



## **BENZODIAZEPINES IN EQUINE OR CANINE URINE FOR GC/MS CONFIRMATIONS**

Part #

XRDAH206 - XtrackT<sup>®</sup> DAU 200 mg, 6 mL Tube

SPPHO6001-10 - Select pH Buffer Pouches 100mM Phosphate pH 6.0

SBSTFA-1-1 – SELECTRA-SIL<sup>®</sup> BSTFA w/1% TMCS

BETA-GLUC-10 - Selectrazyme<sup>®</sup>  $\beta$  - Glucuronidase

### **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 *Haliotis rufescens* in 100 mM acetate buffer (pH=5.0).

Mix/vortex.

Hydrolyze for 3 hours at 65°C.

Centrifuge for 10 minutes at 2000 rpm and discard pellet.

### **2. CONDITION XTRACKT<sup>®</sup> DAU EXTRACTION COLUMN:**

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

1 x 3 mL D.I. H<sub>2</sub>O

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

NOTE: Aspirate at full vacuum or pressure

### **3. APPLY SAMPLE:**

Load at 1 mL/ minute

### **4. WASH COLUMN:**

1 x 2 mL D.I. H<sub>2</sub>O

1 x 2 mL 20% Acetonitrile in 100 mM phosphate buffer (pH= 6.0)

Dry column (5 minutes at full vacuum or pressure)

1 x 2 mL Hexane

### **5. ELUTE BENZODIAZEPINES:**

1 x 5 mL Ethyl Acetate containing 4% NH<sub>4</sub>OH

collect eluate at 1 to 2 mL/minute

### **6. DRY ELUATE:**

Evaporate to dryness at < 40°C

### **7. DERIVATIZE:**

Add 50  $\mu$ L Ethyl Acetate and 50  $\mu$ L BSTFA w/1% TMCS

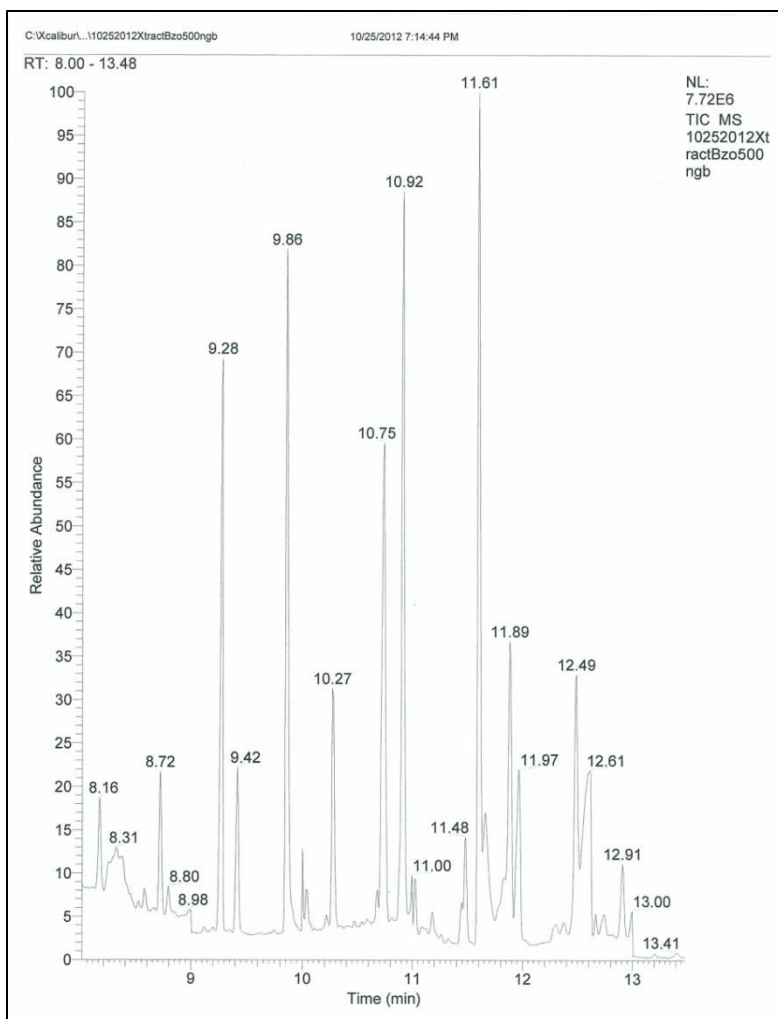
Overlay with Nitrogen and cap. Mix/vortex

React 20 minutes at 70°C. Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

### **8. ANALYZE:**

Inject 1 to 2  $\mu$ L onto gas chromatograph



Compound	Primary Ion*	Secondary Ion	Tertiary Ion
1. Diazepam	256	283	221
2. Nordazepam TBDMS	327	383	369
3. Midazepam	310	325	297
4. Oxazepam - 2TBDMS	457	513	383
Oxazepam - D <sub>5</sub> 2TBDMS <sup>†</sup>	462	519	
5. Temazepam	357	283	385
6. 7-aminoclonazepam TBDMS	342	399	328
7. Lorazepam 2TBDMS	491	513	533
8. Clonazepam	372	326	429
9. Alprazolam	279	204	308
Alprazolam - D <sub>5</sub> <sup>†</sup>	284	313	
10. Alphahydroxyl alprazolam TBDMS	381	423	346

\*Quantitation Ion

†Suggested internal standard for GC/MS