

DETERMINATION OF MANEB IN RAC'S (CROPS)

Reason for Revision: The Quality Assurance Unit requested clarification of the fortification phase of the extraction regarding addition of reagents which determine headspace final volume.

Method References:

1. JAOAC, Volume 52, Number 6, Page 1226 (1969)
2. MTF-88AM-005, 06/15/87, Determination of Maneb in Crops, Morse Laboratories, Inc., Sacramento, CA.
3. ETU-89AM-001, 06/07/89, Determination of Ethylene Bis Dithiocarbamates (EBDC'S) in Crops and Processed Commodities, Morse Laboratories, Inc., Sacramento, CA.

Principle:

Maneb present in the sample is converted to CS₂ during reaction with HCl/Stannous chloride reagent at 100°C in a sealed reaction vial. An aliquot of the headspace is injected into a gas chromatograph where the sample responses are compared to a Maneb standard similarly prepared and injected.

Note: During all analyses, equivalent apparatus, solvents, glassware, or techniques (such as sample concentration) may be substituted for those specified in the method.

Reagents:

Maneb - Analytical grade, Pennwalt Corporation, AgChem Division (ATOCHEM North America) Bryan, TX 77801

GLC Column Packing - 28% Pennwalt 223 + 4% KOH on 80/100 Gas Chrom R, Alltech Associates, Inc., Deerfield, IL 60015 or equivalent.

NOTE: The PT 28% Alltech 223 column is equivalent to the Pennwalt 223 column.

- EDTA (tetrasodium) - over 99% purity, EM Science, Cherry Hill, NJ 08034.
- EDTA Solution - 10% (w/v) in boiled deionized water.
- Hydrochloric Acid - concentrated, J.T. Baker Chemical Co., Phillipsbury, N.J. 08665
- Stannous Chloride - analytical grade, Spectrum Chemical Mfg., Gardena, CA 90248
- Carbon disulfide - reagent grade, J.T. Baker Chemical Co. Phillipsbury, N.J. 08665
- Methanol - nanograde, J.T. Baker Chemical Co. Phillipsbury, N.J. 08665
- Hexane - nanograde, J.T. Baker Chemical Co. Phillipsbury, N.J. 08665
- HCl/Stannous chloride reagent - 8N:3%; weigh 3.6 g $\text{SnCl}_2 \cdot (\text{H}_2\text{O})_2$ into a 100 mL volumetric flask. Add 64 mL concentrated HCl. QS to 100 mL with boiled deionized water.

Apparatus:

- Reaction vials - 160 mL equipped for crimp sealing with teflon-lined septums, Pierce Chemical Co., Rockford, IL 61105, cat. #12995
- Crimp seals with teflon-lined septums - Pierce Chemical Company, Rockford, IL 61105
Teflon/Silicone Disc Septums cat #12720
Aluminum seals (20 mm) cat #13214
- Crimper - Wheaton Instruments, Millville, NJ, part #224303
- Water Bath
- Gas Chromatograph - Microtek MT-220 or equivalent with a flame photometric detector in the sulfur mode.
- Gas Chromatographic Column - 6' x 1/4" o.d. x 4 mm i.d. glass column packed with 28% Pennwalt 223 + 4% KOH on 80/100 Gas Chrom R or equivalent.

Gas tight syringes - 10, 50, 100, 250, 500, 1000, 2500 μL , Hamilton Co., Reno, NV

Waring Blender

Hobart meat grinder
(or equivalent)

Reitz disintegrator
grinder, Wiley mill,
or equivalent

PowrPulse Homogenizer - N-Phase, Austin, Texas

"Airpettor"
Adjustable
pipettes - American SMI "Airpettor", Scientific Products, Sunnyvale, CA 94089 or
equivalent.

Pipettes: 50-200 μL volume range cat. #P5086-2
200-1000 μL volume range cat. #P5086-3

Tips: 2-200 μL cat. #P5059-301
100-1000 μL cat. #P5059-801

Sample Preparation:

Wet crops are ground in the Hobart grinder while frozen with dry ice. Dry crops are ground while frozen using a Reitz disintegrator grinder with dry ice. Individual crops that require further grinding are ground with dry ice in a Waring blender at the discretion of the analyst and with proper documentation.

