



BUPRENORPHINE AND NORBUPRENORPHINE IN EQUINE URINE FOR GC/MS CONFIRMATIONS

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

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 ± 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 XTRACT[®] DAU EXTRACTION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL D.I. H₂O

1 x 1 mL 100 mM Acetate buffer (pH= 5.0)

NOTE: Aspirate at < 3 Inches Hg to prevent sorbent drying

3. APPLY SAMPLE:

Load at 1 to 2 mL/ minute

4. WASH COLUMN:

1 x 2 mL D.I. H₂O

1 x 3 mL 100 mM 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 MeCl₂ / IPA / NH₄OH (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 μ L Ethyl Acetate and 50 μ L BSTFA w/1 % TMCS

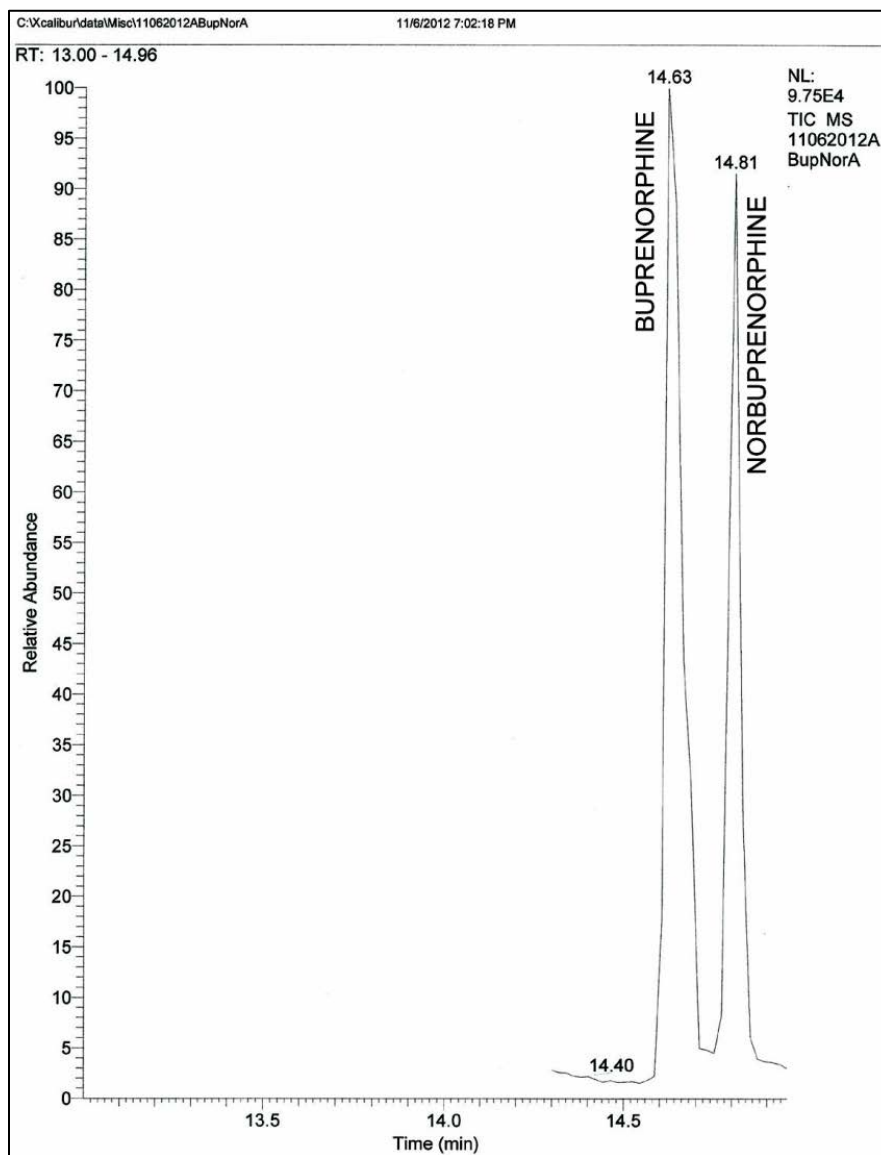
React 20 minutes at 70°C
Remove from heat source to cool
NOTE: Do not evaporate BSTFA

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-D ₄ -TMS [†]	455	470	489
Norbuprenorphine-TMS	468	500	510
Norbuprenorphine-D ₅ -TMS [†]	503	525	542

*Quantitation Ion

[†]Internal Standard