MANEB 3600
Dermal Patch

$C_4H_6N_2S_4Mn$ MW: 265.2 CAS: 12427-38-2 RTECS: OP07000

METHOD: 3600, Issue 1 **EVALUATION: PARTIAL** **Issue 1: 15 March 2003**

OSHA : not applicable
NIOSH: not applicable
ACGIH: not applicable

PROPERTIES: powder, yellow, wettable; sparingly soluble in water and organic solvents; vp not significant

NAMES & SYNONYMS: manganous ethylenebis(dithiocarbamic acid), Manzate; Dithane M-22

SAMPLING

SAMPLER: PATCH
Dry NuGauze®, spun polyester gauze patch (see Figure 1)

FLOW RATE: not applicable

VOL-MIN: not applicable
-MAX: not applicable

SHIPMENT: desorb immediately in shipping bottles, pack in blue ice, cool to 4 °C, ship via overnight express

SAMPLE STABILITY: at least 1 week at 4 °C

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: not applicable

BIAS: not determined

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

ACCURACY: not determined

MEASUREMENT

TECHNIQUE: HPLC, UV detection

ANALYTE: ethylenebisdithiocarbamate anion

EXTRACTION: 40 mL desorbing solution (1% L-cysteine, 3% $Na_4EDTA \cdot 2H_2O$ in water) in 50 to 65-mL wide-mouth bottles with Teflon®-lined screw caps

INJECTION VOLUME: 100 µL

MOBILE PHASE: 0.0675 M phosphate, 0.0525 M $NaClO_4$, pH 6.9, 1 g/L $Na_2EDTA \cdot 2H_2O$, 2 mL/min

COLUMN: Dionex AS7 anion exchange column or equivalent, GS7 guard column

DETECTOR: UV, 285 nm (λ max) or 254 nm

CALIBRATION: solutions of Maneb in 1% L-cysteine, 3% Na_2EDTA

RANGE: 0.02 to 4 mg/sample [1]

ESTIMATED LOD: 0.02 mg/sample (0.5 µg/mL) [1]

PRECISION ($\bar{S}_r$): 0.015 [1]

APPLICABILITY: This method determines Maneb collected on dry Nu Gauze® (spun polyester gauze) dermal patches attached to clothing on the arm(s), leg(s), or torso of agricultural workers. It is applicable to the determination of Zineb, Mancozeb, and Nabam since these all convert to the same analyte when dissolved in EDTA solution.

INTERFERENCES: Not thoroughly investigated. No interferences have been found during method development.

OTHER METHODS: Maneb may also be determined by first methylating followed by HPLC analysis with a C18 column [2-5].

REAGENTS:

1. Maneb,* purity >90%
2. Water, deionized
3. Mobile Phase, 0.0675 M phosphate buffer, 0.0525 M $NaClO_4$, 1 g/L $Na_2EDTA•2H_2O$: In a 1-L volumetric flask, dissolve 4.79 g dibasic sodium phosphate; 4.59 g monobasic potassium phosphate; 6.43 g sodium chlorate; 1 g ethylenediaminetetraacetic acid, disodium salt, dihydrate in 500 mL of deionized water. Bring to volume with deionized water. Adjust pH to 6.9.
4. L-cysteine, reagent grade.
5. Ethylenediaminetetraacetic acid, disodium salt, dihydrate ($Na_2EDTA•2H_2O$), reagent grade.
6. Sodium phosphate, dibasic, anhydrous (Na_2HPO_4), reagent grade.
7. Potassium phosphate, monobasic, anhydrous (H_2PO_4), reagent grade.
8. Sodium Chlorate ($NaClO_3$), reagent grade.
9. Ethylenediaminetetraacetic acid, tetrasodium salt, dihydrate ($Na_4EDTA•2H_2O$)*
10. Desorbing solution, 1% L-cysteine, 3% Na_4EDTA in water: In a 1-L volumetric flask, dissolve 10 g L-cystene and 30 g $Na_4EDTA•2H_2O$ in 500 mL water. Dilute to volume with deionized water. (The pH will be approximately 8.6.*)
11. Calibration stock solution*, 1 mg/mL: Dissolve 10 mg Maneb in 10 mL desorbing solution.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: Polyester gauze, Johnson & Johnson Nu Gauze® 10-cm x 10-cm (4" x 4"), 4-ply or equivalent.
2. Patch holders, aluminized cardboard (see Figure 1).
3. High performance liquid chromatograph (HPLC) with UV detector, 254 nm (or 285 nm if a variable-wavelength UV detector is available), and autosampler. Set chromatograph to conditions on page 1.
4. Columns: Dionex AS7 anion exchange column, AG7 guard column or equivalent.
5. Ultrasonic bath.
6. Forceps.
7. Bottles, wide-mouth, amber, 50- to 65-mL, with PTFE-lined screw caps.
8. Volumetric flasks, 10-mL, 100-mL, 1-L.
9. Graduated cylinder, 50-mL.
10. Vials, autosampler.
11. Pipets, glass, various sizes.
12. Syringes, 100-µL with large bore needle (≥19-gauge).

