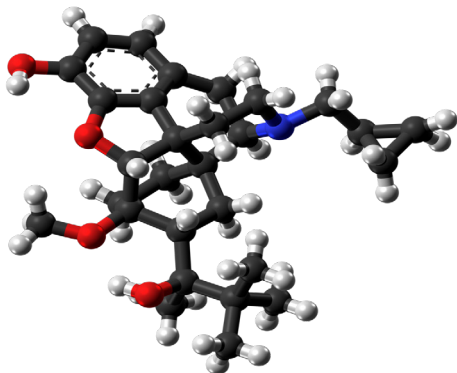


Buprenorphine and Norbuprenorphine In Blood, Urine For GC/MS Confirmations Using: 200 mg Clean Screen® Extraction Column



UCT Part Numbers

CSDAU206
Clean Screen® DAU Extraction
Column 200 mg 6 mL

Procedure:

1. Prepare Sample

- To 1 mL of 100 mM Acetate buffer (pH= 5) add internal standard*. Mix/vortex and add 1 mL of blood, plasma/ serum.
- 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 1-5 mL of blood or 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 Clean Screen® 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 acetic buffer (pH= 5.0).
- 1 x 3 mL Methanol.
- Dry column (5 minutes at > 10 inches Hg).

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 derivitizing efficiency.

6. Dry Eluate

- Evaporate to dryness at <40 °C.

