CAPTAN and THIOPHANATE-METHYL on Dermal Patch

9205

Thiophanate-Methyl:	$C_{12}H_{14}N_4O_4S_2$	MW: 342.40	CAS: 23564-05-8	RTECS:	BA3675000
Captan:	$C_9H_8Cl_3NO_2S$	300.59	133-06-2		GW5075000

METHOD: 9205, Issue 1 **EVALUATION: PARTIAL** **Issue 1: 15 March 2003**

	Captan	Thiophanate-methyl
OSHA:	N/A	N/A
NIOSH:	N/A	N/A
ACGIH:	N/A	N/A

PROPERTIES:
Thiophanate-methyl: Colorless prisms, mp 181.5-182.5°C, soluble in acetone, methanol, chloroform, acetonitrile, insoluble in water.
Captan: Odorless crystals, mp 178°C, soluble in chloroform, practically insoluble in H_2O

NAMES & SYNONYMS:
Thiophanate-Methyl: Topsin-M, [1,2-phenylenebis(iminocarbonothioyl)]bisdimethyl ester carbamic acid
Captan: N-(trichloromethyl)thio-4-cyclohexene-1,2-dicarboximide, Captec

SAMPLING

SAMPLER: DERMAL PATCH (Cleanroom wipe, 4" x 4")

PASSIVE EXPOSURE: place patch in card holder with 7.6-cm diameter circle cut in one side. Affix to worker's clothing or skin.

SHIPMENT: transfer patch to 50-mL centrifuge tubes with caps. Ship cold.

SAMPLE STABILITY: At least 28 days at 4°C.

BLANKS: 2 to 10 field blanks per set.

ACCURACY

RANGE STUDIED: Not determined

BIAS: Not determined

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

ACCURACY: Not determined

MEASUREMENT

TECHNIQUE: HPLC, UV detector

ANALYTE: Captan, Thiophanate-methyl

EXTRACTION: 30 mL 40% isopropanol:60% acetonitrile (V/V) w/ TEA-PO_4 preservative.

INJECTION VOLUME: 5 µL

MOBILE PHASE: A=2% n-propanol in water, 0.02 M TEA-PO_4, pH adjusted to 7.0 +/- 0.1 using phosphoric acid
B=2% n-propanol in acetonitrile.
Gradient from 20% B to 70% B (20 min.), decreasing to 20% B (2 min.), hold at 20% B (5 min.)

COLUMN: reversed phase C-18, 4µm, 250 x 2.00 mm or equivalent.

DETECTOR: UV @200 nm

CALIBRATION: Solutions prepared in extract solvent

RANGE: Table 1

ESTIMATED LOD: Table 1

PRECISION ($\hat{S}_r$): Table 1

APPLICABILITY: This method was developed for the analysis of the two analytes listed above on a dermal patch sampler from orchard workers. (See Figure 1.) In addition, this method may be used to analyze other fungicides and pesticides that may be present as long as they are separated from the two analytes by the analytical conditions of the method. It may be necessary to make adjustments to the gradient conditions of the method to obtain the required separation.

INTERFERENCES: The potential interferences include other organic compounds, in particular other pesticides or fungicides that have the same retention time on a C18 column. Positive identification may be confirmed by dual column chromatography using an appropriate alternative LC column. Possible interferences include: ziram, myclobutanil, mancozeb, imidacloprid, and phosmet.

OTHER METHODS: This method was developed in conjunction with method NMAM 5606, Thiophanate-methyl in air, and NMAM 9202, Captan and Thiophanate-methyl in handrnise.

REAGENTS:

