



# SPEware uSPE (ultraSPE) Faster / Cleaner Sample Preparation

## Benzodiazepines from Whole Blood

For GC-MS or LC-MS confirmations using:  
Trace-B 50mg.

### Blood Prep

1. To 1 ml of Blood add 0.025 ml of ISTD (d7-aminoflunitrazepam, d5-oxazepam, d5-diazepam) 2.0 ml of 0.1 M Sodium Acetate Buffer (pH = 4.5), sonicate for 15 minutes then centrifuge at 3000 rpm for 20 minutes.

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

2. Load sample onto columns at flow rates of 10-20 ml/min

3. Wash each column with 1 ml pH = 9.0 buffer  
(Prepared by dissolving 20 g of  $\text{KHCO}_3$  and 10 g of  $\text{K}_2\text{CO}_3$  in 500 ml of water, adjust the pH to 9.0 then bring the volume to 1000 ml.)

4. Wash each column with 1 ml water.

5. Dry columns for 10 minutes at 25 psig.

6. Elute each column with 2.0 ml Ethyl Acetate:  $\text{NH}_4\text{OH}$  (98:2)

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

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

### Derivatization for GC/MS Analysis

8. Evaporate samples under nitrogen to dryness in a 50 °C heating block.

9. Reconstitute each sample with 50 ul ethyl acetate and transfer to a autosampler vial

10. To each autosampler vial add 25  $\mu\text{L}$  of MTBSTFA + 1% TBDMCS . Place in a 90 °C heating block for 30 minutes.

**Note: It is important to dry samples down immediately, as some Benzos are unstable in this solvent. For LC/MS, reconstitute with 100ul of mobile phase.**

HPLC Column---Targa C-18 5 (TS-0521-C185) 50x2.1mm  
Mobile Phase---35% Acetonitrile, 65% $\text{H}_2\text{O}$  containing 0.1%TFA

### COMPOUNDS EXTRACTED BY THIS METHOD:

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