SPECIAL PRECAUTIONS: Maneb has degradation products which are known to be carcinogenic, mutagenic, and teratogenic [6-9]. Avoid contact with Maneb. Wear appropriate protective equipment. Na_4EDTA is very alkaline. Avoid contact with skin or eyes of either the dry powder or aqueous solutions.

SAMPLING:

NOTE: The sampling procedure has not been thoroughly investigated. This is a prototype method only.

1. Place Nu Gauze® patches in the patch holders.
2. Label each patch holder with name, date, body location, and time of attachment; then attach to clothing on the arm(s), leg(s), or torso of the human subject(s) at location noted on patch label.
3. Label amber wide-mouth glass shipping bottles with the same information.
4. After a 4- to 8-hour time period for the human subject(s) working in the field or with Maneb application equipment, remove the patch holders and record the time.
5. Using forceps, remove the Nu Gauze® patches from the patch holders by folding the corners into the middle. Place patches in shipping bottles with as little disturbance of the collected Maneb as possible.

6. Prepare sufficient desorbing solution for all samples and blanks: 4 g L-cysteine and 12 g $Na_4EDTA•2H_2O$ in 400 mL of deionized water for every 10 samples (40 mL per sample). Prepare this solution in the field to prevent premature oxidation of the L-cysteine preservative.
   NOTE: The EDTA here must be the tetrasodium form, not the disodium form. See SPECIAL PRECAUTIONS.
7. Add 40 mL of desorbing solution to each shipping bottle, cap, and shake well to wet the Nu Gauze® and dissolve the Maneb.
8. Prepare 2 to 10 blank samples by adding 40 mL to clean Nu Gauze® patches in shipping bottles.
9. Pack shipping bottles securely in blue ice or other means to keep at 4 °C, and ship via overnight express.

SAMPLE PREPARATION:

NOTE: Desorption of samples is done on site.

10. On arrival at laboratory, store samples at 4 °C.
11. Ultrasonicate capped sample bottles for 5 to 10 minutes.
12. Transfer 1 to 2 mL of each desorbed sample solution, standards, and blanks to autosampler vials for analysis. Analyze within 24 hours.

CALIBRATION AND QUALITY CONTROL:

13. Calibrate daily with at least six working standards over the range of 0.1 to 100 µg/mL.
    a. Pipet aliquots of calibration stock solution into 10-mL volumetric flasks, and bring to volume with desorbing solution.
    b. Include a calibration blank of unspiked working solution.
    c. Analyze together with field samples, field blanks, and laboratory control samples (steps15 through 17).
    d. Prepare a calibration graph (peak area vs. concentration, µg/mL).
       NOTE: To prepare media standards, prepare a slurry of Maneb in methanol, 1 mg/mL. Ultrasonicate for approximately 2 minutes to pulverize and disperse the particles of Maneb. Shake slurry periodically to keep the Maneb suspended. Up to 20% ethylene glycol can be included to help keep the particles in suspension. Withdraw a measured amount using a 100-µL syringe with a large bore (≥ 19-gauge needle) and spike onto a dry Nu Gauze® patch within a 50- to 65-mL amber wide-mouth bottle. After drying, desorb the Maneb-spiked Nu Gauze® with 40 mL of desorbing solution and ultrasonicate.
14. Prepare laboratory control samples (LCS), in duplicate, with each sample set.
    a. Spike Maneb in methanol onto 10-cm x 10-cm Nu Gauze® patches placed in 50- to 65-mL amber glass bottles in concentrations within the analytical range.
    b. Desorb with 40 mL of desorbing solution.
    c. Analyze along with field samples, standards, and blanks (steps 15 through 17).

MEASUREMENT:

15. Set liquid chromatograph to manufacturer's recommendations and conditions given on page 3600-1.
16. Transfer sample aliquots to injection vials. Inject 100-µL aliquots manually or with an autosampler. Rinse and dry syringe after each injection.
17. Measure peak areas. If sample peak area exceeds the linear calibration range, dilute with aqueous 1% L-cysteine, 3% $Na_4EDTA•2H_2O$ solution, reanalyze, and apply the appropriate dilution factor in the calculations.

CALCULATIONS:

18. From the calibration graph, read the concentration, C (µg/mL), of Maneb found in the sample.
19. Calculate the mass, M, of Maneb in the sample (µg/sample).

$$M(\mu g) = C(\mu g / mL) x 40 mL$$

CONFIRMATION:

No confirmation method has been tested. Possible alternatives are ion-pairing chromatography with a C-18 column [5], or analysis of methylated derivatives on a C-18 column [3,4].

