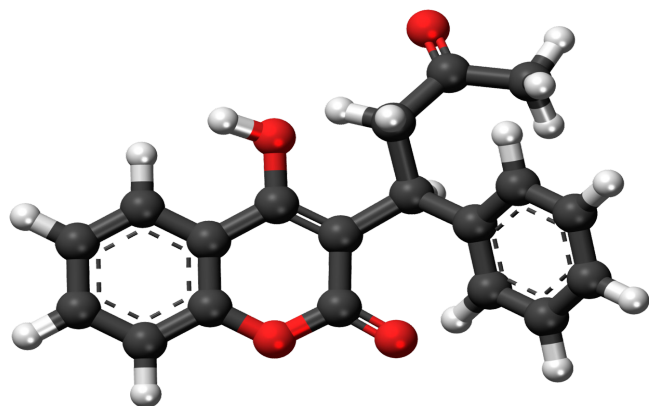


Warfarin In Whole Blood: Manual Method For GC-MS OR LC Confirmations Using: 200mg Clean-Up® C-30 Extraction Column



UCT Part Numbers

CEC30123
200 mg CLEAN-UP®
C-30 Extraction Column

Procedure:

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 CH₃OH
 - 1 x 3 mL DI H₂O
 - 1 x 3 mL 100 mM phosphate buffer, (pH=6.0) aspirate.
- Note:** Aspirate at < 3 inches. Hg to prevent sorbent drying out.

3. Apply Sample

- Load sample at 1-2 mL / minute.

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

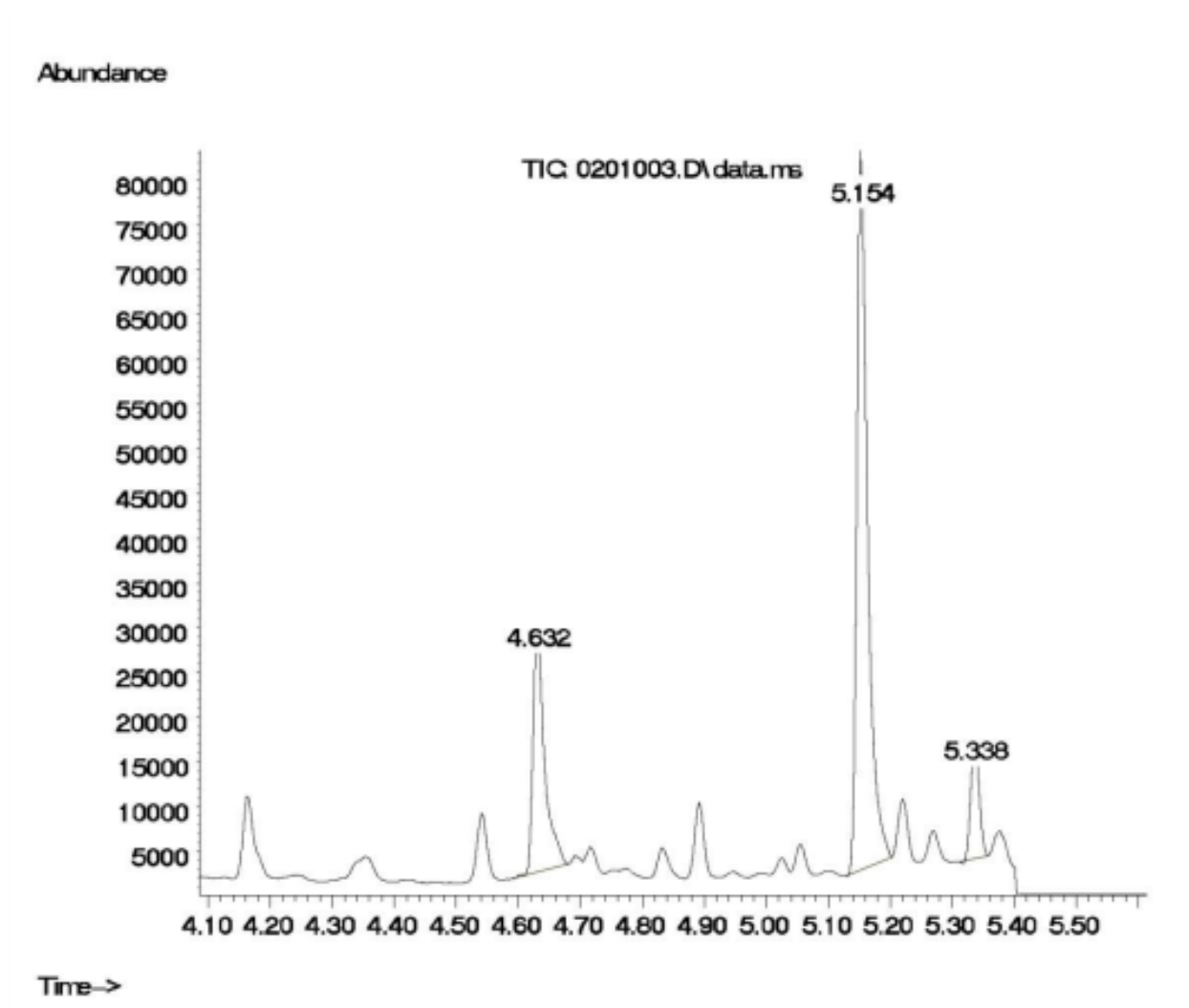
- Evaporate to dryness at < 40°C.
- Add 50 µL of ethyl acetate.
- Add 50 µL of TMAH, and vortex.
- React at for 1 hour at 70 °C.
- Cool and inject 1-2 µL onto GC-MS
- Monitor the following ions:

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



Warfarin Chromatogram

GC-MS (methylation)



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