**RIBAVIRIN**

*Chemical Information*

- **Formula**: C₆H₁₂N₄O₅
- **MW**: 244.21
- **CAS**: 36791-04-5
- **RTECS**: XZ4250000

**METHOD**: 5027, Issue 2  
**EVALUATION**: UNRATED

**OSHA**: no PEL  
**NIOSH**: no REL  
**ACGIH**: no TLV

**PROPERTIES**:
- solid; MP 170 °C; VP negligible;
- sol. (water): 142 mg/mL @ 25 °C

**SYNONYMS**:
- 1-ß-D-ribofuranosyl-1,2,4-triazole-3-carboxamide; Virazole; ICN 1229

**SAMPLING**

- **SAMPLER**: FILTER  
  (1-µm, 37-mm glass fiber)
- **FLOW RATE**: 1 to 4 L/min
- **VOL-MIN**: 5 L @ 0.4 mg/m³  
  **MAX**: 1000 L
- **SHIPMENT**: routine
- **SAMPLE STABILITY**: stable in dark at room temperature [1]
- **BLANKS**: 2 to 10 field blanks per set

**MEASUREMENT**

- **TECHNIQUE**: HPLC, UV DETECTION
- **ANALYTE**: Ribavirin
- **EXTRACTION**: 3 mL H₂SO₄ (pH = 2.5), agitate 30 min
- **INJECTION VOLUME**: 30 µL
- **MOBILE PHASE**: H₂SO₄ (pH = 2.5), isocratic
- **TEMPERATURE**: 65 °C
- **FLOW RATE**: 0.6 mL/min
- **COLUMN**: 30 cm x 7.8 mm, cation exchange resin
- **DETECTOR**: UV, 210 nm
- **CALIBRATION**: standard solutions of Ribavirin in H₂SO₄ (pH = 2.5)
- **RANGE**: 2 µg to 2000 µg per sample [1, 2]
- **ESTIMATED LOD**: 0.7 µg per sample [1, 2]
- **PRECISION (Sₑ)**: 0.057 @ 19 to 112 µg per sample [1, 2]
- **APPLICABILITY**: The working range is 0.04 to 40 mg/m³ for a 50-L air sample.

**ACCURACY**

- **RANGE STUDIED**: not studied
- **BIAS**: unknown
- **OVERALL PRECISION (Sₑ):** unknown
- **ACCURACY**: unknown

**INTERFERENCES**: None known.

**OTHER METHODS**: This method is a modification of a bulk assay procedure developed by Eastman Kodak Company [3].
REAGENTS:
1. Ribavirin,* reagent grade.
2. H2SO4,* conc.
3. Mobile phase: Add conc. H2SO4,* to deionized, distilled water until pH is 2.5 ±0.1 as measured by a pH meter.
4. Calibration stock solution: Dilute 5 mg Ribavirin to 10 mL in a volumetric flask using mobile phase as solvent. Prepare fresh daily.
5. Standard buffer solutions (pH 7.00 and 3.00) for calibrating pH meter. Available as a reference standard from USP (Cat. No. 60270-6).

EQUIPMENT:
1. Sampler: 1-µm, 37-mm glass fiber filter (Type A/E; Cat. No. 61652, Gelman Sciences, Inc., Ann Arbor, MI 48106, or equivalent) with a cellulose backup pad in a 2-piece cassette.
2. Personal sampling pump capable of operating for 8 hours at 1 to 4 L/min, with flexible connecting tubing.
3. High performance liquid chromatograph, isocratic, with water jacket (or equivalent) to maintain column temperature at 65 °C; UV detector (210 nm); peak integrator; and cation exchange resin column (Cat. No. HPX-87H, Bio-Rad Laboratories, Richmond, CA 94804 or equivalent).
4. Vials, 10-mL, glass with PTFE-lined caps.
5. Culture tubes, PTFE-lined screw caps, 13-mm x 100-mm.
6. Syringe filters, disposable, 0.45-µm pore size, for filtering samples.
7. Pipets, 1- to 10-mL.
8. Volumetric flasks, 10-mL.
10. Shaker, mechanical, wrist-action
11. pH meter.

* See SPECIAL PRECAUTIONS.

SPECIAL PRECAUTIONS: Ribavirin has been found to be teratogenic in animals. [4, 5] Use protective gloves when handling. Work only in a fume hood. Women of childbearing age should exercise extreme caution. Avoid skin contact with concentrated sulfuric acid.

SAMPLING:
1. Calibrate each personal sampling pump with a representative sampler in line. Attach sampler to personal sampling pump with flexible tubing.
2. Sample at an accurately known flow rate between 1 and 4 L/min for a total sample size of 5 to 1000 L. Avoid overloading the filter (ca. 2 mg total dust maximum loading).
3. Seal the samplers and pack securely for shipment.
4. Collect a bulk sample (ca. 1 g) in a glass vial and ship it separately.

SAMPLE PREPARATION:
5. Carefully remove the filter from the cassette. Use forceps to fold the filter in half, and insert into a culture tube. Discard the backup pad.
6. Add 3 mL mobile phase. Seal tightly with screw cap and agitate samples with a shaker for 30 minutes.
   NOTE: Although Ribavirin is stable as a solid, it degrades after 12 h in the mobile phase necessitating daily preparation of standards and prompt analysis of extracted samples [3].
7. Filter the sample solution through a syringe filter.
CALIBRATION AND QUALITY CONTROL:

8. Calibrate daily with at least six working standards over the range 0.2 to 700 µg/mL.
   a. Add known amounts of calibration stock solution to mobile phase in 10-mL volumetric flasks and dilute to the mark.
   b. Analyze with samples and blanks (steps 11 through 13).
   c. Prepare calibration graph (peak area vs. µg Ribavirin).
9. Determine recovery (R) at least once for each lot of filters used for sampling in the range of interest. Prepare three filters at each of five levels plus three media blanks.
   a. Deposit a known amount of Ribavirin onto the filter. Allow filters to air dry.
   b. Store samples overnight in the dark.
   c. Prepare (steps 5 through 7) and analyze with working standards.
   d. Prepare a graph of R vs. µg Ribavirin spiked.
10. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graph and R graph are in control.

MEASUREMENT:

11. Set liquid chromatograph system according to manufacturer’s recommendations and to the conditions given on page 5027-1.
12. Inject sample aliquot using syringe, fixed volume sample loop or autosampler.
   NOTE: If peak area is above linear range of calibration graph, dilute, reanalyze, and apply appropriate dilution factor in calculations.
13. Measure peak area.

CALCULATIONS:

14. Determine the mass, µg (corrected for R) of Ribavirin found in the sample (W), and in the average media blank (B), from the calibration graph.
15. Calculate concentration, C, of Ribavirin in the air volume sampled, V (L):

\[ C = \frac{(W - B)}{V}, \text{ mg/m}^3. \]

EVALUATION OF METHOD:

This method is a modification of a bulk assay procedure developed by Eastman Kodak Company [3]. Measurement precision, $S_r$, was 0.057 with average recovery of 100% representing no bias, based on 16 samples ranging from 19.2 to 112 µg per filter. Sampling precision was not determined. The calibration curve was shown to be linear between 0.63 and 666 µg of Ribavirin/mL of extraction solution. A least-squares fit of the calibration curve yielded a limit of detection of 0.7 µg per filter and a limit of quantitation of 2 µg per filter [1,2].

REFERENCES:


METHOD WRITTEN BY:

Barry R. Belinky and G. David Foley, NIOSH/DPSE.