

# SPE Extraction of Benzodiazepines From Urine



## UCT Part Numbers

**WSH96XCE11**  
Clean Screen® Xcel I  
100 mg 96 Well Plate

**WSH48XCE11**  
Clean Screen® Xcel I  
100 mg 48 Well Plate

## Procedure:

### 1. Sample Preparation (Hydrolysis Step)

- To 1-2 mL urine sample add 500  $\mu$ L of acetate buffer (pH= 5.0) containing 5,000 units/mL  $\beta$ -glucuronidase.
- Or, add 500  $\mu$ L of acetate buffer and 25  $\mu$ L of concentrated  $\beta$ -glucuronidase.
- Add appropriate volume and concentration of internal standards.
- Vortex and heat for 1-2 hours at 65°C.
- Allow sample to cool.

**Note:** Do not adjust pH- sample is ready to be added to extraction column.

### 2. Apply Sample to Column

- Load sample directly to column without any preconditioning.
- Pull sample through at a rate of 1-2 mL/ minute.
- Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for 1 minute.

### 3. Wash Column

- Wash sample with 1 mL of methylene chloride.
- Dry column thoroughly under vacuum (10 mm Hg) or positive pressure (~ 80-100 psi) for a minimum of 5-10 minutes.

**Note 2:** It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.

### 4. Elution

- Elute samples with 1 mL ethyl acetate/ammonium hydroxide (98/2).
- Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

### GC/MS Analysis

It is recommended to add 50  $\mu$ L of ethyl acetate to 50  $\mu$ L of derivatization agent and react at ~70 °C for 15 minutes. Inject 1-2  $\mu$ L of cooled (50:50) solution in the GC/MS system for analysis.

### LC/MS Analysis

Reconstitute in methanol or appropriate mobile phase.

### Benzodiazepine Analytes Extracted

Diazepam	Clonazepam	Alprazolam	Midazolam
Nordiazepam	7-aminoclonazepam	$\alpha$ -OH Alprazolam	$\alpha$ -OH Midazolam
Temazepam	Oxazepam	Lorazepam	



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