PENTAMIDINE ISETHIONATE 5032

$C_{23}H_{36}N_4S_2O_{10}$ MW: 592.75 CAS: 140-64-7 RTECS: CV6500000

METHOD: 5032, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 May 1989**
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OSHA : no PEL
NIOSH: no REL
ACGIH: no TLV

PROPERTIES: solid; MP 192.3 - 193.7 °C; soluble in water and ethanol

SYNONYMS: 4,4'-[1,5-pentanediylbis(oxy)]bisbenzenecarboximidamide bis(2-hydroxyethanesulfonate); Pentam 300

SAMPLING

SAMPLER: FILTER (37-mm, PVC in opaque cassette)

FLOW RATE: 1 to 2 L/min

VOL-MIN: 50 L @ 1 µg/m^3
-MAX: 1500 L

SHIPMENT: routine

SAMPLE STABILITY: ≥ 27 days @ 25 °C in the dark

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: none

BIAS: not determined

OVERALL PRECISION ($\hat{S}_{rT}$): not determined

ACCURACY: not determined

MEASUREMENT

TECHNIQUE: HPLC, FLUORESCENCE DETECTION

ANALYTE: pentamidine isethionate

RECOVERY: 3 mL of 50:50 ethanol:water, 0.085% H_3PO_4 and 0.04% TMAC; ultrasonic bath 10 min

INJECTION VOLUME: 100 µL

MOBILE PHASE: 19.6:80.4 CH_3CN: water, 0.085% H_3PO_4 and 0.04% TMAC; 1 mL/min

COLUMN: Ultrasphere C-8, 5-µm particles, 25 cm x 4.6-mm; ultrasphere C-8 guard

DETECTOR: fluorescence excitation/emission: 270/340 nm

CALIBRATION: pentamidine isethionate in eluent

RANGE: 50 to 3,000 ng per sample

ESTIMATED LOD: 18 ng per sample

PRECISION ($\hat{S}_r$): 0.065

APPLICABILITY: The working range is 0.5 to 30 µg/m^3 for a 100-L air sample. This method has been used to measure pentamidine isethionate in air in a hospital [1].

INTERFERENCES: None identified.

OTHER METHODS: None published for air samples. The method for measurement in solution is a variation of the method of Lin et al. [2].

REAGENTS:

1. Pentamidine isethionate, 99+% pure.
2. Water, deionized.
3. Ethanol, undenatured, chromatographic quality.
4. Acetonitrile, chromatographic quality.
5. Phosphoric acid, 42.5% solution. Dilute 85% solution 1:1 with water.
6. Tetramethylammonium chloride (TMAC), 10% solution. Dissolve 10.3 g of 97% pure reagent in water to make 100 mL of solution.
7. Eluent. Place 1 L of ethanol, 4 mL of 42.5% H_3PO_4, and 8 mL of 10% TMAC solution into a 2-L flask. Add water to make 2 L of solution.
8. Mobile phase. Place 4 mL of 42.5% H_3PO_4 and 8 mL of 10% TMAC into a 2-L flask. Add 1608 mL of water and 392 mL of acetonitrile.
9. Recovery solutions, 1.5, 35, and 1000 ng/µL. Dissolve 25 mg of pentamidine isethionate in ethanol to make 25 mL of solution. Make serial dilutions with ethanol. Recovery solutions are stable at least 6 days in the dark at 0 °C.
10. Disinfectant. 70% Ethanol or 70% isopropanol in water.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: two-piece filter cassette holder (opaque), 37-mm PVC membrane filter, 5-µm pore size.
2. Personal sampling pump, 1 to 2 L/min, with flexible connecting tubing.
3. High performance liquid chromatograph with a guard column packed with Ultrasphere C8, an analytical column (Beckman Instruments) (page 5032-1), and a fluorescence detector.
4. Volumetric flasks, 2-L and 25-mL.
5. Syringes, 1- and 0.5-mL, readable to 10 µL; 10 µL, readable to 0.2 µL.
6. Beakers, 50-mL.
7. Film, plastic, flexible, water-resistant.
8. Pipets, disposable.
9. Forceps.
10. Ultrasonic bath.
11. Biological safety cabinet [3].
12. Gloves, disposable.
13. Gauze pads.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Attach the sampler to personal sampling pump with flexible tubing.
3. Sample at an accurately known flow rate between 1 and 2 L/min for a total sample size of 50 to 1500 L. Limit the loading of particulate matter on the filter to a maximum of about 1 mg.
4. Seal the inlet and outlet of the sampler with plugs.
5. Wet a gauze pad with disinfectant, and wipe the exterior surface of the sampler.
6. Pack the sampler securely for shipment.

SPECIAL PRECAUTIONS: This method includes safety steps to minimize exposure of the analyst to tuberculosis bacteria (Mycobacterium tuberculosis). A fraction of the patients with acquired immunodeficiency syndrome (AIDS) have tuberculosis. Consequently, live tuberculosis bacteria may be collected during air sampling.

Inhalation of tuberculosis bacteria in aerosols is the only notable means of transmission which results in infection. Infection does not result from skin contact. Thus, the analyst is safe as long as aerosols are not produced. The use of alcohol to kill tuberculosis bacteria simply increases the degree of safety.

SAMPLE PREPARATION:

NOTE: Sample preparation should be performed in a biological safety cabinet. Gloves should be worn.

