CAPTAN - 03

232/237

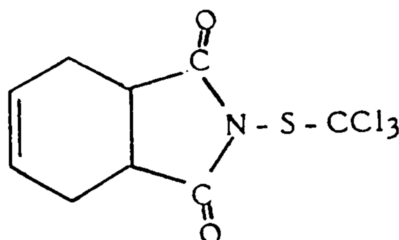
CHEVRON CHEMICAL COMPANY
AGRICULTURAL CHEMICALS DIVISION
RESEARCH AND DEVELOPMENT DEPARTMENT
RICHMOND, CALIFORNIA

DETERMINATION OF CAPTAN AND THPI RESIDUES IN CROPS (RM-1N)

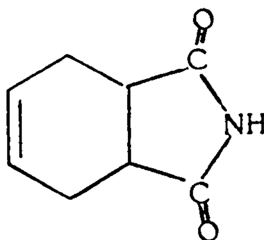
DATE: APRIL 4, 1986
FILE NO: 740.01/CAPTAN

INTRODUCTION

Captan [cis-N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide] is a fungicide with a wide spectrum of activity.



4-Cyclohexene-1,2-dicarboximide (or tetrahydrophthalimide hereafter referred to as THPI) has been shown to be a principal metabolite of captan.



This analytical method determines both the parent compound captan, and the metabolite, THPI, and is a revision of RM-1K-2. This revision describes the procedure as carried out by Morse Laboratories and uses a Coulson electrolytic conductivity detector for analysis which allows the elimination of the florisil column step for all samples and of the GPC step for non-oily crops.

REAGENTS

Acetone - nanograde

Acetonitrile - nanograde

Ethyl acetate - nanograde

Hexane - nanograde

Methanol - nanograde

Dichloromethane - nanograde

Florisil - activated at 1200°F - Mix 3 parts by weight of 60-100 mesh with 2 parts 100-200 mesh. Heat approximately 16 hours at 110°C prior to using.

Nuchar - S-N, Westvaco, Covington, Virginia

Phosphoric acid - (85%) AR

Silica gel - chromatographic grade, E. Merck Darmstadt, Germany, 75-235 mesh, Cat. No. 7734

Sodium Sulfate - Anhydrous, granular, reagent grade, washed with acetone (nanograde) and dried thoroughly before using.

Fractogel® PVA-2000 available from EM Laboratories, Inc., 500 Executive Blvd., Elmsford, New York, 10523, Cat. No. 9375

Captan - reference standard - (Prepare stock solutions in acetone).

THPI - reference standard - (Prepare stock solutions in acetone).

SPECIAL EQUIPMENT: (NOTE: Use of plastic equipment should be avoided)

Automatic Gel Permeation Chromatograph - Model 1002 GPC (available from Analytical Bio Chemistry Laboratories, Inc., Columbia, MO 65205 equipped with 25 x 600 mm gel permeation column, packed with 35g Bio Beads S-X3, 200-400 mesh, after swelling in chloroform overnight. Eluting solvent - chloroform. Flow rate: 5 ml/min.

Osterizer and/or Hobart food chopper.

Omni-Mixer with adaptor for use with pint and quart Mason jars.

Rotary vacuum evaporators equipped with 40°C water bath for evaporation steps.

Liquid chromatographic columns - 25 x 400 millimeters equipped with Teflon® stopcocks.

Centrifuge tubes, glass-stoppered, 15 ml.

B-D Multifit Syringe #2152 with Luer-lok-10cc capacity.

Filter holder for 13 mm filters (Gelman #4310)

Gas Chromatograph (Varian 2100 or equivalent) equipped with a Coulson electrolytic conductivity detector in the halogen mode and the following parameters for captan analysis:

Column A: 3% SP-2401 on Supelcoport, 100/120 mesh, 2' x 1/4" od glass column

Column B: 10% DC-200 on Chromosorb W HP, 80/100 mesh, 4' x 1/4" od glass column

	Column A	Column B
Carrier gas (H_2) flow rate:	90 ml/min.	90 ml/min.
Pyrolysis temperature:	810°C	810°C
Oven temperature:	155°C	205°C
Injector temperature:	240°C	240°C
Detector temperature:	--	--
Retention time:	2.4 min.	2.4 min.

Gas Chromatograph (Varian 2100 or equivalent) equipped with a Coulson electrolytic conductivity detector in the nitrogen mode and the following parameters for THPI analysis:

Column A: 5% EG ss-x on Gas Chrom Q, 100/120 mesh, 3' x 1/4" od glass column.

Column B: 20% OV-11 on Chrom W HP, 100/120 mesh, 2' x 1/4" od glass column.

Column C: 1% HiEff 8BP on Gas Chrom Q, 100/120 mesh, 2' x 1/4" od glass column.

	Column A	Column B	Column C
Carrier gas (H_2) flow rate:	130 ml/min.	120 ml/min.	140 ml/min.
Pyrolysis temperature:	810°C	810°C	810°C
Oven temperature:	180°C	160°C	145°C
Injector temperature:	240°C	240°C	240°C
Detector temperature:	--	--	--
Retention time:	2.4 min.	2.5 min.	2.4 min.

