BETA BLOCKERS IN BLOOD OR URINE FOR GC/MS CONFIRMATIONS USING 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #:
ZSDAU020 without Tips
or
ZCDAU020 with CLEAN-THRU® Tips

1. **PREPARE SAMPLE:**
   To 1 mL of Acetate buffer (pH 4.5) add 1 mL of blood or urine. Add 2 mL of Acetate buffer (pH 4.5).
   Mix/vortex
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 3 mL 100 mM Acetate Buffer (pH 4.5).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/ minute.

4. **WASH COLUMN:**
   2 x 1 mL Acetone/ Methanol (1:1) aspirate.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BETA BLOCKERS:**
   1 x 1 mL CH_2Cl_2/ IPA/NH4OH (78:20:2).
   Collect the eluate by gravity.
   NOTE: Prepare elution solvent fresh daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

7. **DERIVATIZE:**
   Derivatization Solution: Methaneboronic acid at 5 mg/mL
   prepared in dry Ethyl Acetate (use molecular sieve).
   Store this solution at –20 °C (freezer conditions) until use.

   Reaction Mixture
   Add 100 µL of the Methaneboronic acid solution (see above).
   Mix/vortex.
   React 15 minutes at 70 °C. Remove from heat source to cool.

   NOTE: Do not evaporate this solution.

8. **ANALYSIS:**
   Inject 1 to 2 µL sample.

Reference:
Branum G, Sweeney S, Palmeri A, Haines L and Huber C
The Feasibility of the Detection and Quantitation of ß Adrenergic Blockers By Solid Phase Extraction and Subsequent Derivatization with Methaneboronic Acid. Journal of Analytical Toxicology 22: 135-141 (1998)

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM

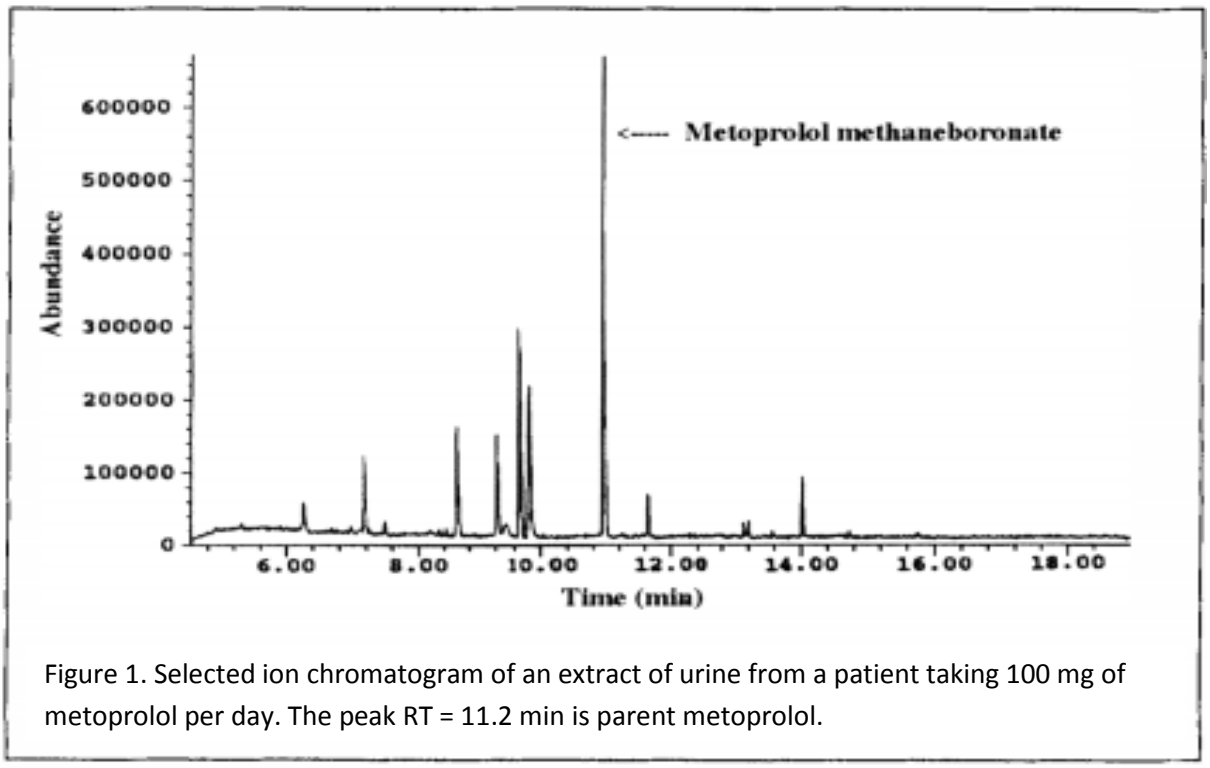

Figure 1. Selected ion chromatogram of an extract of urine from a patient taking 100 mg of metoprolol per day. The peak RT = 11.2 min is parent metoprolol.