7. Place the 37-mm PVC filter with the exposed side facing upward into a 50-mL beaker.
8. Add 3.0 mL of eluent to the beaker. Seal the mouth of the beaker with plastic, flexible, water-resistant film.
9. Place beaker into ultrasonic bath for 10 min. Use disinfectant as medium in ultrasonic bath.
10. Transfer solution to a sample vial with a disposable pipet.

    NOTE: Filtration of the sample solution is not recommended because fluorescent material could be leached from the filter or the housing for the filter, causing interference during analysis. Filtration is unnecessary because the guard column protects the analytical column from particulate matter.
11. Recover pentamidine isethionate from the interior surface of the front piece of the cassette filter holder.
    a. Add 3.0 mL of eluent to this cassette piece (original plug remains in the inlet).
    b. Wet much (about 80%) of the interior surface by tilting the cassette piece. Avoid bringing eluent into contact with glove being worn. Complete this step in about 15 seconds to minimize evaporation.
    c. Transfer solution to a sample vial with a disposable pipet.

CALIBRATION AND QUALITY CONTROL:

12. Calibrate daily with at least eight working standards over the range 2 to 1000 ng of pentamidine isethionate per mL of solution.
    a. Prepare a series of working standards in eluent by serial dilution.

       NOTE: Working standards in eluent are stable for more than 3 months when stored in the dark at 0 °C.
    b. Analyze together with samples and blanks (steps 15 through 17).
    c. Prepare a calibration graph (peak height or area versus concentration of pentamidine isethionate).
13. Determine recovery (R) at least once for each lot of PVC membrane filters used for sampling in the calibration range (step 12). Prepare three filters at each of five levels plus three media blanks.
    a. Place filters into 50-mL beakers.
    b. By means of a 10-µL syringe, fortify each filter with recovery solution.
    c. Seal the mouth of each beaker with plastic, flexible, water-resistant film.
    d. Store each sample overnight in the dark at 25 °C.
    e. Prepare samples (steps 7 through 10) and analyze with working standards (steps 15 through 17).
    f. Prepare a graph of R vs. ng of pentamidine isethionate recovered.
14. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graph is in control.

MEASUREMENT:

15. Set liquid chromatograph to manufacturer's recommendations and to conditions given on page 5032-1.
16. Inject 100-µL sample aliquot manually or with autosampler.
17. Measure peak area or peak height.

CALCULATIONS:

18. Determine the mass, ng (corrected for R), of pentamidine isethionate found on the filter (W_f), on the average media blank (B_f), and the mass, ng, of pentamidine isethionate found on the interior surface of the cassette filter holder (W_c) and on the interior surface of a blank cassette filter holder (B_c).
19. Calculate concentration, C, of pentamidine isethionate in the air volume sampled, V (L):

$$C = \frac{(W_f + W_c - B_f - B_c)}{V}, \ \mu g/m^3.$$

EVALUATION OF METHOD:

Average recoveries after fortification of 37-mm PVC membrane filters with 50-, 99.9-, 300-, and 8816-ng quantities of pentamidine isethionate were 0.76, 0.81, 0.84, and 0.91, respectively; precision ($\bar{S}_r$) was 0.065 (24 samples, pooled). The average recovery of 324-ng quantities of pentamidine isethionate from PVC filters after 27 days of storage at room temperature in the dark was 0.97; s_r was 0.045 (6 samples). This method was not evaluated with generated atmospheres in a laboratory. However, the method was employed for measurement of pentamidine isethionate in air in a hospital [1]. Significant quantities [10 - 3,810 ng (3 - 11% of the totals)] of pentamidine isethionate were found on interior surfaces of the front pieces of eight cassette filter holders.

The LOD of pentamidine isethionate in solution was 7 ng per 3 mL. According to a curve of average recovery of pentamidine isethionate from PVC filters versus average concentration in solution, a conservative estimate of recovery was 0.40 when the concentration in solution was at the LOD. Thus, the LOD for pentamidine isethionate on a PVC filter was 18 ng (7 ng divided by 0.4). The LOQ for pentamidine isethionate on a PVC filter (50 ng) was the lowest level at which recovery was acceptable (>75%).

The LOD and LOQ for pentamidine isethionate on the interior surface of the front piece of a cassette filter holder were 7 and 24 ng, respectively.

A standard solution of pentamidine isethionate in eluent at a concentration of 100 ng/mL was stable for a period of more than 3 months during storage at 0 °C in the dark.

Smith states that for killing wet and dry tuberculosis bacteria, 95% ethanol and 50% ethanol, respectively, are most active . Also, perhaps 70% ethanol would be the best single solution for all purposes [4]. However, injection of a solution of pentamidine isethionate in 70% ethanol resulted in poor chromatography. Consequently, a 50% ethanol solution was selected for recovery of pentamidine isethionate from PVC filters.

REFERENCES:

[1] Tucker, S.P., B.R. Belinky, T.A. Seitz, and G.D. Foley, American Industrial Hygiene Association Journal 54 (10), 628-632 (1993).
[2] Lin, J. M.-H., R. J. Shi, and E. T. Lin, Journal of Liquid Chromatography , 9 (9), 2035-2046 (1986).
[3] Biosafety in Microbiological and Biomedical Laboratories, 2nd ed., Appendix A, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and National Institutes of Health, Washington, D.C. (1988); HHS Publication No. (NIH) 88-8395.
[4] Smith, C. R. Public Health Reports (U.S.), 62, 1285-1295 (1947).

METHOD WRITTEN BY:

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