

## 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 300uL of 1.0 ug/mL for urine or 100ul for blood 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 and fall between and approximate concentration of 75 and 400 ng/ml.*

##### 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 or 300 µL working 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.

# WORK INSTRUCTIONS

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

### SAMPLE PREPARATION:

