

# Buprenorphine and Norbuprenorphine in Equine Urine for GC/MC Confirmations



## UCT Part Numbers

### XRDAH206

200 mg XtrackT® DAU Extraction  
Column in 6 mL cartridge

### SPHACE5001-10

Select pH Buffer Pouches  
100mM Acetate pH 5.0

### SBSTFA-1-1

BSTFA w/1% TMCS

## Procedure:

### 1. Prepare Sample

- To 1 mL of 100 mM Acetate buffer (pH= 5) add internal standard.
- Mix/ vortex and add 1 mL of Equine Urine.
- Add 2 mL of 100 mM Acetate buffer (pH= 5) and mix/ vortex.
- Sample pH should be  $6.0 \pm 0.5$ .
- Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
- Centrifuge as appropriate.
- Enzyme Hydrolysis of Glucuronides. To 1 mL of 100 mM Acetate buffer add internal standard.
- Add 1 mL of Equine Urine. Mix/vortex.
- Add 2 mL of 100 mM Acetate buffer (pH= 5).
- Hydrolyze with Helix Pomatia (5,000 units/mL), heat for 3 hours at 60°C. Cool before proceeding.

### 2. Condition XTRACKT® 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 = 5.0).

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

### 3. Apply Sample

- Load sample at 1-2 mL / minute.

### 4. Wash Column

- 1 x 3 mL D.I. H<sub>2</sub>O
- 1 x 3 mL 100 acetate buffer (pH=5.0)
- 1 x 3 mL Methanol
- Dry column (5-10) minutes at full vacuum or pressure.

### 5. Elute Buprenorphine / Norbuprenorphine

- 1 x 3 mL methylene chloride / iso-propano / ammonium hydroxide (78/20/12). (Make elution solvent fresh)
- Collect eluate at 1 to 2 mL/minute

**Note:** Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency.

### 6. Dry Eluate

- Evaporate to dryness at < 40°C

### 7. Derivatize

- Add 50 µL ethyl acetate and 50 µL BSTFA w/1 % TMCS
- React 20 minutes at 70°C
- Remove from heat source to cool

**Note:** Do not evaporate BSTFA

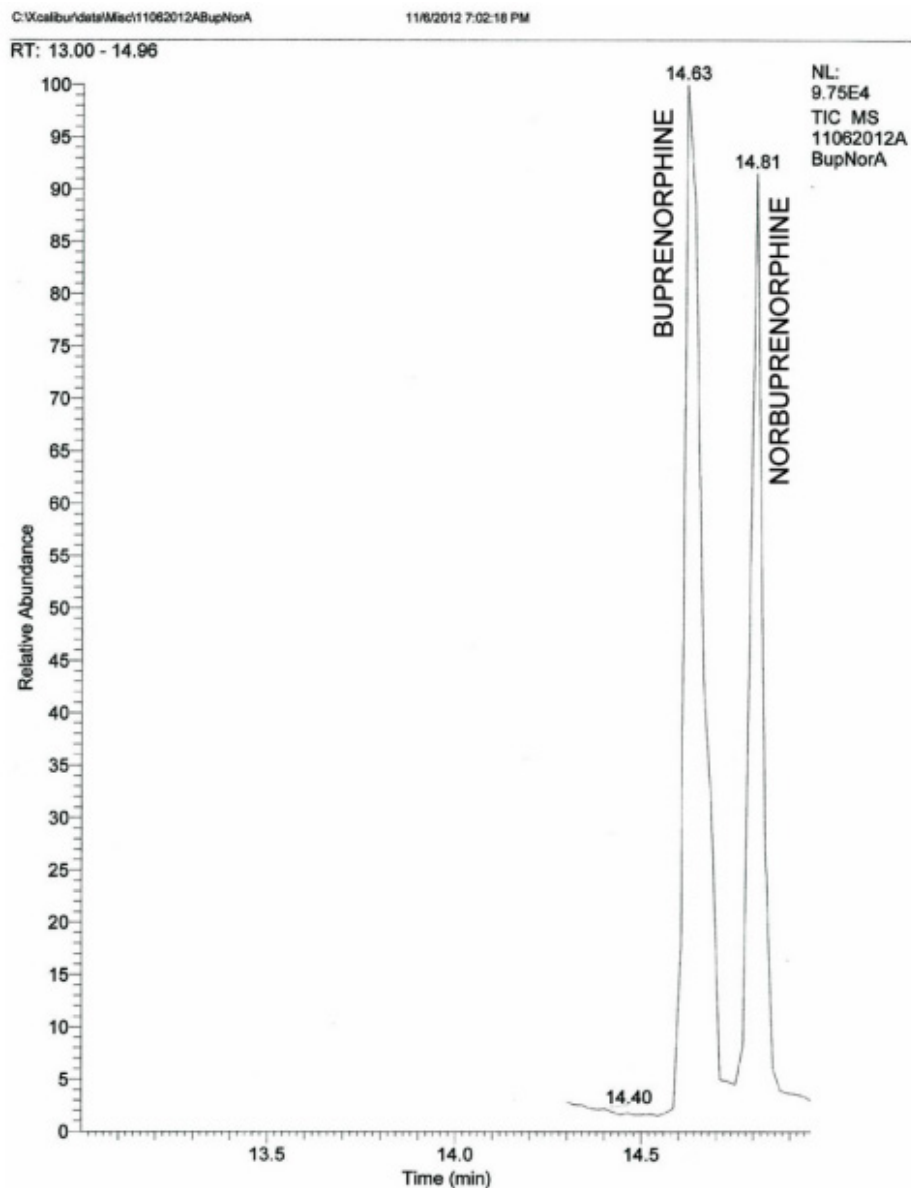
### 8. Quantitative

- Inject 1 to 2 µL onto gas chromatograph/mass spectrometer



## Chromatogram

### Buprenorphine and Norbuprenorphine



**Mass Spec Table**

| Compound                             | Primary Ion* | Secondary Ion | Tertiary Ion |
|--------------------------------------|--------------|---------------|--------------|
| Buprenorphine-TMS                    | 452          | 467           | 487          |
| Buprenorphine-D4-TMS <sup>†</sup>    | 455          | 470           | 489          |
| Norbuprenorphine-TMS                 | 468          | 500           | 510          |
| Norbuprenorphine-D5-TMS <sup>†</sup> | 503          | 525           | 542          |

\*Quantitation Ion

<sup>†</sup>Internal Standard



**4107-04-01**

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