7. Derivatize

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

Note: Do not evaporate BSTFA

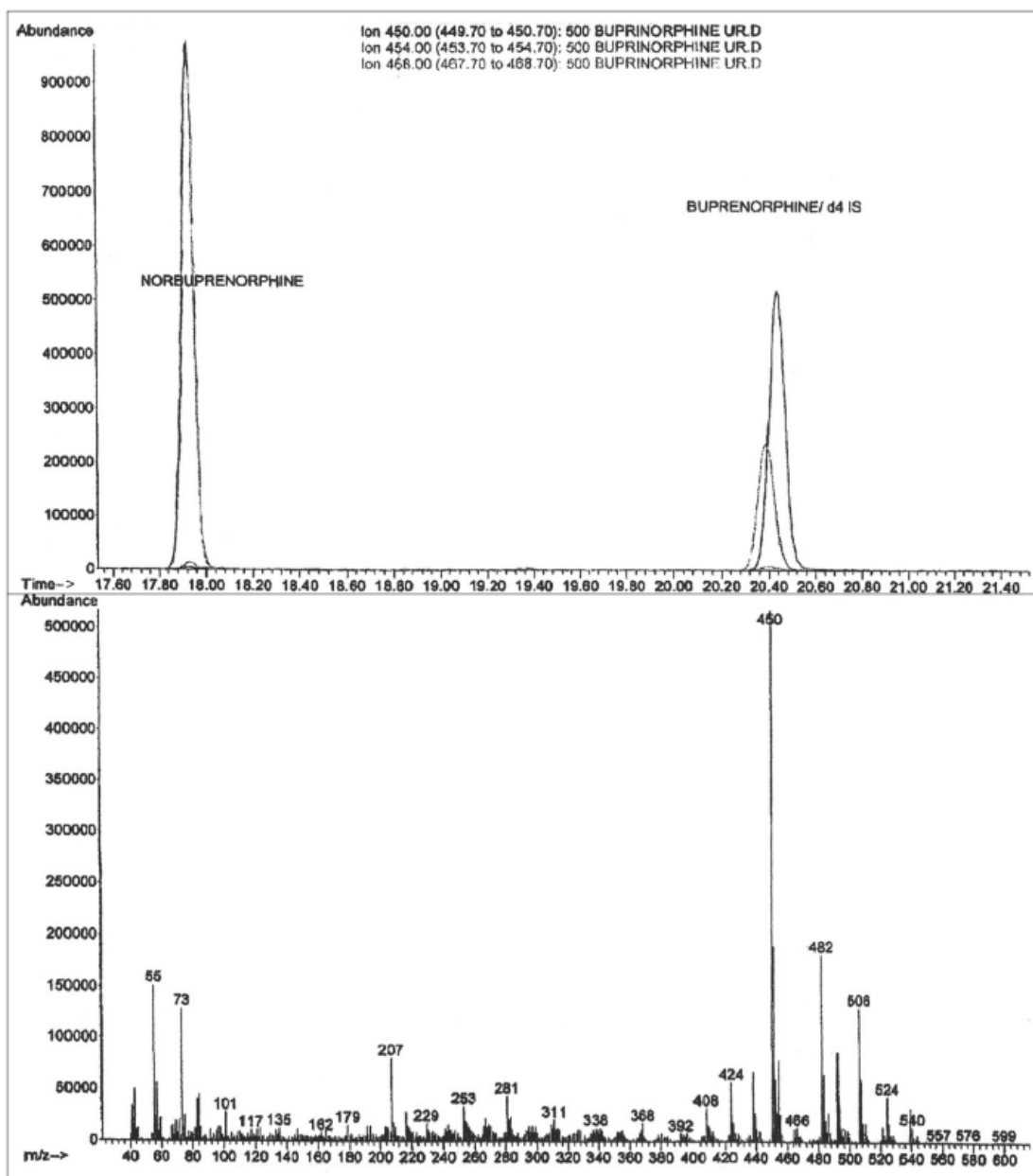
8. Quantitate

- Inject 1 to 2 µL onto gas chromatograph/mass spectrometer
- For MSD monitor the following ions:

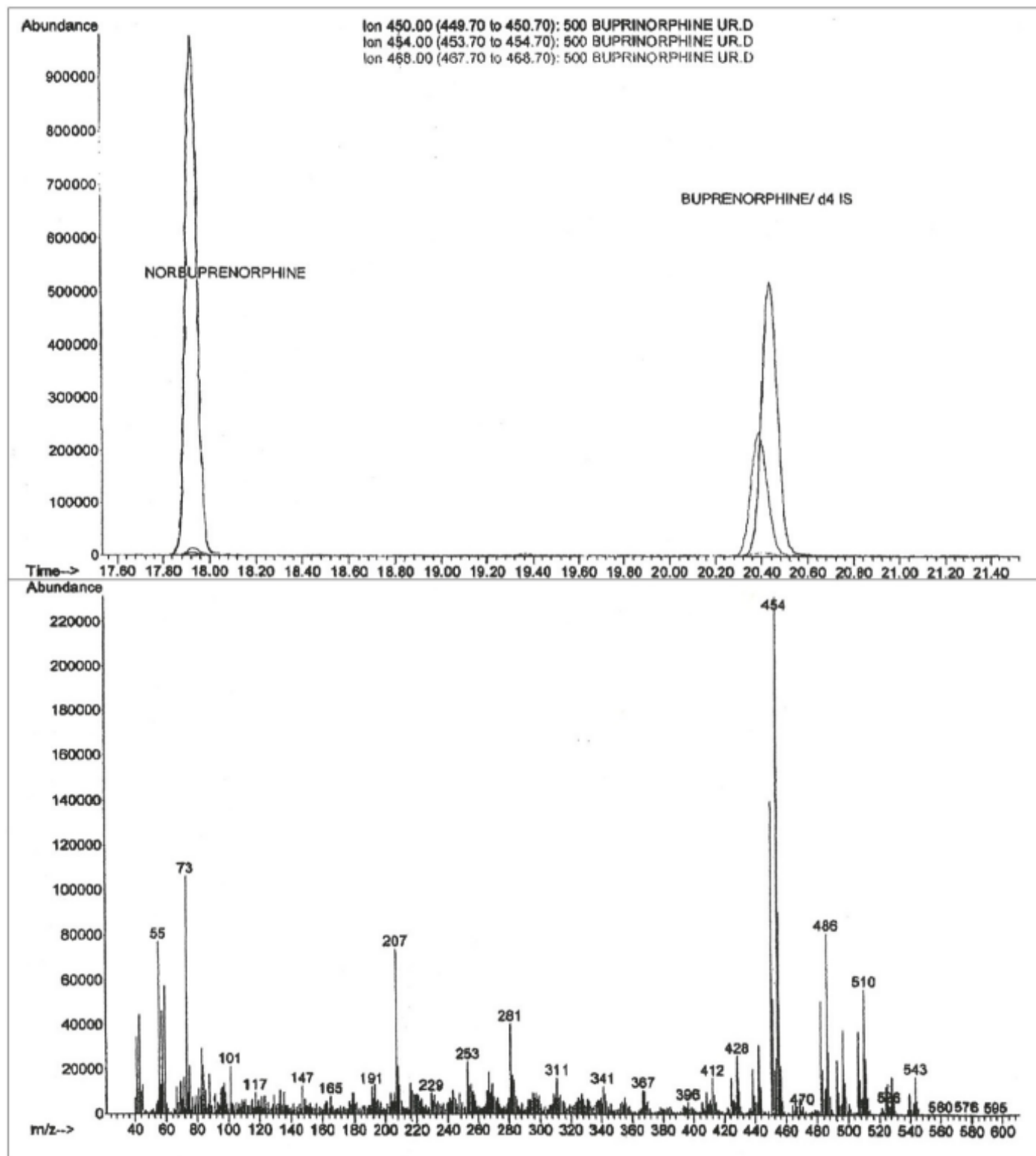


Compound	Primary Ion	Secondary	Tertiary	Cerilliant #
*Buprenorphine-D4-TMS	454	486	510	B-901
Buprenorphine-TMS	450	482	506	B-902
Norbuprenorphine-TMS	468	500	524	N-912
*Norbuprenorphine-D3-TMS	471	503	527	N-920

