CAFFEINE, THEOPHYLLINE AND THEOBROMINE IN BLOOD, PLASMA/SERUM, AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020

LC-PDA

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM acetic acid add internal standard.*
   Add 1 mL Blood, Serum/ Plasma, or Urine. Add 2 mL of 100 mM acetic acid.
   Vortex and centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM acetic acid.
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

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

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE CAFFEINE/THEOBROMINE/THEOPHYLLINE:**
   1 x 3 mL Ethyl Acetate : Methanol (90:10)
   Collect eluate at 1-2 mL / minute.

6. **EVAPORATION:**
   Combine eluates
   Evaporate eluates under a gentle stream of nitrogen < 40 °C.

7. **RECONSITITUE: sample in 1000 µL of 0.1 % Formic Acid (aq).**
   Inject 20 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) Gold C_{18} (Thermofisher)

Mobile phase: Acetonitrile: 0.1% Formic Acid aqueous (10:90).

Flowrate: 0.1 mL/ minute

Column Temperature: ambient

Detector: Diode Array (200-350 nm)

CHROMATOGRAM OF SHOWING:

Compound

Theobromine: 7.5 minutes

Theophylline: 9.5 minutes

Caffeine: 14.5 minutes

*8-Chlorotheophylline: 18.0 minutes