VALIDATION SUMMARY

Procedure Name	**Exclusionary Drug Screen by UPLC-ESI-FTMS**

Validation Summary

Note: Due to inconsistent performance on Exactive 2, this supplemental validation was performed solely on Exactive 1.

<u>Ion Suppression/Enhancement</u>

Ion suppression/enhancement from the original validation was recalculated, and is available in CU records for posterity.

Ion suppression was evaluated in ten lots of both blood and urine at a low (3 ng/mL) and high (100 ng/mL) level.

Very high ion suppression/enhancement (>25%) was noted for most compounds. Additionally, high variability among matrix lots was noted. These limitations will be noted in the technical procedure, and this method will be used in tandem with other screening procedures to ensure coverage for multiple analytes. Replacement of this procedure over the next year will be prioritized.

Results of the Ion Suppression/Enhancement studies follow:

Average Ion S&E						
	Blood			Urine		
	Low	High	%CV>20%	Low	High	%CV>20%
d3-BE	-18.6	1.18	Yes	-60.4	-46.2	Yes
d5-oxazepam	-14.9	-15.9	No	25.9	2.71	No
d6-oxycodone	1.88	2.17	No	-62.4	-52.6	Yes
d6-zolpidem	-9.13	-33.2	No	-48.3	-58.8	Yes
d3-diphenhydramine	25.6	-3.37	No	-45.2	-52.8	Yes
d3-hydrocodone	-10.9	24.6	Yes	-53.7	-37.5	Yes
d3-morphine	2.43	6.15	No	-31.7	-28	Yes
d4-aminoclonaz	-51.3	-45.1	Yes	-82.5	-76.5	Yes
d4-clonaz	-55.6	-61.1	Yes	-25.5	-47.8	Yes
d5-alprazolam	-19.8	-13.3	Yes	-34.3	-23.8	Yes
d5-diazepam	-53.1	-62.6	No	-47.6	-67.7	Yes
d5-OHalprazolam	11.1	7.55	No	48.1	31.3	No
6-AM	-39.7	-36.9	No	-64.4	-58.7	Yes
7-aminoclonaz	-59.2	-47.3	No	-92.5	-86.9	Yes
7-aminoflun	-55.3	-51.4	Yes	-81.4	-74.7	Yes
alprazolam	-42.7	-31.1	No	-65	-51.7	Yes
BE	-44.4	-36.6	Yes	-71.2	-71.5	Yes
bromazepam	-55.1	-38	No	-49.6	-37.9	Yes
brompheniramine	-60.6	-40.9	Yes	-68.4	-74.5	Yes
chlordiazepoxide	-59.4	-51.2	Yes	-82.5	-74.4	Yes
chlorpheniramine	-58.1	-41.7	Yes	-61.1	-72.3	Yes
clonazepam	-74.9	-69	No	-48.7	-65.9	Yes
cocaethylene	20.1	-1.71	No	-20.8	-44.1	Yes
cocaine	12.1	-12.7	No	-31.5	-55.5	Yes
codeine	-10.1	-13.1	Yes	-46.6	-40	Yes

desalkylflurazepam	-79.6	-77	No		-79.9	-74.7	Yes
desmethylflunitrazepam	-49.9	-47.9	No		-46.1	-49.5	Yes
DXM	-43.5	-51.3	Yes		-72	-77.4	Yes
dextrorphan	-44.9	-48.6	Yes		-72.6	-74.5	Yes
diazepam	-62.9	-61.8	No		-65.6	-63.2	No
dihydrocodeine	-63.7	-56.3	No		-73.5	-68	Yes
diphenhydramine	0.34	-28.3	Yes		-65.6	-71	Yes
doxylamine	-54.3	-35.3	Yes		-35.8	-57.2	Yes
duloxetine	-44.7	-67.2	Yes		-78.1	-86.8	Yes
EME	-10.3	-15.6	No		-46.7	-74	Yes
EDDP	32.2	-9.5	Yes		-14.6	-42.8	Yes
estazolam	-54.9	-31.8	No		-67.4	-44.9	No
etizolam	-26.8	-33.5	No		-36.9	-39.5	Yes
flunitrazepam	-49.9	-40.1	No		-50.4	-41.1	No
flurazepam	0.176	-27.1	Yes		-62.3	-70.2	Yes
hydrocodone	-35.2	-65.7	Yes		-72.1	-80.5	Yes
hydromorphone	-63.5	-61.9	Yes		-86.5	-79.2	Yes
OHalprazolam	-22.9	-25.7	No		-20	-28.4	No
OHmidazolam	-63.8	-52.8	No		-69.8	-60.5	Yes
OHtriazolam	-35.2	-36	No		-35.9	-40.8	No
hydroxyzine	-13.3	-47.8	Yes		-67.5	-78.2	Yes
lorazepam	-70.2	-68.4	No		-67.7	-67.3	Yes
lormetazepam	-62.5	-62.8	No		-61.6	-60.1	No
medazepam	-43.9	-50.2	Yes		-91.2	-90.1	Yes
midazolam	-64.4	-63.7	Yes		-90	-87.6	Yes
morphine	-20.8	-19.9	No		-37.8	-65.8	Yes
norchlorcyclizine	-54.1	-63.6	Yes		-76.4	-84.6	Yes
norcodeine	-46.9	-30.5	No		-45.2	-36.4	No
nordiazepam	-81	-80.5	Yes		-81.2	-79.3	No
normorphine	-17.9	-9.33	No		-85.6	-53.8	Yes
noroxycodone	-20.9	-20	Yes		-61.2	-55.1	Yes
oxazepam	-34.1	-36.3	Yes		-19	-36.6	Yes
oxycodone	-21.7	-27.2	Yes		-85.8	-77.4	Yes
oxymorphone	-29.2	-13.5	No		-74.8	-61.4	Yes
phenazepam	-97.5	-95.3	Yes		-97.2	-93.1	Yes
pheniramine	-52.2	-30.3	Yes		-56.4	-62.5	Yes
prazepam	-90.7	-91.6	Yes		-79.7	-84.2	Yes
temazepam	-47.5	-49.3	No		-43.9	-46	Yes
tetrahydrozoline	6.81	-5.64	No		-30.5	-50.3	Yes
tetrazepam	-66.5	-63.9	Yes		-78.3	-73.9	Yes
triazolam	-50.4	-41.7	Yes		-67.1	-55.3	Yes
zaleplon	-29.9	-36.5	No		-42.4	-57.5	No
zolpidem	-13.4	-36	No		-61.6	-68.6	Yes

