

# Extraction Of Benzodiazepines From Urine Using SPE Cartridges



## UCT Part Numbers

**CSXCE106**  
130 mg, 6 mL  
Clean Screen® XCEL I Column

**CSXCE103**  
130 mg, 3 mL  
Clean Screen® XCEL I Column

## SPE Procedure:

### 1. Sample Preparation (Hydrolysis Step)

- To 1-5 mL urine sample add 1-2 mL of 0.1M 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 internal standards
  - Vortex and heat for 1-2 hours at 65°C
  - Allow sample to cool
- Note:** Do not adjust the pH. The sample is ready to be loaded on to the extraction column.

### 2. Load Sample

- Load at 1 to 2 mL/minute
- Dry for 1 minute under full pressure or vacuum

### 3. Wash Column

- 1 x 1 mL DCM or MeOH

### 4. Dry Column

- Dry column for 5 to 10 minutes at full pressure or vacuum

### 5. Elute

- 1 x 1 mL EtOAc:NH<sub>4</sub>OH (98:2)

### 6. Evaporate

- Evaporate eluate to dryness at about 35°C

### 7. Reconstitute

- LC/MS: Reconstitute in appropriate volume of mobile phase
- GC/MS: Add 50  $\mu$ L of EtOAc to 50  $\mu$ L of derivatizing reagent and react at 70°C for 15 minutes

## Benzodiazepine Analytes Extracted

Diazepam	Clonazepam	Alprazolam	Midazolam
Nordiazepam	7-aminoclonazepam	a-OH Alprazolam	a-OH Midazolam
Temazepam	Oxazepam	Lorazepam	Flurazepam

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