

## WORK INSTRUCTIONS

### 6.1.1 Confirmation of Benzodiazepines and Z drugs in blood and urine

#### REFERENCE MATERIAL

##### Benzodiazepine Positive Control

- Stock Solutions  
1 mg/mL single component benzodiazepine class reference solutions.
- Calibrator Solution  
The calibrator solution must contain 7-aminoclonazepam, 7-aminoflunitrazepam, zopiclone, zolpidem, chlordiazepoxide, quetiapine, midazolam, flurazepam, nitrazepam, alpha-hydroxyalprazolam, alpha-hydroxytriazolam, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, flunitrazepam, temazepam, and diazepam
  - 1.0 µg/mL Target mix in methanol  
Add 10µL each 1mg/mL or 25µL 250µg/mL Stock Solution to  $\cong$  6mL MeOH (LCMS grade) in 10mL ball flask. Bring up to volume with MeOH. Solution is stable for one-year when stored under refrigeration.
  - 10.0 µg/mL Target mix in methanol  
Add 250µL each 1mg/mL Stock Solution to  $\cong$  20mL MeOH (LCMS grade) in 25mL ball flask. Bring up to volume with MeOH. Solution is stable for one-year when stored under refrigeration.
- Positive Control:
  - *Negative urine or blood can be spiked with 250uL of 1.0 ug/mL (or 25uL of 10.0 ug/mL) of a working solution, the compounds in that solution **cannot** be the same lot as was used for the calibrators. At minimum, the control must contain two compounds included in the scope of the method.*

##### Conjugated Controls (Urine samples only)

- Spiked Negative urine (must be the same lot for both positive and negative Glucuronide controls or
- Stock Solution  
100µg/mL Oxazepam Glucuronide or Lorazepam Glucuronide
- Direct spiking  
Spike negative urine with 30µL of 100µg/mL stock solution.
- Working Glucuronide Solution (10ng/µL)  
Add 1mL 100µg/mL Stock Solution to 10mL MeOH. Solution is stable for one year when stored under refrigeration.

##### Internal Standard

- Stock Solution  
100 ug/mL 7-aminoflunitrazepam-D7, alphahydroxyalprazolam-D5, oxazepam-D5, nordiazepam-D5, clonazepam-D4, temazepam-D5, diazepam-D5
- Working Internal Standard Solution (1.0 µg/mL ISTD mix in methanol)  
Add 100 µl of 100ug/mL stock solution to  $\cong$  9mL MeOH in 10mL ball flask. Bring up to volume with MeOH. Solution is stable for one year when stored under refrigeration.

#### COMMENTS

This method has instructions for the preparation of both urine and blood casework samples.

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### SAMPLE PREPARATION:

