$C_{23}H_{22}O_6$ MW: 394.43 CAS: 83-79-4 RTECS: DJ2800000

METHOD: 5007, Issue 2 **EVALUATION: FULL** **Issue 1: 15 February 1984**
Issue 2: 15 August 1994

OSHA : 5 mg/m^3
NIOSH: 5 mg/m^3; Group II Pesticide
ACGIH: 5 mg/m^3

PROPERTIES: solid, MP 163 °C or 181 °C; BP 220 °C @ 0.5 mm Hg; d ca. 1 g/cm^3; VP not significant

SYNONYMS: tubatoxin; cube

SAMPLING

SAMPLER: FILTER (1-mm PTFE membrane)

FLOW RATE: 1 to 4 L/min

VOL-MIN: 8 L
-MAX: 400 L

SHIPMENT: routine

SAMPLE STABILITY: at least 7 days @ 25 °C in dark

BLANKS: 2 to 10 field blanks per set

BULK SAMPLE: desirable; 1 g

ACCURACY

RANGE STUDIED: 1 to 11 mg/m^3 [1] (100-L sample)

BIAS: - 0.6%

OVERALL PRECISION ($\hat{S}_{rT}$): 0.079

ACCURACY: ± 13.5%

MEASUREMENT

TECHNIQUE: HPLC; UV DETECTION

ANALYTE: Rotenone

EXTRACTION: 4 mL acetonitrile; 30 min

INJECTION VOLUME: 10 µL

MOBILE PHASE: 60% methanol/40% water, 2 mL/min

DETECTOR: UV @ 290 nm; 0.1A full-scale; 1-cm cell

COLUMN: µ-Bondapak C_{18} (30 cm x 3.9-mm ID stainless steel); ambient temperature

CALIBRATION: solutions of Rotenone in acetonitrile

RANGE: 0.04 to 1 mg per sample

ESTIMATED LOD: 4 µg per sample [1,2]

PRECISION ($\bar{S}_r$): 0.024 [1]

APPLICABILITY: The working range is 0.4 to 10 mg/m^3 for a 100-L air sample and the method is applicable to commercial formulations.

INTERFERENCES: None known. Rotenone, a naturally occurring insecticide, is adequately separated by HPLC from other compounds (e.g., sumatrol, α-toxicarol, deguelin, elliptone, malaccol, and tephrosin [3]) present in commercial cube root extracts [4]. Rotenone is sensitive to photodecomposition.

OTHER METHODS: This is Method S300 [2] in a revised format.

REAGENTS:

1. Acetonitrile, HPLC grade.*
2. Methanol, HPLC grade.
3. Rotenone, 97% purity.
4. Water, distilled, HPLC grade.
5. Calibration stock solution, 3 mg/mL. Dissolve 0.075 g Rotenone in 25 mL acetonitrile. Prepare fresh daily in duplicate.
6. Recovery stock solution, 50 mg/mL. Dissolve 0.500 g Rotenone in acetone. Dilute to 10 mL. Prepare fresh daily.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: 37-mm, two-piece cassette containing 1-µm PTFE membrane filter with backup pad.
   NOTE: Use an opaque cassette or otherwise shield the filter from light to minimize photodecomposition of Rotenone during and after sampling.
2. Personal sampling pump, 1 to 4 L/min, with flexible connecting tubing.
3. HPLC, UV detector, integrator and column (page 5007-1).
4. Jars, ointment, 60-mL, with PTFE-lined caps.
5. Vials, 4-mL, with PTFE-lined caps.
6. Syringes, 5-mL.
7. Filtration device, Swinney 13-mm with 1-µm PTFE filters, or PTFE syringe filters.
8. Volumetrics, 10- and 25-mL.
9. Syringes, microliter, for sample injection and standard preparation.
10. Pipet, 4-mL, with pipet bulb.

SPECIAL PRECAUTIONS: Avoid breathing acetonitrile vapors; may cause skin irritation.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Sample at an accurately known flow rate between 1 and 4 L/min for a total sample size of 8 to 400 L. Do not exceed 2 mg total dust loading on the filter.
3. Collect a bulk sample (1 g) in a glass vial with PTFE-lined cap; ship separately from filters.

SAMPLE PREPARATION:

4. Open filter cassette; transfer filter to ointment jar.
5. Add 4.0 mL acetonitrile; gently swirl for 30 min.
6. Filter each sample using a 5-mL syringe with PTFE syringe filter or Swinney filtration device. Deliver filtrate to a 4-mL vial.

CALIBRATION AND QUALITY CONTROL:

7. Prepare at least six working standards daily in the range 0.01 to 1 mg Rotenone per sample.
   a. Add known amounts of calibration stock solution to acetonitrile in 10-mL volumetric flasks and dilute to the mark.
   b. Analyze together with samples and blanks (steps 9 and 10).
   c. Prepare calibration graph (peak area vs. mg Rotenone).
8. Check recovery (R) with at least three spiked media blanks per sample set in the calibration range (step 7).
   a. Add aliquots of recovery stock solution to blank filters with a microliter syringe. Air dry.
   b. Analyze together with working standards (steps 4 through 6, 9 and 10).
   c. Calculate recovery [(mg recovered - mg blank)/mg added].
   d. Prepare recovery graph (R vs. mg Rotenone).

MEASUREMENT:

9. Set HPLC system according to manufacturer's recommendations and to conditions given on page 5007-1. Inject 10-µL sample.
   NOTE: If peak area is above linear range of calibration graph, dilute, reanalyze, and apply appropriate dilution factor in calculations.
10. Measure peak area.

CALCULATIONS:

11. Read the mass, mg (corrected for recovery) of Rotenone found on the filter (W) and average media blank (B) from the calibration graph.
12. Calculate the concentration, C (mg/m^3), of Rotenone in the air volume sampled, V (L):

$$C = \frac{(W - B) \cdot 10^3}{V}, \ mg/m^3.$$

EVALUATION OF METHOD:

Method S300 [2] was issued on May 11, 1979, and validated over the range 1.16 to 11.1 mg/m^3 at 25°C and 760 mm, using 100-L samples [1, 5]. Overall precision, $\hat{S}_{rT}$, was 0.079 with average recovery 100.4%, representing a non-significant bias. The concentration of Rotenone (generated by Wright dust feeder using Ortho Rotenone Dust [1%; Chevron Chemical Co.] enriched to 10% Rotenone with analytical grade Rotenone [Aldrich Chemical Co.]) was independently verified by collection in dioxane and HPLC analysis. Recovery was 0.98 in the range 250 to 1000 µg Rotenone per sample. Collection efficiency of the PTFE filter was found to be greater than 99% and no detectable Rotenone (LOD = 4 µg) was found on Chromosorb 102 tubes placed behind the PTFE filters at 11.8 mg/m^3. No loss was seen from spiked filters stored in the dark at room temperature for seven days.

REFERENCES:

[1] Backup Data Report prepared under NIOSH Contract 210-76-0123, available as Order No. PB 82-114729 from NTIS, Springfield, VA 22161.
[2] NIOSH Manual of Analytical Methods, 2nd. ed., V. 5, S300, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 79-141 (1979).
[3] Gunther, F. A., and R. G. Blinn. Analysis of Insecticides and Acaricides, 419-420, Interscience, NY (1955).
[4] Bushway, R. J., B. S. Engdahl, B. M. Colvin, and A. R. Hanks. J. Assoc. Official Anal. Chemists, 58, 965 (1975).
[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:

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