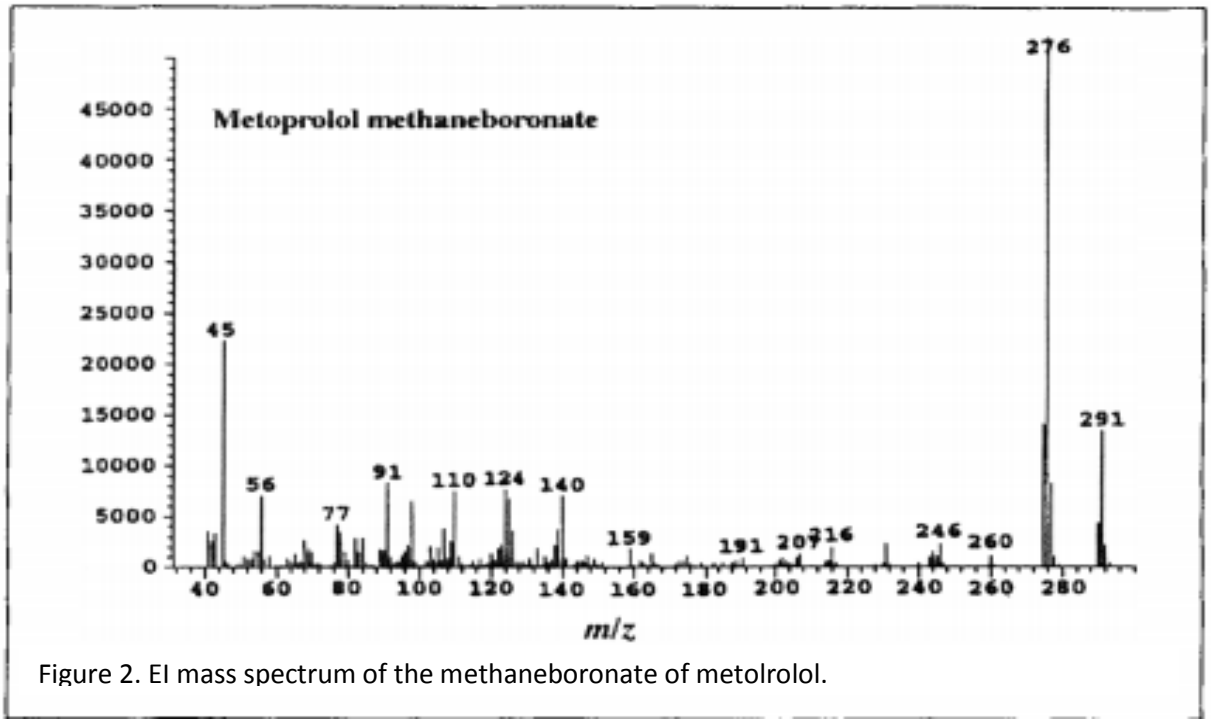

Figure 2. EI mass spectrum of the methaneboronate of metolrolol.

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Acebutolol	246	299	360
Alprenolol	258	273	138
Atenolol	275	290	164
Betaxolol	316	331	246
Bisoprolol	230	334	349
Carteolol	301	316	218
Isoproteronol (IS)	202	244	259
Metoprolol	276	291	140
Propranolol	283	268	128
Soltalol	281	296	239

PARAMETERS

GC/MS: Agilent - 5971/ 5890 GC/MS System with 7683B ALS System

GC capillary column: Rtx-5sil MS 30m X 0.25mm, 0.25µm

Injector: 2µL Splitless, 250°C

Oven temperature program: 110°C for 1 min; 20°C/min to 170°C; 7°C/min to 225°C; 24°C/min to 290°C for 10 min

Carrier gas: Helium