



**WARFARIN IN WHOLE BLOOD:  
MANUAL METHOD FOR GC-MS OR LC CONFIRMATIONS  
USING: 200 mg CLEAN-UP® C-30 EXTRACTION COLUMN**  
Part #: CEC30203

**1. PREPARE SAMPLE**

To 9 mL of 100 mM phosphate buffer (pH= 6.0) add internal standard. Add 1mL of whole blood) and Mix/vortex.

Sample pH should be 6.0 + 0.5.

Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.

Centrifuge as appropriate

**2. CONDITION EXTRACTION COLUMN**

1 x 3 mL CH<sub>3</sub>OH

1 x 3 mL DI H<sub>2</sub>

1 x 3 mL 100 mM phosphate buffer, (pH=6.0) aspirate.

**NOTE:** Aspirate at < 3 inches. Hg to prevent sorbent drying.

**3. APPLY SAMPLE**

Load at 1-2 mL/min.

**4. WASH COLUMN**

Add 1 x 3 mL of phosphate buffer (0.1 M pH 6)

Dry under full vacuum for 10 mins

Add 1 x 3 mL of Hexane

Dry under full vacuum for 10 mins

**5. ELUTE WARFARIN:**

Add 2 x 3 mL of methanol/ ethyl acetate (12:88)

**Note:** Prepare elution solvent daily.

**6. Collect eluates at approx 1-2 mL/minute**

**7. Dry samples**

Evaporate to dryness at <40°C

Add 50 µL of ethyl acetate.

Add 50 µL of TMAH, and vortex.

React at for 1 hour at 70°C.

Cool and inject 1-2 µL onto GC-MS

Monitor the following ions:

<u>Compound</u>	<u>Primary</u>	<u>Secondary</u>	<u>Tertiary</u>	<u>Cerilliant #</u>
Warfarin	279	322	280	W-003
*p-chlorowarfarin	313	315	356	

# WARFARIN CHROMATOGRAM

GC-MS (methylation)

