



**CLONAZEPAM & 7-AMINOCLONAZEPAM IN  
URINE FOR GC/MS CONFIRMATIONS USING:  
200 mg CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN**

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU<sup>®</sup> Tips

**1. PREPARE SAMPLE:  $\beta$ -GLUCURONIDASE HYDROLYSIS.**

To 2 mL of urine, add internal standard(s)\* and 1 mL of  $\beta$ -Glucuronidase solution.

$\beta$ -Glucuronidase solution contains 5,000 F units/mL *Patella vulgata* in 100 mM acetate buffer (pH 5.0).

Mix/vortex.

Hydrolyze for 3 hours at 65°C.

Cool before proceeding.

**2. CONDITION CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN**

1 x 3 mL CH<sub>3</sub>OH.

1 x 3 mL deionized water.

1 x 1 mL 100 mM phosphate buffer (pH 6.0).

**NOTE:** Aspirate at < 3 inches Hg to prevent sorbent drying.

**3. APPLY SAMP**

Load at 1 to 2 mL/ minute.

**4. WASH COLUMN**

1 x 2 mL deionized water.

1 x 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0).

Dry column (5 minutes at > 10 inches Hg).

1 x 2 mL hexane.

**5. ELUTE CLONAZEPAM / 7-AMINOCLONAZEPAM**

1 x 3 mL ethyl acetate with 2% NH<sub>4</sub>OH:

Collect eluate at 1 to 2 mL/minute.

**NOTE:** Prepare fresh daily.

**6. DRY ELUATE**

Evaporate to dryness at < 40°C.

**7. DERIVATIZE**

Add 50  $\mu$ L ethyl acetate and 50  $\mu$ L MTBSTFA (with 1% TBDMCS)\*\*\*\*.

Mix/vortex.

React 20 minutes at 90°C.

Remove from heat source to cool.

**NOTE:** Do not evaporate MTBSTFA solution.

**8. ANALYSIS**

Inject 1 to 2  $\mu$ L sample.

For MSD monitor the following ions:

<u>Compound</u>	<u>Primary Ion**</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerilliant#</u>
Clonazepam-TBDMS	372	374	326	C-907
7-Aminoclonazepam-TBDMS	342	344	399	A-915
Clonazepam-D4-TBDMS	376	378	377	C-905
7-Aminoclonazepam-D4-TBDMS	346	348	403	A-917

\*Suggested internal standard for GC/MS: Clonazepam-D4, 7-aminoclonazepam-D4.

\*\*Quantitation ion

\*\*\*\*Part # SMTBSTFA-1-1,10, 25, 100