, equal weighting), Zolpidem (linear curve, 1/x weighting), PCP (linear curve, 1/x weighting), Methadone (linear curve, 1/x weighting)

Bias:

N=15 for all analytes

	Low	Medium	High	Dilution Control
Ketamine	-5.53%	0.42%	-1.63%	2.45%
Norfentanyl	-13.77%	-7.64%	-8.50%	-8.72%
Tramadol	-6.09%	1.96%	0.06%	2.45%
Zolpidem	-5.75%	-5.25%	-7.23%	-0.74%
PCP	3.51%	1.68%	-4.28%	-0.85%
Methadone	-13.80%	-1.09%	0.69%	2.48%

Precision:

Repeatability:

N=15 for all analytes

	Low	Medium	High	Dilution Control
Ketamine	1.95%	0.73%	2.38%	1.11%
Norfentanyl	1.73%	1.41%	2.44%	1.85%
Tramadol	1.69%	1.13%	2.81%	1.35%
Zolpidem	3.24%	1.44%	3.32%	1.81%
PCP	2.95%	1.53%	1.38%	2.12%
Methadone	2.88%	0.97%	2.63%	1.37%

Intermediate Precision:

N=15 for all analytes

	Low	Medium	High	Dilution Control
Ketamine	1.95%	0.81%	2.63%	2.68%
Norfentanyl	2.33%	1.79%	2.44%	3.61%
Tramadol	2.16%	1.50%	2.91%	2.99%
Zolpidem	3.24%	1.56%	3.32%	5.43%
PCP	2.95%	2.43%	1.91%	2.51%
Methadone	2.88%	0.97%	2.63%	3.61%

	Carryover was assessed by analyzing blank matrices immediately after the highest calibrators for the 1st 3 calibration curves. No carryover was detected for the following: ketamine, norfentanyl, tramadol and zolpidem. Carryover for pcp, fentanyl, and methadone was < 1%. Ionization suppression and enhancement (post-column approach) was performed. Ten lots of blank blood (in duplicate) were extracted and reconstituted with low and high control solutions mimicking 100% recovery. Low and high control solutions were also injected neat at each level 6 times for comparison with a matrix reconstituted controls. Suppression was less than the preferred 25% for ketamine, norfentanyl, tramadol, and zolpidem. However suppression was > 25% for the following: pcp (-63.7% low, -51.9% high), fentanyl (-65.3% low, -48.3% high), and methadone (-50.8% low, -55.4% high). Due to the higher ionization suppression for pcp, fentanyl, and methadone, limit of detection studies were conducted with 9 lots of negative blood (in duplicate) for 3 batches. The following LOD's were verified per TOX425-5: ketamine, methadone, & tramadol (10 ng/mL), zolpidem & pcp (1 ng/mL), norfentanyl (5 ng/mL). The LOD for fentanyl was determined to be 0.5ng/mL (a decrease from the previous value in TOX425-5). Limit of quantitation is as follows: ketamine & tramadol (50 ng/mL), pcp, zolpidem, & norfentanyl (10 ng/mL), methadone (20 ng/mL), and fentanyl is continuing to be evaluated. Processed sample stability samples were analyzed as 12 pooled low and high controls (3 each set per analysis) at the following times after extraction: 1st set (~ 0.75 days), 2nd set (~ 3.2 days), 3rd set (~ 7.2 days), and 4th set (~ 9.3 days). All seven drugs at both control levels were within +/- 20% of the initial areas.

APPROVALS			
Technical Approval	Redacted	Date	12/7/2023
Unit Chief Approval		Date	12/7/2023