zopiclone	-95	-79.2	Yes		-96.5	-84.6	Yes

LOD

Nine urine samples and nine blood samples were fortified with Tox 215 analytes at the previously established LODs, were extracted per the SOP, and analyzed on Exactive 1. (Exactive 2 was excluded from these experiments against the original plan as it was not performing as consistently as Exactive 1 on testmix.) This was repeated on two days for a total of three days. The matrix lots chosen for these experiments were rather old, representing "worse case scenario" case samples. Unbeknownst to the analyst, some of the matrix lots were actually natively positive for Tox 215 analytes. The LOD study was repeated at 3x the procedure's original LOD and 10x the procedure's original LOD.

Data was initially run through the Tox 215 data analysis method after estimating LOD based on peak shape and instrument response. Then LOD data and blank matrix samples were evaluated to set a threshold limit for each compound. The area thresholds were added to the method, and the data was reanalyzed. LODs were set at a level where 27 of 27 matrix samples were indicated as positive in the resulting datafiles, which was determined by having a peak present that would not be mistaken for noise. Multiple Forensic Toxicology Examiners reviewed the data, and if there were any discrepancies, the TL went back to the data and either verified the LOD that the majority had determined, or raised the LOD if there was any question of the veracity of a peak in the sample(s) pointed out by the outlier reviewer. LODs were not evaluated higher than 10x the original LODs.

High variability among LOD was seen from lot to lot of matrix. This limitation will be noted in the technical procedure, and this method will be used in tandem with other screening procedures to ensure coverage for multiple analytes. Replacement of this procedure over the next year will be prioritized.

Updated LODs are summarized below:

Analyte	Blood LOD (ng/mL)	Urine LOD (ng/mL)
Benzodiazepines and Metabolites		
α-hydroxyalprazolam	1	1
α-hydroxymidazolam	3	3
α-hydroxytriazolam	3	1
7-aminoclonazepam	1	>10
7-aminoflunitrazepam	1	>10
alprazolam	1	1
bromazepam	10	3
chlordiazepoxide	10	10
clonazepam	>10	>10
desalkylflurazepam	10	>10
desmethylflunitrazepam	>10	10
diazepam	1	1
estazolam	1	1
etizolam	1	1
flunitrazepam	3	3
flurazepam	1	1
lorazepam	>10	>10
lormetazepam	3	3
medazepam	5	10

midazolam	1	10
nordiazepam	3	3
oxazepam	1	1
phenazepam	>10	>10
prazepam	>50	3
temazepam	10	3
tetrazepam	3	3
triazolam	1	1
Opioids and Metabolites		
6-acetylmorphine	1	9
codeine	1	3
dihydrocodeine	3	1
dihydromorphone	1	3
EDDP	1	>10
hydrocodone	1	>10
hydromorphone	1	3
morphine	1	3
norcodeine	3	30
normorphine	3	10
noroxycodone	1	10
oxycodone	1	3
oxymorphone	1	3
Cocaine and Metabolites		
ecgonine methyl ester	1	15
benzoylecgonine	1	3
cocaethylene	1	3
cocaine	1	3
Antihistamines and Related Compounds		
brompheniramine	3	3
chlorpheniramine	1	>10
dextromethorphan	10	1
dextrorphan	5	1
diphenhydramine	5	1
doxylamine	5	1
hydroxyzine	50	>10
norchlorcyclizine	>50	>10
pheniramine	1	>10
tetrahydrozoline	>10	10
Hypnotics		
zaleplon	1	10
zolpidem	3	>30

	zopiclone	>10	>10
	Antidepressants		
	duloxetine	>500	>50

Interferences

Interferences from stable-isotope IS were evaluated.

Results from matrix samples with IS only: Peaks for benzoylecgonine (blood) and alprazolam (urine) met decision criteria for true peaks, but area counts were less than 1% of that of the IS.

Results from matrix samples with non-labeled IS compounds only: Peaks for d3-BE and d3-hydrocodone (both in urine) met decision criteria, but area counts were less than 1% of the area for the non-labeled drug.

Additionally, ten postmortem blood samples were run through the procedure along with 10 urine specimens to gather new blank matrix samples in order to set data thresholds for data analysis.

Redacted

APPROVALS			
Technical Approval	Redacted	Date	12/19/23
Unit Chief Approval		Date	12/19/23