VALIDATION SUMMARY	
Procedure Name	**TOX 201 – Direct Solvent Extraction of Acid/Neutral Drugs from Biological Fluids (Supplemental Validation)**
Validation Summary	**Carryover** Carryover was evaluated in extracted blood and urine. Appreciable carryover was identified for naproxen (~2.3%), phenytoin (~1.5%), and phenobarbital (~0.53%) in blood. Appreciable carryover was identified for naproxen (2.4~%) and phenytoin (1.7~%), and phenobarbital (~0.66%) in urine. It was noted that theophylline carryover values in urine may be artificially inflated by the baseline presence of theophylline in the blank lot of urine used in this study. **Limit of Detection (LOD)** Previously established LOD values were evaluated in both blood and urine. The LOD values for levetiracetam (2.5 µg/mL) and acetaminophen (25 µg/mL) in blood require additional evaluation. LOD values for amobarbital (0.1 µg/mL), carbamazepine (0.1 µg/mL), pentobarbital (0.1 µg/mL), phenobarbital (0.1 µg/mL), secobarbital (0.1 µg/mL), phenytoin (0.25 µg/mL), carisoprodol (0.5 µg/mL), meprobamate (0.5 µg/mL), butalbital (2.5 µg/mL), theophylline (2.5 µg/mL), ibuprofen (5 µg/mL), and naproxen (50 µg/mL) in blood were confirmed. The LOD values for phenobarbital (0.1 µg/mL), carisoprodol (0.1 µg/mL), meprobamate (0.25 µg/mL), levetiracetam (2.5 µg/mL), theophylline (0.1 µg/mL), ibuprofen (1 µg/mL), and acetaminophen (1 µg/mL) in urine require additional evaluation. LOD values for amobarbital (0.1 µg/mL), carbamazepine (0.1 µg/mL), pentobarbital (0.1 µg/mL), secobarbital (0.1 µg/mL), phenytoin (0.1 µg/mL), butalbital (0.5 µg/mL), and naproxen (2.5 µg/mL) in urine were confirmed. Data collected on MSD-09 for the purpose of instrument verification will be available for processing at a later date, if needed. **Processed Sample Stability** For the purposes of this validation, an analyte was defined as stable if the average area of the most abundant characteristic ion over three injections of aged extract was within ± 50% of the average of the same ion for three injections of an extract injected immediately on the date of extraction. The validation plan called for evaluation of extracts on Day 0, Day 1, and Day 3. Due to the long run time on the instrument for each sequence, the extracts were analyzed on Day 0, Day 2, and Day 6. Overall, most drugs were stable in both blood and urine extracts for up to 6 days when stored in an unpunctured, screw-top ALS vial and stored at 2-8 degrees. Ibuprofen appears to have stability problems at higher concentrations in blood when stored at 2-8 degrees. When vials were stored at room temperature on the autosampler, after vials were punctured during first analysis, and later reconstituted, many of the drugs remained stable, however carbamazepine has marked stability issues in blood at lower concentrations. In addition, the NSAIDs in general had more stability problems in the reconstituted extracts.

	Please see the "Summary" tab of the table named "Extract Stability Results_Average_rev 2" for a quick and detailed overview of results. **Matrix Interference** A review of matrix interference raw data from 2011 was thoroughly reviewed. No interfering peaks were observed in blank lots of blood. One (1) lot of urine appeared to have an interfering peak for ibuprofen. This is not an unexpected finding as these urine samples were donated from in-house volunteers. **Analyte Interference** One solution containing multiple drugs at ~25ppm in methanol was injected on MSD-08. The resultant data file was analyzed for the presence of all drugs known to have been spiked into the sample. Cetirizine was not detected. Acetaminophen appears to co-elute and suffer from interference from amobarbital. Guaifenesin appears to have interference from butalbiltal due to co-elution. Oxycodone appears to have interference from flunitrazepam and temazepam as a result of co-elution. 100 ppm solutions of available drugs were injected individually on MSD-08 and each data file was evaluated to determine whether or not it may cause a false positive result for any of this assay's target compounds. While many drugs will yield detectable signals for the most abundant ions frequently seen in the target drugs, they all had different expected retention times and different characteristic mass spectra and therefore do not interfere.

APPROVALS				
Technical Approval	Redacted		Date	12/13/23
Unit Chief Approval			Date	12/13/2023