

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 10µL 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.

Conjugated Controls (Urine samples only)

- Commercially obtained conjugated control (example: Bio-Rad C3)
- Stock Solution
1 mg/mL or 100µg/mL Oxazepam Glucuronide or Lorazepam Glucuronide
- Direct spiking
Spike negative urine with 3µL of 1mg/mL stock solution or 30µL of 100µg/mL stock solution.
- Working Glucuronide Solution (10ng/µL)
Add 100µL of 1mg/mL Stock Solution (or 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 100ug/mL stock solution on 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:

