$C_{19}H_{16}O_4$ MW: 308.33 CAS: 81-81-2 RTECS: GN4550000

METHOD: 5002, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 February 1984**
Issue 2: 15 August 1994

OSHA : 0.1 mg/m^3
NIOSH: 0.1 mg/m^3; Group I Pesticide
ACGIH: 0.1 mg/m^3; STEL 0.3 mg/m^3

PROPERTIES: solid, MP 161 °C; VP not available

SYNONYMS: 3-(α-acetonylbenzyl)-4-hydroxycoumarin.

SAMPLING

SAMPLER: FILTER (1-µm PTFE membrane)

FLOW RATE: 1 to 4 L/min

VOL-MIN: 200 L @ 0.1 mg/m^3
-MAX: 1000 L

SHIPMENT: routine

SAMPLE STABILITY: 93% recovery after 7 days @ 25 °C [1]

BLANKS: 2 to 10 field blanks per set

BULK SAMPLE: desirable; 1 to 5 g

ACCURACY

RANGE STUDIED: 0.054 to 0.24 mg/m^3 [1] (408-L samples)

BIAS: none identified [1]

OVERALL PRECISION ($\hat{S}_{rT}$): 0.056 [1]

ACCURACY: ± 11.0%

MEASUREMENT

TECHNIQUE: HPLC, UV DETECTION

ANALYTE: Warfarin

EXTRACTION: 5 mL methanol; swirl

INJECTION VOLUME: 20 µL

MOBILE PHASE: 30% 0.0025 N phosphoric acid and 70% methanol, isocratic, 1.5 mL/min, ambient temperature

COLUMN: C_{18} reverse phase, 10-µm packing, 25 to 30 cm

DETECTOR: UV absorption @ 280 nm

CALIBRATION: standard solutions of Warfarin in methanol

RANGE: 20 to 200 µg per sample

ESTIMATED LOD: 2.5 µg per sample

PRECISION ($\bar{S}_r$): 0.016 [1]

APPLICABILITY: The working range is 0.05 to 0.5 mg/m^3 for a 400-L air sample.

INTERFERENCES: No interferences have been studied.

OTHER METHODS: This is P&CAM 313 [2] in a new format. Analytical methods for Warfarin in some rodenticides [3] and in drugs [4] are available; these are spectrophotometric methods for bulk materials.

REAGENTS:

1. Warfarin, 99%.*
2. Methanol, HPLC grade.*
3. Water, HPLC grade.
4. Phosphoric acid, 85%.*
5. Calibration stock solution, 2 mg/mL.* Dissolve 20.0 mg Warfarin in 10 mL methanol.
6. Mobile phase component, 0.0025 N phosphoric acid. Dissolve 0.06 mL 85% phosphoric acid in 1 L water.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: 37-mm diameter PTFE membrane filter, 1-µm pore size, and cellulose backup pad in two-piece filter holder held together with tape or shrinkable band.
2. Personal sampling pump, 1 to 4 L/min, with flexible connecting tubing.
3. High pressure liquid chromatograph with 20 to 50-µL injection loop; 25 to 30 cm, 10-µm C_{18} column; UV absorption detector at 280 nm; and integrator.
4. Jars, squat form, 60-mL, ointment, with PTFE film gaskets and screw caps.
5. Tweezers.
6. Pipet, 5-mL, and pipet bulb.
7. Syringes, microliter.
8. Volumetric flasks, 10-mL and 1-L.

SPECIAL PRECAUTIONS: Methanol is flammable and toxic. Use in fume hood and away from ignition sources.

Warfarin is toxic and can be absorbed through the skin.

Phosphoric acid is corrosive. Use gloves, goggles and other appropriate equipment to prevent eye contact and repeated or prolonged skin contact. Wash skin with water and change clothes if contact occurs.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Sample at an accurately know flow rate between 1 and 4 L/min for a sample size of 200 to 1000 L. Do not exceed a total loading of 2 mg on the filter.

SAMPLE PREPARATION:

3. Transfer filter with tweezers to jar.
4. Add 5.0 mL methanol. Cap the jar. Swirl to wet filter thoroughly.

CALIBRATION AND QUALITY CONTROL:

5. Calibrate daily with at least six working standards covering the range 4 to 120 µg Warfarin per sample.
   a. Add aliquots (2 to 60 µL) of calibration stock solution to 5 mL methanol in ointment jars.
   b. Analyze the working standards together with the samples and blanks (steps 7 and 8).
      NOTE: Duplicate injections should differ by no more than 3% in peak area.
   c. Prepare a calibration graph (peak area vs. µg Warfarin).
6. Determine recovery at least once in the calibration range for each lot of filters. Prepare three filters at each of five levels plus three media blanks.
   a. Inject known volumes of calibration stock solution onto blank filters with a microliter syringe. Include media blanks.

b. Allow the filters to air dry overnight.
c. Analyze the filters (steps 3, 4, 6 and 7).
d. Prepare a graph of recovery vs. µg of Warfarin recovered.

MEASUREMENT:

7. Set liquid chromatograph according to manufacturer's recommendations and to conditions given on page 5002-1.
8. Make duplicate injections of each sample and working standard. Measure peak area.

CALCULATIONS:

9. Read the mass, µg (corrected for recovery) corresponding to the sample peak area of the sample (W) and average media blank (B) from the calibration graph.
10. Calculate the concentration, C (mg/m^3), of Warfarin in the volume of air sampled, V (L):

$$C = \frac{W - B}{V}, \ mg/m^3.$$

EVALUATION OF METHOD:

Method P&CAM 313 was issued on April 13, 1979 [2]. Lab testing was done with spiked samples and atmospheres dynamically generated by Wright dust feeder from commercial formulation; no adequate independent method was used for verification [1,5]. Storage stability was 93.5% for 60-µg samples stored seven days at ambient conditions. Collection efficiency = 100% for 408-L samples at 0.24 mg/m^3; no evidence of vapor in Tenax backup. Precision was as given on page 5002-1.

REFERENCES:

[1] Backup Data Report, P&CAM 313, prepared under NIOSH Contract 210-76-0123 (unpublished, April, 1979).
[2] NIOSH Manual of Analytical Methods, 2nd. ed., V. 6, P&CAM 313, U.S. Department of Health and Human Services, Publ. (NIOSH) 80-125 (1980).
[3] Horwitz, W., Ed. Official Methods for Analysis of the AOAC, 13th ed., p. 85 (1980).
[4] Ibid, 628.
[5] NIOSH Research Report-Development and Validation of Methods for Sampling and Analysis of Workplace Toxic Substances, U.S. Department of Health and Human Services, Publ. (NIOSH) 80-133 (1980).

METHOD REVISED BY:

James E. Arnold, NIOSH/DPSE; P&CAM 313 developed under NIOSH Contract 210-76-0123.