Validation Plan for the Addition of Xylazine to the Acid/Base/Neutral Drug Screen and Quantitation by GC and GC-MS using the Liquid-Liquid Extraction Option

Validation Criteria to be Evaluated

- Sensitivity (Estimated Limit of Detection (LOD))
- Interferences
    - Endogenous
    - Standard
    - Internal Standard
    - Commonly Encountered Drugs
- Carryover
- Stability

Sensitivity (Estimated LOD)

The estimated limit of detection will be assessed at 0.010 mg/L and 0.005 mg/L in all matrix types (blank blood, antemortem blood, postmortem blood, urine). Xylazine will be spiked into, at minimum, three different blank matrix sources, per matrix type. The three different matrix sources, per matrix type, shall be analyzed over three different batch analyses.

Predetermined acceptance criteria:

Retention Time: ±2%

Signal to Noise: ≥3

Presence of major indicative ions (i.e., m/z 205, 220, 130, 145)

Interferences

Endogenous

The potential interference from endogenous compounds from the matrices will be evaluated. A minimum of ten matrix sources, per matrix type (blank blood, antemortem blood, postmortem blood, urine) shall be extracted without the addition of standards or internal standards. Samples will be evaluated for the presence of xylazine via the extracted ion chromatogram for the major indicative ions (i.e., m/z 205, 220, 130, 145).

Standard

A xylazine standard will be fortified into a single blank matrix sample, per matrix type at 1.0 mg/L with no addition of the internal standard (methapyrilene). The extracted samples will be evaluated for the presence of the internal standard.

Internal Standard

The internal standard (methapyrilene) will be fortified into a single blank matrix sample, per matrix type. without the addition of xylazine. The extracted samples will be evaluated for the for the presence of xylazine via the extracted ion chromatogram for the major indicative ions (i.e., m/z 205, 220, 130, 145).

Commonly Encountered Drugs

Commonly encountered base drugs will be fortified into a single blank matrix blank, per matrix type at approximately 1.0 mg/L without xylazine and analyzed for the presence of xylazine.

The same commonly encountered drugs will be fortified into the same matrices with the addition of the xylazine standard at 1.0 mg/L. The samples will be analyzed for any interference with the presence of xylazine.

Carryover

Carryover will be evaluated by running injections of 1.0 mg/L and 2.0 mg/L xylazine followed by a matrix blank. For each concentration, there will be triplicate analyses with a minimum of three sources per matrix type. The matrix blanks will be evaluated for carryover. Analyte carryover is indicated by a response greater than 10% of the LOD.

Stability

The stability of extracted samples will be evaluated by extracting a single fortified blank matrix, per matrix type, at 1.0 and 0.10 mg/L xylazine with internal standard. The samples will be analyzed every 24 hours for seven days with triplicate injections at each time point (or until the samples are consumed). The responses will be averaged and all other responses from subsequent time points will be evaluated against the average response. The average instrumental responses for each time point will be compared to the day one instrumental response and plotted. Compounds are considered stable if the average signal response of the triplicate injections for a time point falls within the method's predefined acceptable bias (i.e., ±20%). For example, if the peak area increases above 120% or decreases below 80% of the original response the compound is no longer deemed stable. Alternatively, the ratio of peak area of analyte to internal standard may be utilized in the stability evaluation as opposed to peak area.