



LC/MS METHOD FOR EXTRACTING ETHYL GLUCURONIDES FROM URINE USING: 200 mg CLEAN SCREEN[®] EXTRACTION COLUMN

Part #: CSETG203

1. PREPARE SAMPLE:

Add 50 μ L of formic acid to 1 mL of urine. (Internal standard EtG -d5 at 200 ng/mL.)

Centrifuge for 10 minutes at 3000 rpm.

Decant solution onto SPE cartridge previously conditioned with 2 mL of 1% formic acid.

Wash sample column with 2 mL DI water and dry at 10 mm Hg for 10 minutes.

Elute the EtG with 2 mL of 1% formic acid/ Methanol solution.

Evaporate to dryness under stream of nitrogen.

Reconstitute with 1 mL of Methanol. The solution

should be filtered through a 0.2 μ m filter for LC/MS analysis.

2. SUGGESTED LC/MS PROCEDURE:

Prepare 1.0 M ammonium acetate buffer by weighing 3.8 g ammonium acetate and dilute to 5L.

(Option: 0.77 g diluted to 1L DI water). This solution should be filtered through 0.2 μ m filter for LC use.

LC Mobile Phase –ammonium acetate/ acetonitrile (10/90) at a flow rate of 0.2 mL/minute.

Injection Volume – 10 mL.

Detection Limit – 10 ng/mL

3. SUGGESTED LC/MS/MS PARAMETERS:

Tuning the MS:

Tune MS using PPG

Tune MS using 500 ng/mL EtG, mobile phase 0.2 mL/min, EtG solution 1 μ L/min. Optimize ion source and mass analyzer to signal 221 m/z. Determine the collision voltage for ion 75 m/z and reference ions 85 and 113 m/z. Tune file uses scan rate of 0.3 s; acquisition time 6 minutes.

Quantifier ion is 75 and qualifier ions are 85 and 113. Collision voltage 75(16), 85(16) / and 113 (14.5).

NOTES:

The prepared buffer should be filtered 30-45 minutes (equilibrated) before analysis for constant results.

After sample elution from the column, the LC must be programmed to flush the column using an acetonitrile / DI water gradient (50/50 to 90/10) to avoid carryover from previous specimen.

ETHYL GLUCURONIDES CHROMATOGRAM

