



SPEware uSPE (ultraSPE) Faster / Cleaner Sample Preparation

Benzodiazepines from Urine

For GC-MS or LC-MS confirmations using:
Trace-B 35mg.

Pre-Extraction

1. To 1 ml of urine add 0.025 ml of ISTD (d7-aminoflunitrazepam, d5-oxazepam, d5-diazepam) 1.0 ml of 1 M Sodium Acetate Buffer (pH = 4.5), and 0.050 ml of glucuronidase (H. Pomatia, type H-2). Mix well.
2. Loosely stopper and incubate at 56 °C for 2 Hours.
3. Allow tubes to cool then centrifuge for 5 minutes at 2000 rpm.

Extraction

Note: For Full Scan Mode, rinse columns with 2ml Ethyl Acetate followed by 2ml MeOH and 15 sec. of drying at 25 psig

1. Load sample onto columns at flow rates of 10-20 ml/min
2. Wash each column with 1ml pH = 9.0 buffer
(Prepared by dissolving 20 g of KHCO₃ and 10 g of K₂CO₃ in 500 ml of water, adjust the pH to 9.0 then bring the volume to 1000 ml.)
3. Wash each column with 1 ml water.
4. Dry columns for 10 minutes at 25 psig.
5. Elute each column with 2.0 ml Ethyl Acetate: NH₄OH (98:2)

Note: If you see water droplets in the bottom of the vial decant into a new vial leaving water droplets behind.

6. Evaporate samples to dryness using nitrogen blow down at 40 °C.

Derivatization for GC/MS Analysis

1. Evaporate samples under nitrogen to dryness in a 50 °C heating block.
2. Reconstitute each sample with 50 ul Ethyl Acetate and transfer to a autosampler vial
3. To each autosampler vial add 25 µL of MTBSTFA+1% TBDMCS . Place in a 90 °C heating block for 30 minutes.

For LC/MS, reconstitute with 100ul of mobile phase.

COMPOUNDS EXTRACTED BY THIS METHOD:

Alprazolam, aHydroxylalprazolam-, Chlordiazepoxide, Clonazepam, Clonopin, Diazepam, Demoxepam, Estazolam, Flurazepam, Flunitrazepam, Nordiazepam, Norchlordiazepoxide, Nitrazepam, Desalkylflurazepam-, Hydroxyethylflurazepam-, Lorazepam-, Midazolam, Oxazepam, Prazepam, Temazepam, Triazolam, aHydroxytriazolam