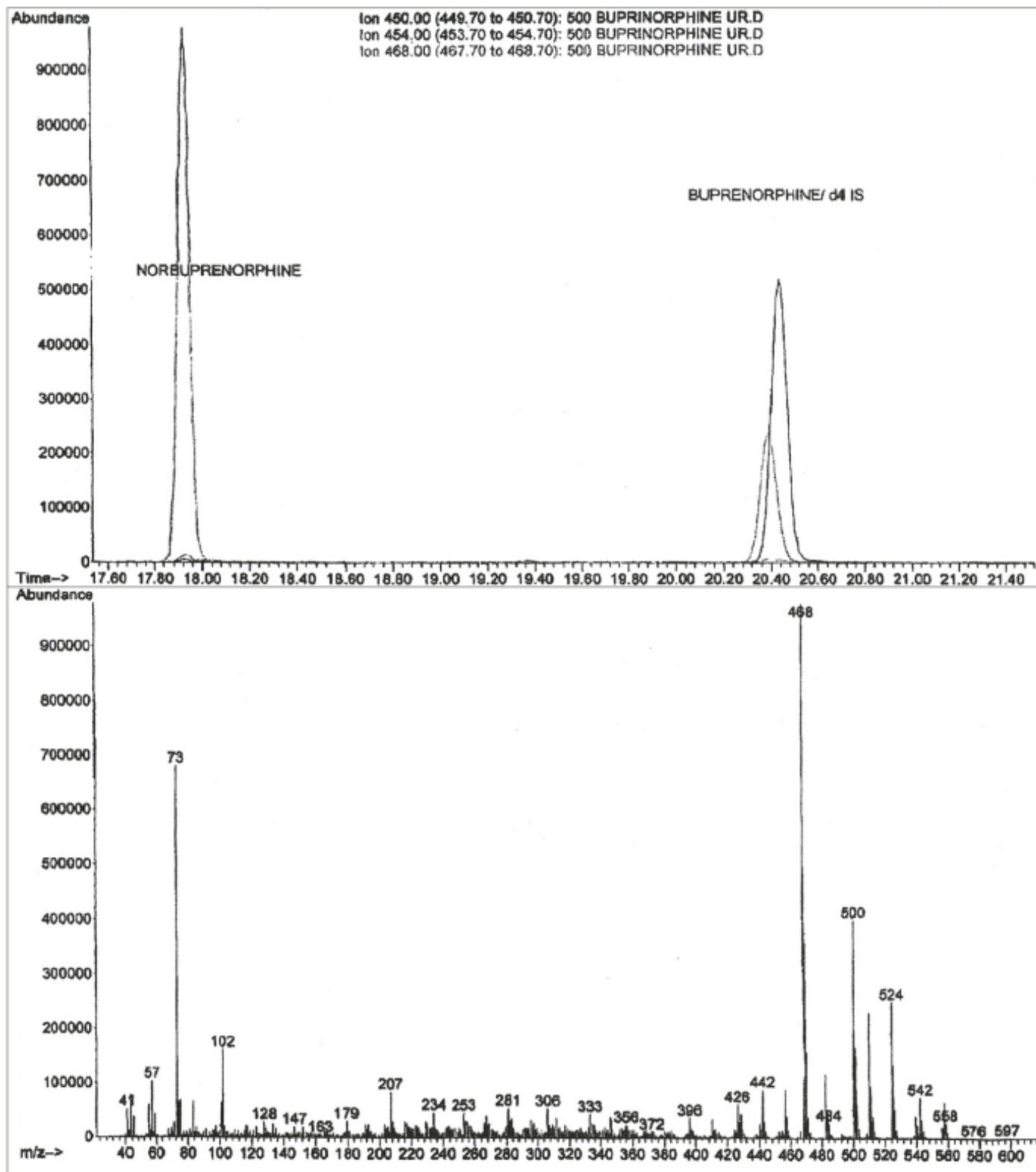
Buprenorphine and Norbuprenorphine Chromatogram I



Buprenorphine and Norbuprenorphine Chromatogram II



Buprenorphine and Norbuprenorphine Chromatogram III



UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255

www.unitedchem.com Email: methods@unitedchem.com

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