1. Isopropanol, HPLC pesticide grade.*
2. Acetonitrile, HPLC grade.*
3. Triethylamine (TEA).*
4. Ortho-phosphoric acid >85% by weight, ACS grade or better.*
5. Deionized water.
6. Extraction solution: 40% isopropanol/60% acetonitrile w/2 mL TEA-PO_4 preservative for every liter of extraction solution prepared.
7. Thiophanate-methyl * stock solution, 10 mg/mL. Prepare in acetonitrile.
8. Captan* (Chem Service) stock solution, 5 mg/mL. Prepare in acetonitrile.
9. TEA-PO_4 Preservative. Dissolve 1.4 mL of TEA in 90 mL of deionized water in a 100 mL volumetric flask. Add phosphoric acid to lower pH to 7.0(± 0.1) as indicated by a calibrated pH meter. Bring volume to 100 mL with water. Keep tightly capped and refrigerated. Solution stable for 12 months.
10. Mobile phase A. Combine 20 mL of n-propanol and 2.8 mL TEA in a 1 L volumetric flask and bring to volume using deionized water. Adjust pH to 7.0 (+/- 0.1) with phosphoric acid using a pH meter. Final concentrations: 2% n-propanol, 0.02 *M* TEA-PO_4. Degas prior to use.
11. Mobile phase B. Add 20 mL of n-propanol to acetonitrile in a 1 L volumetric flask and bring to volume. Degas prior to use.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Dermal Patch: Texwipe™ AlphaWipe® polyester cleanroom wipe (4" x 4") in holder. The wipe is available commercially.
2. Patch Holder: White, 2-side chipboard, 0.08 SBS with a 7.6 cm diameter circle cut in one side (Wellman Container Corp, Fairfield, Ohio).
3. High Performance Liquid Chromatograph (HPLC).
4. Autosampler capable of 5 µL injections.
5. Analytical column: Phenomenex® Synergi™ 4µ Hydro-RP 80A (250 mm x 2.00 mm) or equivalent.
6. UV detector at 200 nm.
7. Vials, 2 mL, PTFE-lined caps.
8. Centrifuge tubes, polypropylene, 50 mL.
9. Syringes, 50-µL, 1-mL, and 5-mL.
10. Volumetric flasks, 5-mL, 100-mL, and 1-L.
11. PTFE syringe filter, 4-mm, 0.45-µm pore.
12. Large vial/tube rotator.
13. pH meter.
14. Graduated cylinders, 50-mL.
15. Pipettes, glass, disposable, 2-mL.
16. Forceps.
17. Bagged refrigerant.

SPECIAL PRECAUTIONS: Thiophanate-methyl: Avoid inhaling vapors or dust; avoid skin contact. Wear gloves and suitable clothing when handling pure material. Solvents: Avoid skin contact and open flame. Use in a hood. Phosphoric acid: Avoid skin contact. Captan: Use in hood. Avoid contact with skin, eyes, and clothing and ingestion or inhalation. Eye protection should be worn.

SAMPLING:

1. Secure the patch material in the patch holder with staples at two opposite corners. Attach the patch and holder to the appropriate location of the skin or clothing and sample for designated time period.
2. At the end of the sampling time, remove the patch from holder using clean forceps and transfer to a 50-mL centrifuge tube.
3. Label and pack the tube securely for shipment. These are shipped cold with other samples.
4. Samples should be stored under refrigeration until needed for analysis.

SAMPLE PREPARATION:

5. Add 30 mL of extraction solvent to each centrifuge tube and recap.
6. Mix by rotating the tubes end-over-end for at least one hour.
7. Filter an extract aliquot into a 2-mL autosampler vial through a 4-mm, 0.45 µm pore, PTFE filter.

CALIBRATION AND QUALITY CONTROL:

8. Determine retention times for analytes using the column and chromatographic conditions as outlined on pages 9205-1 and 9205-2. The approximate retention time of thiophanate-methyl is 14 minutes and captan is 21 minutes. (See Figure 1.)
9. Calibrate daily with at least six working standards containing each of the two analytes and covering the analytical range (Table 1) for thiophanate-methyl and captan.
10. Prepare QC samples by placing a new patch in a 50 mL tube and spiking with known amounts of the two analytes. Allow the samples to set open until the solvent has evaporated. Cap the tube and prepare for analysis in the same manner as the field samples in steps 5-7.

MEASUREMENT:

11. Set LC according to manufacturer's recommendations. Set the wavelength for detection at 200 nm and flow rate at 0.200 mL/min.
12. Inject 5 µL aliquot of the sample extract with autosampler.
    NOTE: If peak area of a sample is greater than the area of the highest standard, dilute with extracting solvent and reanalyze.
13. Measure peak area of the analyte.

CALCULATIONS:

14. Perform a separate regression analysis of the peak areas vs. quantity of standard for each of the two analytes. Determine the concentration, µg/mL, (corrected for DE) of the analyte in each sample patch (C_P) and in the media blank (B_b) from the calibration graph.
15. Calculate the mass of each analyte, M (µg), on a sample patch by adjusting for the volume , V (mL) of extraction solvent.

$$M = (C_P V_P - B_b V_b), \mu g$$

EVALUATION OF METHOD:

This method was evaluated with a recovery study at room temperature over the range of 306.0 - 6120 µg/sample for thiophanate-methyl and 300.4 - 6220 µg/sample for captan using spiked laboratory samples with respective average recoveries in the range of 89.1-95.5% and 89.6-96.0%. [1] The storage study at 4°C was completed at 6000 µg/sample for thiophanate-methyl, and 1500 µg/sample for captan with respective recovery averages of 86.2-92.2%, and 87.6-95.7% over the 28 days of the study.

