BUPRENORPHINE AND NORBUPRENORPHINE IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

CSDAU206 – CLEAN SCREEN® DAU 200 mg, 6 mL Tube

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

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

1. PREPARE SAMPLE:

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
Add 1 mL of blood, plasma/ serum, or 1 g (1:4) tissue homogenate.
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

Urine: PREPARE ENZYME HYDROLYSIS OF GLUCURONIDES:
To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0)
containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated
β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool.
Do not adjust pH~ sample is ready to be added to the extraction column.

2. CONDITION CLEAN SCREEN® EXTRACTION COLUMN:

1 x 3 mL CH_3OH
1 x 3 mL D.I. H_2O
1 x 1 mL 100 mM Acetate buffer (pH 5.0)
NOTE: Aspirate at full vacuum or pressure

3. APPLY SAMPLE:

Load at 1 to 2 mL/minute

4. WASH COLUMN:

1 x 3 mL D.I. H_2O
1 x 3 mL 100 mM acetate buffer (pH 5.0)
1 x 3 mL CH_3OH
Dry column (5-10 minutes at full vacuum or pressure)

5. ELUTE BUPRENORPHINE/NORBUPRENORPHINE:

1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2 v/v)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

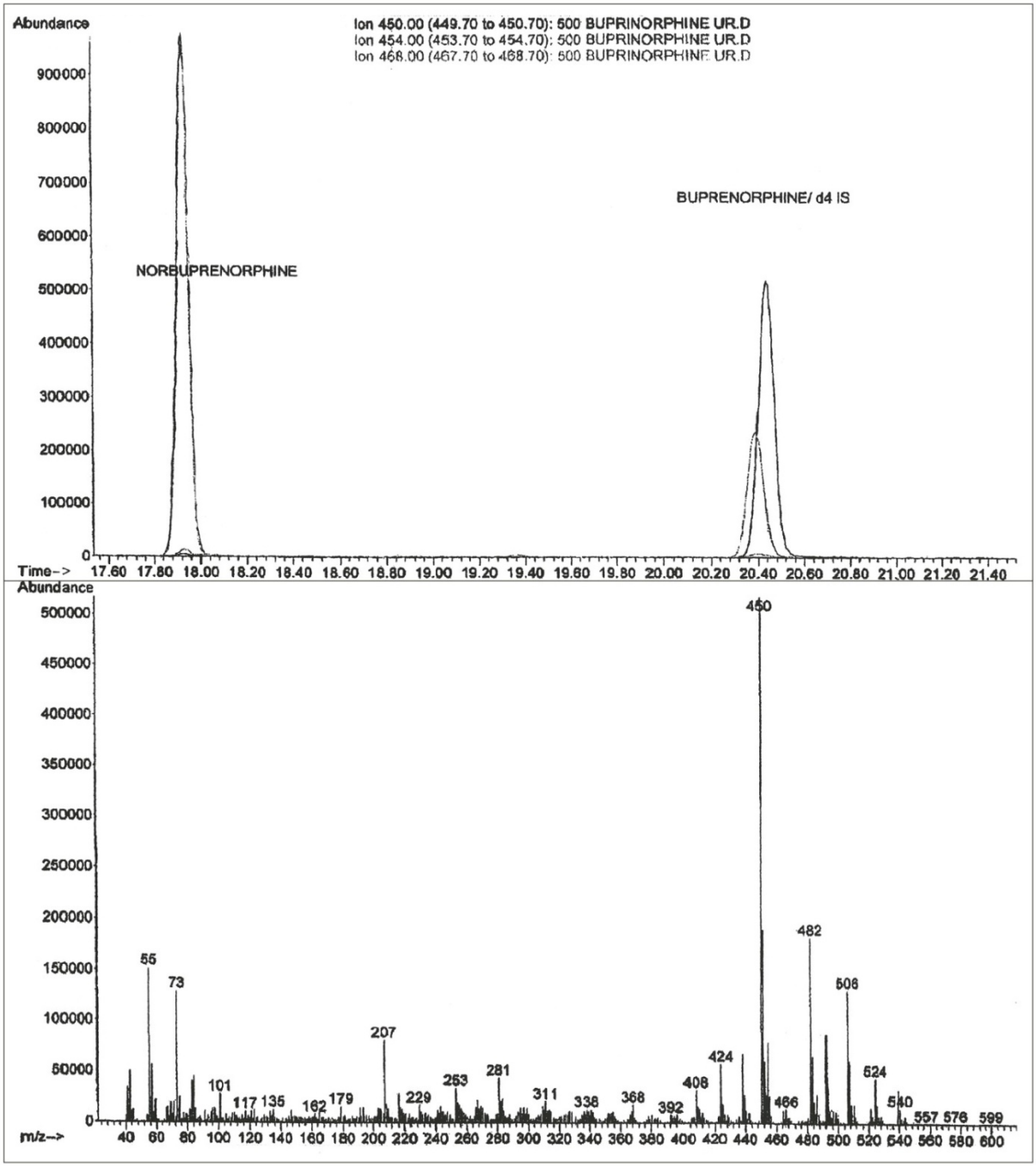
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