EVALUATION OF METHOD:

This method has been evaluated in the laboratory only. No field samples have been analyzed to date.

LOD/LOQ

A series of media standards (See Note above under step 13.d.) from 0.1 to 100 µg/mL were prepared in duplicate, analyzed, and fitted with a linear curve. The Limit of Detection (LOD), 0.02 mg/sample for a 40-mL desorbing volume, and Limit of Quantitation (LOQ), 0.066 mg/sample, were estimated using NIOSH SOP 018 [10].

PRECISION AND ACCURACY

Twenty-four media standards were prepared, six at each of four levels: 3xLOQ, 10xLOQ, 30xLOQ, and 100xLOQ, as described above in Note under step 13.d. The media standards were desorbed with 40 mL of desorbing solution and analyzed. Levels 3xLOQ, 10xLOQ, and 100xLOQ were used to obtain a pooled relative standard deviation. One of the six samples at the 100xLOQ level was shown to be an outlier by Grubbs' test and was not included in the calculations. Bias, Accuracy, and Overall Precision were not determined. The recoveries were all greater than 90% except for Level 3xLOQ, which was 88% [1].

STABILITY

Stability studies were performed at 528 µg Maneb per dermal patch. At room temperature (24 °C), Maneb on dry Nu Gauze® patches was not stable, with less than 60% recovery on Day 2. At 4 °C, however, Maneb was stable on dry Nu Gauze® to at least Day 8. Maneb which was spiked onto Nu Gauze® and allowed to stand approximately two hours while the solvent dried and was then desorbed in a 1% L-cysteine, 3% Na_4EDTA solution, was stable to Day 22 with recoveries greater than 90%. At Day 30 the recovery was 87% [1].

COMMENTS:

Most ethylene-bis-dithiocarbamates (EBDC) containing divalent (or higher) metals (*e.g.*, Maneb, Mancozeb, and Zineb) dissolve with difficulty in almost every solvent. If the metal ion is removed by complexation with EDTA (at high pH), the EBDC will dissolve but will begin to degrade rapidly, presumably by oxidation, to disulfides or other products. Adding an antioxidant such as L-cysteine greatly inhibits this oxidative degradation. Excluding headspace air has been shown to assist in reducing oxidation; however, cooling to 4 °C is one of the most important factors in sample preservation.

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METHOD WRITTEN BY:

Paul C. Gillespie and John M. Reynolds, DataChem Laboratories, Salt Lake City, UT.

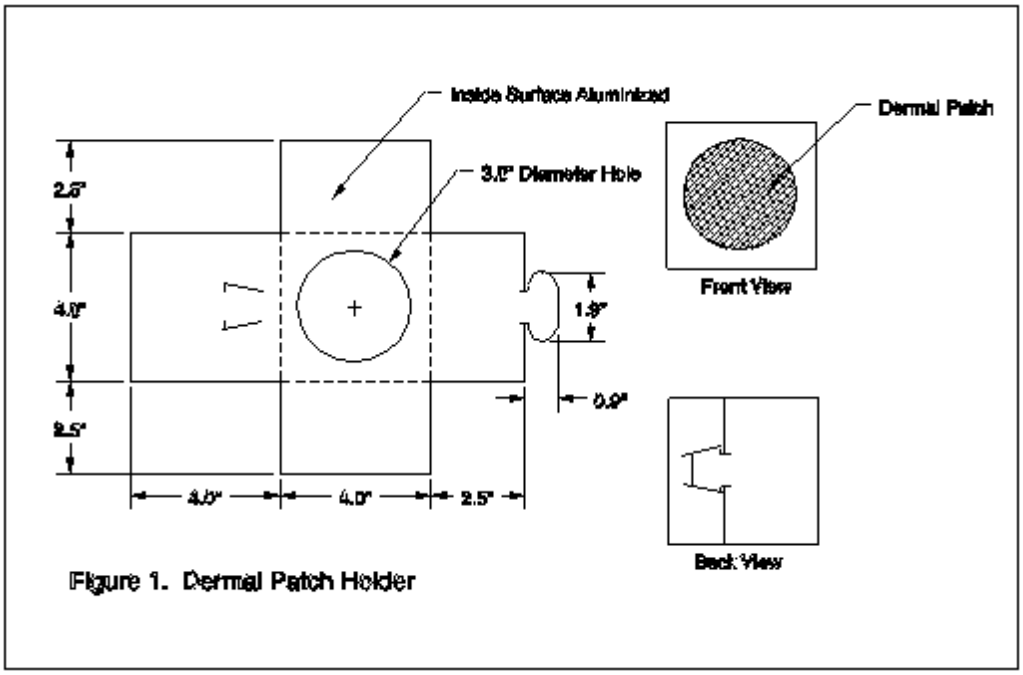

Figure 1. Dermal Patch Holder