Standard Preparation:

Carbon disulfide (CS_2):

1. 100 μL CS_2 is dissolved in 100 mL hexane.
2. 4 μL of the 0.1% solution is placed in a reaction flask, which is immediately sealed and heated to 100°C to allow all hexane and CS_2 to equilibrate in headspace.

Note: This standard is used to locate the gas chromatographic CS₂ response the first time this analysis is attempted. Thereafter, given similar column and GC conditions, only the active ingredient standard needs to be prepared.

Maneb:

1. Correcting for purity, weigh 10.0 mg active ingredient of Maneb analytical standard into a 250 mL beaker.
2. Using a 100 mL volumetric pipet, deliver 100 mL boiled deionized water (cooled) into the beaker containing the Maneb standard.
3. With a PowrPulse homogenizer (or equivalent, i.e., Polytron homogenizer), homogenize the Maneb suspension for 10 minutes.

Note: The resulting suspension (100 µg/mL) must be homogeneous and devoid of heavy particles.

4. **While the suspension is being homogenized, remove 10.0 mL with a graduated pipet and dilute to 100 mL with boiled deionized water.**

Note: 10 mL aliquot must be withdrawn quickly and accurately on first attempt. If the initial withdrawal overshoots or undershoots 10 mL mark, the withdrawal procedure must be reinitiated. This produces a 10 µg/mL solution.

5. The instrumentation standard is prepared by adding 1.35 mL of the 10 µg/mL suspension (using an Airpettor) to a 160 mL vial.

Note: Two 675 µL aliquots can be used in place of 1.35 mL.

6. Add 8.65 mL 10% EDTA solution and 15 mL HCl/Stannous chloride reagent to the reaction vial and immediately crimp seal.

7. React contents of vial in the same fashion as samples, as discussed later. Resulting concentration of prepared Maneb standard for gas chromatography is 0.1 µg/mL headspace.

Note: Based on the stability of similar compounds (EBDC's) in water suspension, the least stable of which is **analytically stable (<3% degradation)** for 30 minutes, all manipulations with suspension standards must be completed within this time frame.

Sample Extraction:

1. Place 4.0 g of frozen, ground sample into a 160 mL reaction vial.
2. Add 10% EDTA solution to make a total volume of 10 mL (sample plus 10% EDTA solution). This volume must be determined for each crop prior to analysis, based on the volume of 4 g ground sample (see discussion).
3. Add 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal the reaction vial.
4. Place vial in boiling waterbath for 2 hours, hand shaking vials approximately every 5 minutes for the first 30 minutes, then every 30 minutes for the remaining 1 1/2 hours.
5. After reaction, maintain sample at 100°C in the waterbath during GLC analysis.

Note: Some crops, such as corn forage, may require reaction for more than 2 hours or a reduction in sample size. This modification is only needed on matrices where the procedure produces apparently poor recoveries at the standard reaction time of two hours and the standard sample size of 4.0 g.

Sample Fortification:

1. Spike samples are fortified at the correct level by adding the appropriate volume from either the 1.0 µg/mL, 10 µg/mL or 100 µg/mL fortification solutions.

Note: 1.0 µg/mL is only prepared if necessary. While the 10 µg/mL suspension is being homogenized, remove 10.0 mL and dilute to 100 mL with boiled deionized water.

2. Due to the fact that the Maneb standard is in a suspension rather than a solution, use only pipettes or "Airtettors" to add the fortification solutions to the sample. **DO NOT USE MICROLITER SYRINGES.**
3. Place 4.0 g frozen ground sample into a 160 mL reaction vial.
4. Fortify the spike samples with the appropriate concentration while the suspensions are being homogenized.
5. Add 10% EDTA solution to make a total volume of 10 mL (sample plus fortification solution plus 10% EDTA solution). See Discussion.

6. Add 15.0 mL HCl/Stannous Chloride (8N/3%) reagent. Immediately seal.
7. Treat as samples from this point on, heating the fortification at 100°C for the same time as the samples.

Gas Chromatographic Analysis:

1. Equilibrate the gas chromatograph as follows:

Injector Temperature - 210°C
Detector Temperature - 160°C
Column Temperature - 135°C
Nitrogen Carrier Gas Flow - 45 mL/min.

