LC/MS Method For Extracting Ethyl Glucuronides From Urine Using: 200 mg Clean Screen® Extraction Column

UCT Part Numbers

CSETG203

Clean Screen® ETG 200 mg in a 3 mL SPE cartridge

Procedure:

1. Prepare Sample

- a) Add 50 µL of formic acid to 1 mL of urine. (Internal standard EtG –d5 at 200 ng/mL.)
- b) Centrifuge for 10 minutes at 3000 rpm.
- c) Decant solution onto SPE cartridge previously conditioned with 2 mL of 1% formic acid.
- d) Wash sample column with 2 mL DI water and dry at 10 mm Hg for 10 minutes.
- e) Elute the EtG with 2 mL of 1% formic acid/ Methanol solution.
- f) Evaporate to dryness under stream of nitrogen.
- g) Reconstitute with 1 mL of Methanol. The solution should be filtered through a 0.2 µm filter for LC/MS analysis.

2. Suggested LC/MS Procedure

- a) Prepare 1.0 M ammonium acetate buffer by weighing 3.8 g ammonium acetate and dilute to 5L.
- b) (Option: 0.77 g diluted to 1L DI water). This solution should be filtered through 0.2 µm filer for LC use.
- c) LC Mobile Phase –ammonium acetate/ acetonitrile (10/90) at a flow rate of 0.2 mL/minute.
- d) Injection Volume 10 mL.
- e) Detection Limit 10 ng/mL.

3. Suggested LC/MS/MS Parameters

Tuning the MS:

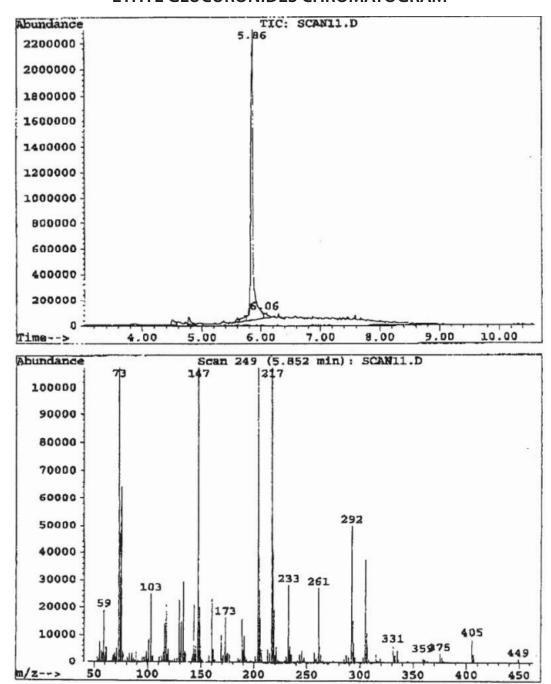
- a) Tune MS using PPG
- b) Tune MS using 500 ng/mL EtG, mobile phase 0.2 mL/min, EtG solution 1 μL/min. Optimize ion source and mass analyzer to signal 221 m/z. Determine the collision voltage for ion 75 m/z and reference ions 85 and 113 m/z. Tune file uses scan rate of 0.3 s; acquisition time 6 minutes.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



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