Carbendazim, which is a decomposition product of thiophanate-methyl, may also be found in the chromatogram along with the other two analytes. An attempt was made to quantitatively analyze carbendazim as a part of this method. However, reproducibility problems for carbendazim in the presence of thiophanate-methyl has necessitated that the analysis be only qualitative for carbendazim. The reproducibility problem was more pronounced when the ratio of thiophanate-methyl to carbendazim was greater than about eight to one. Additionally, this problem is compounded when other materials, such as benomyl, which also decomposes to produce carbendazim, are present in the sample. Because this method is unable to distinguish the decomposition source of the carbendazim, it can only provide qualitative data for carbendazim. Under the method conditions, its approximate retention time is 9 minutes.

Field samples have been analyzed by this method. Because the field samples contained other organic compounds and/or interferences, it was necessary to make adjustments to the analysis parameters. For example, it was necessary to insert a longer column rinse at the end of the solvent gradient to elute higher molecular weight compounds and/or other material from the column. It was also necessary to change the end of the gradient solvent run to have a greater organic phase ratio to help rinse the column of these materials.

Based on the needs of the laboratory and the complexity of the samples to be analyzed, it may be necessary to make other changes to the analysis parameters to get the best resolution of the compounds in the samples.

REFERENCES:

[1] Jaycox LB, Andrews RN [2002]. Backup data report for Captan and Thiophanate-methyl on Dermal Patch method development, Cincinnati, OH: National Institute for Occupational Safety and Health, DART/NIOSH (unpublished, June).
[2] NIOSH [1998]. Method 9201: Chlorinated organonitrogen herbicides (dermal patch). In: Cassinelli ME, O'Connor PF, eds. NIOSH Manual of Analytical Methods (NMAM), 4th ed., 2nd supplement. Cincinnati, OH: National Institute for Occupational Safety and Health, DHHS (NIOSH) Publ 98-119.

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Table 1.

Analyte	Range	Estimated LOD	Precision ($\bar{S}_r$)
Captan	67.5-6220 µg/sample	20.2 µg/sample	0.0256
Thiophanate-methyl	62.0-6120 µg/sample	18.6 µg/sample	0.0229

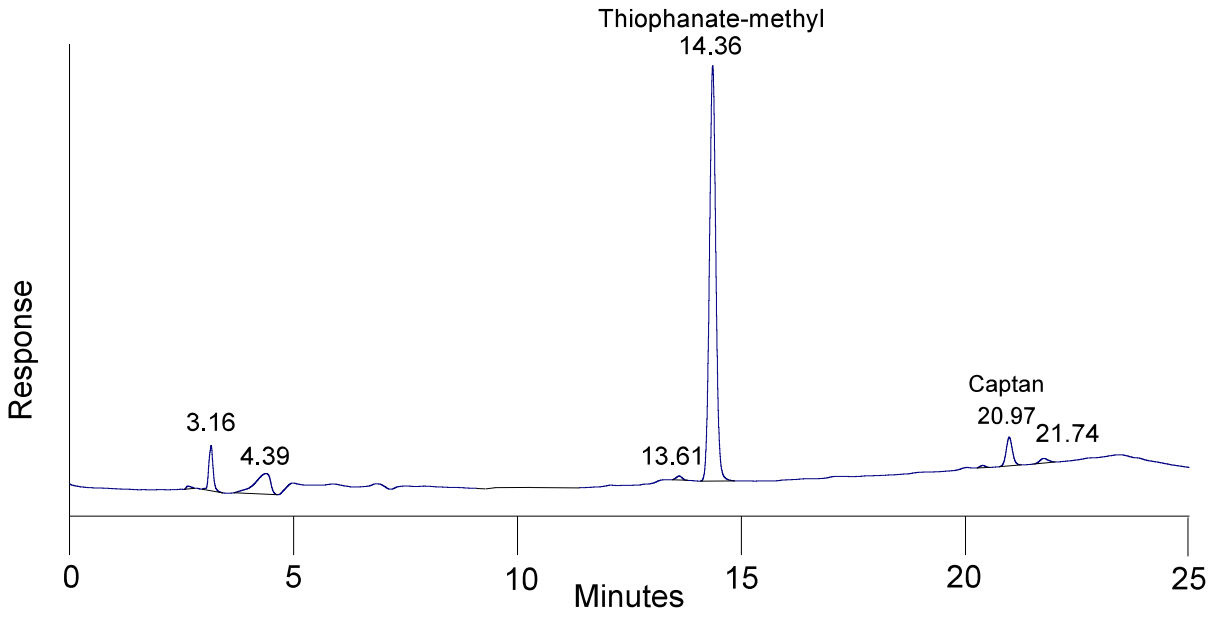

Figure 1 This chromatogram shows results from a spiked patch sample that was extracted and analyzed by this method. The analyte quantities were: thiophanate-methyl - 200 µg/sample and captan- 50 µg/sample.