2. Condition column overnight at 200°C with 30 mL/min carrier gas flow.
3. Prior to analysis, make several injections (10-100 µL) of Maneb standard (0.1 µg/mL headspace) to sensitize the column for CS₂.

Note: Under these gas chromatographic conditions, the retention time for Maneb as CS₂ is 2-3 minutes.

4. Prepare a 4-point standard curve by chromatographing 0.4 ng through 2.5 ng of appropriate Maneb standard.

Note: 2.5 ng may produce an off-scale response on some FPD detectors due to their inherent differences in logarithmic response. In such cases, inject an amount of standard which produces approximately a 90% full scale deflection (FSD) as the high point of the curve.

5. In any case, 0.4 ng, representing a 0.0135 ppm response when 1000 µL of sample is injected, must be included as the lowest point on the standard curve and should produce a response peak height of at least 5 mm. Identification of the peak produced by the Maneb standard as CS₂ is achieved by demonstrating its retention time to be identical to that of the peak produced by an injection of CS₂ standard. This identification process needs to be conducted only if the CS₂ retention time needs to be identified (i.e., when a new GC column is employed or instrument repairs which might have affected retention time have occurred).
6. Inject 2 - 1000 µL of airspace from the samples into the gas chromatograph using airtight syringes. Compare sample responses to those produced in the standard curve.

7. Calculation is made by use of the following equation:

$$\text{ppm} = \frac{\text{ng Maneb}}{\text{mg sample injected}}$$

where: *ng Maneb is derived from standard curve*

mg sample injected =

$$\frac{4.0 \text{ g}}{\text{headspace volume of sample-containing reaction vial}} \times \mu\text{L injected}$$

where: *headspace volume of sample-containing reaction vial = 135 mL*

Notes:

1. Standard curve is prepared by plotting ng injected vs. peak height on full logarithmic graph paper.
2. Historically, the column and conditions stated in the method have been satisfactory for the matrix being analyzed. The specific column packing, carrier gas, column temperature and flow rate listed are typical conditions for this analysis. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

Discussion:

- The gas chromatographic method described herein has a limit of quantitation of 0.02 ppm for Maneb.
- In order to eliminate a possibility of CS₂ contamination, reaction vials were rinsed with methanol and baked at 135°C for 45 minutes. Teflon-lined septums were rinsed with methanol and air dried. The use of rubber or plastic utensils was avoided.
- The volume of 4.0 g of matrix is determined by weighing 4.0 g of sample into a 25 mL graduated cylinder and adding 10 mL water. The total volume minus 10 mL is the volume of the 4 g sample. (Note: Better results may be achieved with some matrices if 20 mL of water is used in place of 10 mL.)

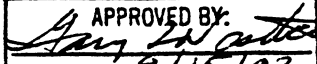
- Standard curves are prepared by injection of variable volumes of a single standard preparation at a concentration of 0.1 $\mu\text{g}/\text{mL}$ headspace.
- Samples must be kept frozen at all times until addition of extraction/reaction reagents. This includes weighing and fortification (preparation of spikes) processes. Keep samples on dry ice before and after weighing and during fortification.
- Samples must be reacted immediately following addition of reaction reagents. Once reacted, the samples (now containing in the form of CS_2 any Maneb that may have been present) may be stored at room temperature overnight. Simply reheat the samples the following day at 100°C for approximately 30 minutes with shaking.
- When the volume of fortification solution added to the reaction vial is less than 1.0 mL, the volume of 10% EDTA added to obtain a final headspace volume of 135 mL is not adjusted to account for the addition of the fortification solution.

The addition of less than 1.0 mL of solution would account for at most, 0.74% of the total volume of headspace. It is felt by Morse Laboratories, Inc. that this percentage is insignificant and subtracting the fortification solution volume from the volume of 10% EDTA to be added is not necessary.

However, because volumes of fortification solutions greater than 1.0 mL do approach significance (1.35 mL would equate to a 1.0% error), volumes ≥ 1.0 mL are accounted for when calculating the volume of 10% EDTA to be added to obtain a final volume headspace of 135 mL.

SOP prepared by:

Gary Westberg
&
Kim Tufts

APPROVED BY: 
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