EXTRACTION OF ANTICOAGULANTS FROM URINE USING CEC30 SORBENT IN 96 WELL PLATE FORMAT

LC-MS/MS

April 13, 2011

1. **PREPARE SAMPLE:**
   To 200 µL of 100 mM phosphate buffer (pH 6) add internal standard.*

   Vortex mix

   Add 100-500 µL of urine and vortex mix

   Add 200 µL of 100 mM phosphate buffer and vortex mix.

   Centrifuge as appropriate

2. **CONDITION 96 WELL PLATES:**
   1 x 1 mL CH_3OH

   1 x 1 mL D.I. H_2O

   1 x 0.5 mL 100 mM phosphate buffer (pH 6)

   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

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

4. **WASH 96 WELL PLATES:**
   1 x 1 mL 100 mM phosphate buffer (pH 6).

   Dry column (5 minutes at > 10 inches Hg).

   1 x 1 mL Hexanes

   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE ANTICOAGULANTS:**
   2 x 2 mL of ethyl acetate: methanol (75:25)

   Collect eluate at 1-2 mL /minute.

6. EVAPORATION:

Evaporate eluates to dryness under a gentle stream of nitrogen (<40ºC)

Alternative:

Transfer eluate to a clean glass tube with the aid of a disposable glass pipette.

Wash 96 well collection rack with 1 x 1 mL of elution solvent.

Transfer washing to glass tube containing eluate

Evaporate eluates to dryness under a gentle stream of nitrogen (<40ºC)

7. LC-MS/MS

Reconstitute sample in 100 µL CH_3OH Inject 5 µL.
Column: 50 x 2.1 (3 µm) SELECTRA Phenyl (UCT, Inc.)

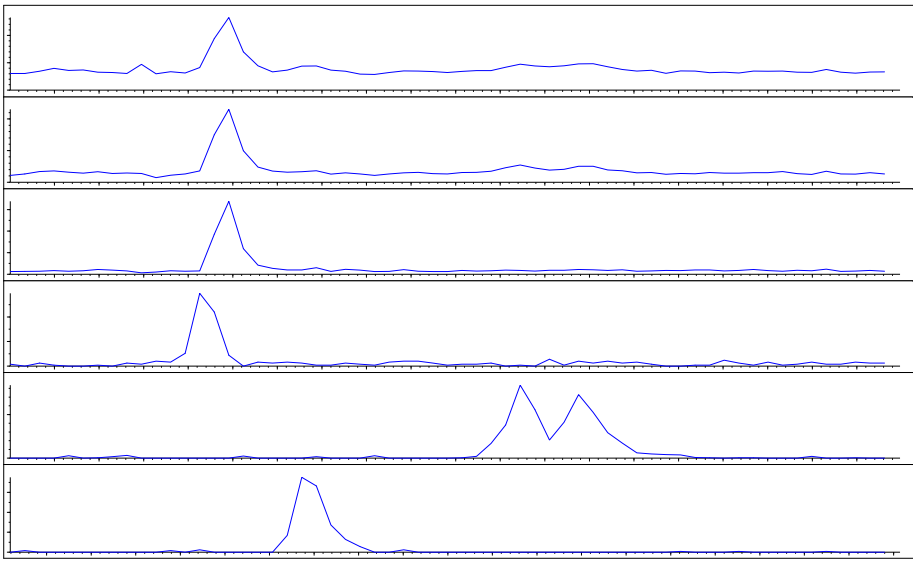
MOBILE PHASE

Time / min	% Acetontitrile	%0.1% Formic Acid
0	60	40
10	60	40

Flowrate: 0.3 mL/minute.
Column Temperature: 40ºC.
Detector: API 4000 QTRAP MS/MS

Compound	(+) MRM Transition	(-) MRM Transition
Warfarin	309.1/163	306.9/161
*p-Chlorowarfarin	343.1/163	340.9/160.0
Broadificoum	524.3/256	520.8/78
Bromodione	527.8/250.5	524.7/249
Coumatetralyl	293.8/175.0	
Flocoumafen	542.4/159.1	

CHROMATOGRAM OF ANTICOAGULANTS
(500 ng) extracted from urine (500 µL)



Recovery > 90%

Matrix effects < 10%

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