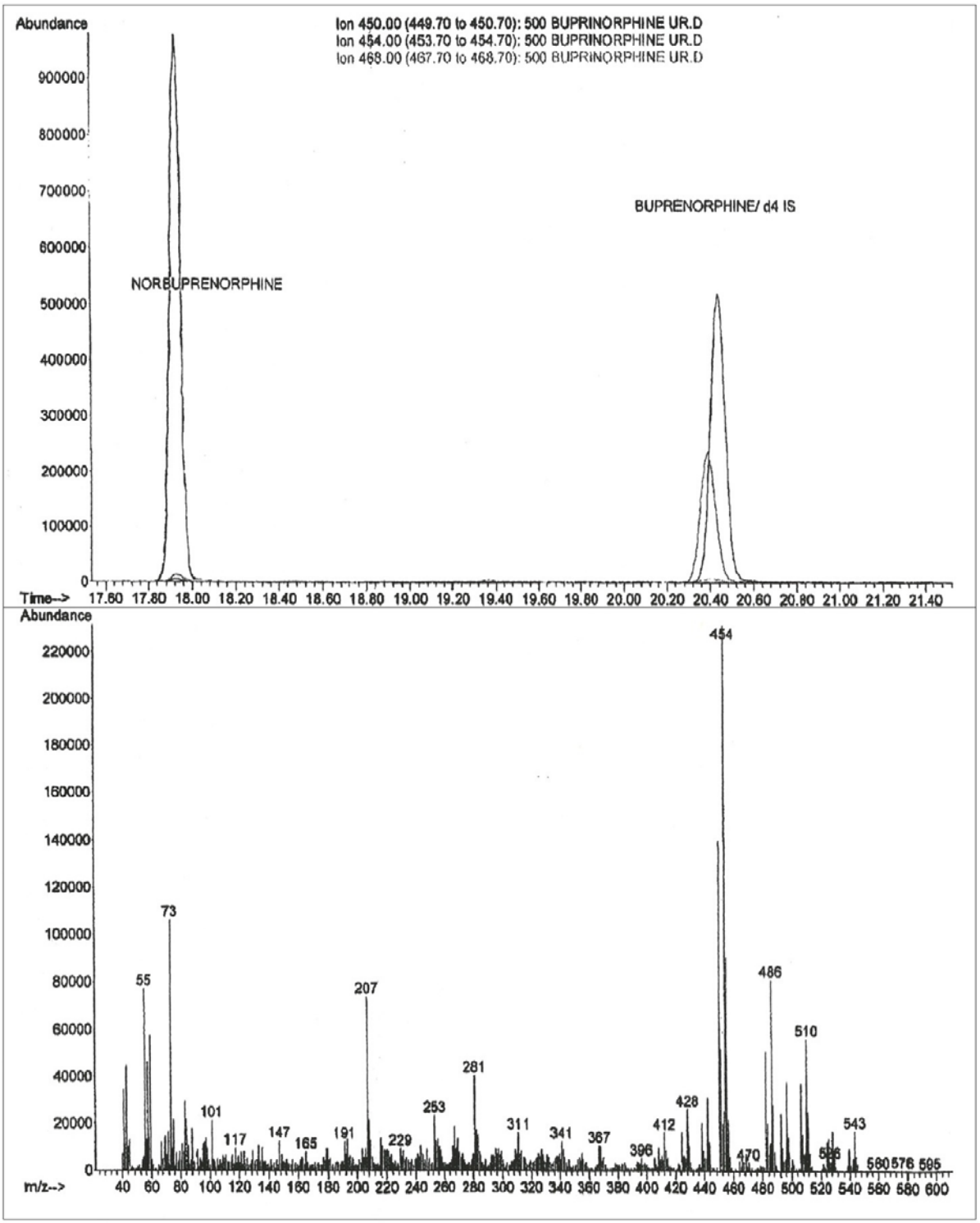
Alternate Derivatization

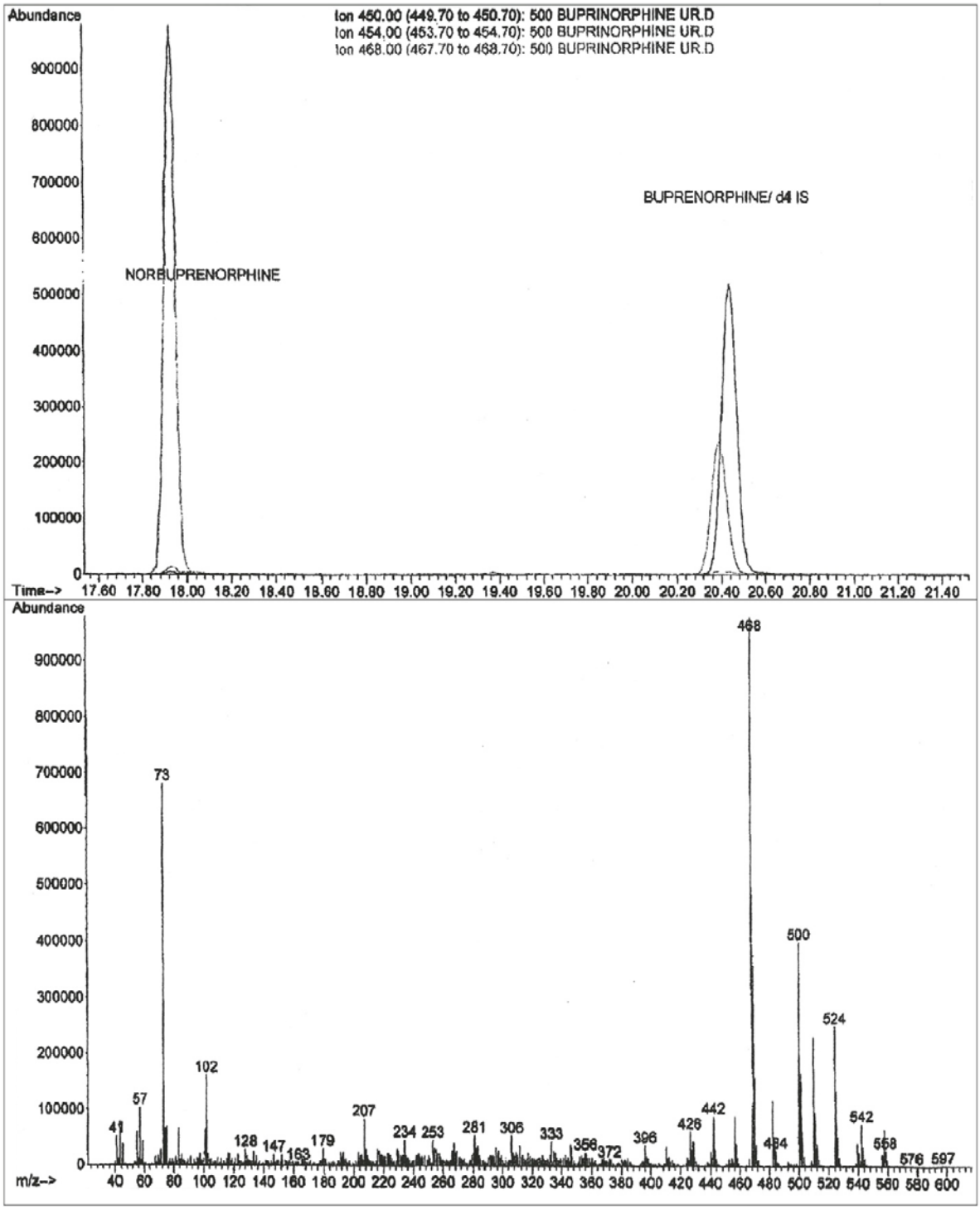
- Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of 50 µL BSTFA w/ 1% TMCS react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAMS




Abundance
Ion 450.00 (449.70 to 450.70): 500 BUPRINORPHINE UR.D
Ion 454.00 (453.70 to 454.70): 500 BUPRINORPHINE UR.D
Ion 468.00 (467.70 to 468.70): 500 BUPRINORPHINE UR.D
NORBUPRENORPHINE
BUPRENORPHINE/ d4 IS
Time-->
Abundance
m/z-->



Analyte	Primary Ion	Secondary Ion	Tertiary Ion
Buprenorphine-D_4-TMS	454	486	510
Buprenorphine-TMS	450	482	506
Norbuprenorphine-TMS	468	500	524
Norbuprenorphine-D_3-TMS	471	503	527