



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

Part #:

CEC30203 – CLEAN-UP[®] C30 200 mg, 3 mL Tube

STMPAH-0-1 – SELECTRA-SIL[®] TMPAH

1. PREPARE SAMPLE:

To 9 mL of 100 mM phosphate buffer (pH 6.0.0) 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 CLEAN-UP[®] COLUMN:

1 x 3 mL CH₃OH
1 x 3 mL D.I. H₂O
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>
Warfarin	279	322	280
p-chlorowarfarin (internal standard)	313	315	356

WARFARIN CHROMATOGRAM

GC-MS (methylation)

