

# Extraction of Anticoagulants From Urine Using CEC30 Sorbent In 96 Well Plate Format

## UCT Part Numbers

**CEC30111**

Clean-Up C30 - Endcapped  
100 mg 1 mL

**VMF96WP**

Well Plate Manifold System - 96



## Procedure:

### 1. Prepare Sample

- To 200  $\mu\text{L}$  of 100 mM phosphate buffer ( $\text{pH} = 6$ ) add internal standard.\*
- Vortex/mix
- Add 100-500  $\mu\text{L}$  of urine and vortex/mix
- Add 200  $\mu\text{L}$  of 100 mM phosphate buffer and vortex/mix
- Centrifuge as appropriate

### 2. Condition 96 Well Plates

- 1 x 1 mL  $\text{CH}_3\text{OH}$
  - 1 x 1 mL D.I.  $\text{H}_2\text{O}$
  - 1 x 0.5 mL 100 mM phosphate buffer ( $\text{pH} = 6$ )
- Note:** Aspirate at < 3 inches Hg to prevent sorbent drying.

### 3. Apply Sample

- Load sample at 1 to 2 mL/minute

### 4. Wash 96 Well Plates

- 1 x 1 mL 100 mM phosphate buffer ( $\text{pH} = 6$ )
- 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

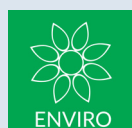
- Evaporate eluates to dryness under a gentle stream of nitrogen (<40°C)

**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 (<40°C)

### 7. LC-MS/MS

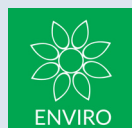
- Reconstitute sample in 100  $\mu\text{L}$   $\text{CH}_3\text{OH}$ . Inject 5  $\mu\text{L}$



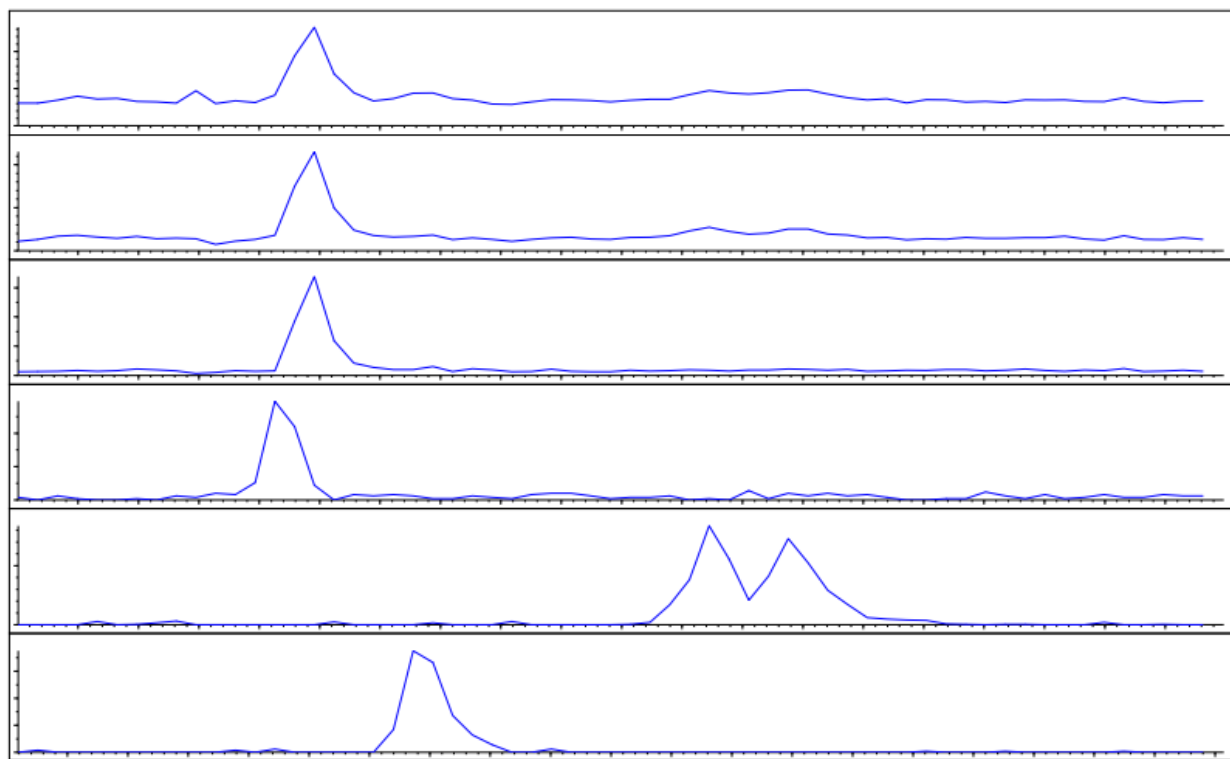
| Instrument Conditions (LC-MS/MS) |   |
|----------------------------------|---|
| Column                           | 50 x 2.1 (3 µm) SELECTRA Phenyl (UCT, Inc.) |
| Column Temperature               | 40°C  |
| Flowrate                         | 0.3 mL/minute                               |
| Detector                         | API 4000 QTRAP MS/MS                        |

| Mobile Phase |                |                     |
|--------------|----------------|---------------------|
| Time (min)   | % Acetonitrile | % 0.1 % Formic Acid |
| 0            | 60             | 40                  |
| 10           | 60             | 40                  |

| Compound          | (+) MRM Transition | (-) MRM Transition |
|-------------------|--------------------|--------------------|
| Warfarin          | 309.1/163          | 306.9/161          |
| *p-Chlorowarfarin | 343.1/163          | 340.9/160.0        |
| Broadifcoum       | 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 $\mu$ L)



Recovery > 90%

Matrix effects < 10%

**DCN-113140-215**

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