EXTRACTION

Captan is reported to be unstable in a macerated crop matrix even when stored at -20°C. This instability requires analysis of captan-treated crops immediately following maceration and sub-sampling, and precludes re-analysis of samples in cases where the initial analysis is unsuccessful. To insure that acceptable residue data is obtained for all residue trial samples, the following order of analysis is to be followed. Untreated control samples which are a part of all scheduled residue trials are analyzed first. If no captan or THPI peaks are found (<0.05 ppm), proceed with the analysis of the treated samples. In cases where captan residues are confirmed by THPI residues, assume that the control sample is field contaminated and obtain an alternate clean control sample or fortify the contaminated check sample at levels 4 times higher (not to exceed 2x expected level in treated samples) than the ppm level found in the check sample for recovery purposes. For situations where apparent residues of captan or THPI are not due to field contamination, re-analyze control samples by RM-1L as an alternative procedure in an attempt to eliminate the interference peak. If interference peaks are not eliminated, obtain an alternate control sample.

Non-Oily Crops

Macerate the sample using a Hobart food chopper or Osterizer blender. Transfer a 50 gram sample to a quart Mason jar. Immediately add 3 ml 85% phosphoric acid and stir. (Fortify the untreated samples for recovery purposes with 1.0 ml of an acetone solution of mixed reference standards containing 5 µg/ml of each chemical species.) To dry crop samples, add up to 50 ml water, as needed, to thoroughly wet the sample. Add 150 ml ethyl acetate, 200 grams sodium sulfate and blend on an Omni-Mixer for 5 minutes. Filter the extract through sodium sulfate into a round-bottom flask. Repeat the extraction and filtration step two more times using 100 ml of ethyl acetate each time. (Note: If thorough agitation is not obtained during the blending step, additional ethyl acetate may be required.) Proceed with the ethyl acetate:water partition step.

Oily Crops

Proceed as for non-oily crops above. Evaporate the combined extracts to dryness and proceed with the acetonitrile:hexane partition step.

SAMPLE CLEANUP

Ethyl Acetate:Water Partition Cleanup for Non-oily Crops

Transfer the combined extract to a 1000 ml separatory funnel and wash, by gently swirling, with three 50 ml portions of 0.5-1% phosphoric acid. Filter the ethyl acetate through sodium sulfate. Evaporate the extract to dryness and proceed with the Nuchar:Silica Gel column separation.

Acetonitrile:Hexane Partition Cleanup for Oily Crops

Transfer the residue to a separatory funnel using a total of 25 ml acetonitrile and 100 ml hexane. If necessary, add sodium sulfate to the round-bottom flask to aid in removal of the residue from sides of the flask. Gently swirl the separatory funnel for one minute. Transfer the acetonitrile to a second separatory funnel. Wash the acetonitrile with two 100 ml portions of hexane. Extract each hexane wash, in turn, with two 25 ml portions of acetonitrile. Combine the acetonitrile extracts, evaporate to dryness, and proceed with the GPC cleanup.

Gel Permeation Chromatography (on oily crops only)

Dissolve the residue in 10.0 ml dichloromethane:acetone - 3:7. (If necessary, add a few grams of sodium sulfate and swirl to remove the solid material from the walls of the flask.) Either (1) pour the solution into the barrel of a 10 cc syringe with filter attached and force the solution through the filter into a vial by depressing the syringe plunger or (2) transfer the solution to a centrifuge tube, centrifuge at 2000 rpm for 10 minutes, and decant the supernatant solution into a vial.

Load the 5 ml sample loops of the GPC with clear solutions. Adjust the chromatographic cycle to dump for 14 minutes and collect for 15 minutes. (These parameters will vary and must be established by each individual laboratory.) Evaporate the eluate to dryness and proceed with the Nuchar-silica gel column separation.

Nuchar:Silica Gel Column Separation (for all samples)

Place a glass wool plug in the bottom of the column. Add 15 grams of the Nuchar:silica gel (5:95 w/w) mixture and cover the mixture with a glass wool plug. Wash the column with 100 ml 5% ethyl acetate in dichloromethane, followed by 2 x 25 ml dichloromethane rinses.

Dissolve the residue in 10 ml dichloromethane and transfer to the column with two 10 ml dichloromethane rinses. Allow the dichloromethane to drain to the top of the glass wool plug, then add 20 ml dichloromethane and elute. Adjust flow rate to obtain discrete drops of column eluate. Discard the dichloromethane eluates and elute captan residues with 150 ml 5% ethyl acetate in dichloromethane. Replace the flask with a second round-bottom flask and elute the THPI with 125 ml 15% acetone in dichloromethane. Evaporate the eluate containing captan to dryness and proceed with the Florisil column chromatography step. Evaporate the eluate containing the THPI to dryness and proceed with the measurement for THPI.

MEASUREMENT

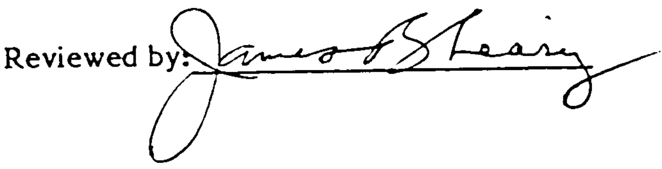
Captan

Dissolve residue in volume of solvent to make a concentration of 10 g/ml. Inject suitable amount of reference standard solutions (0.1, 0.4, 0.8, 1.0 µg/ml captan in hexane) into the gas chromatograph to contruct a standard curve. Because captan response is not linear, a "Shipman's Curve" is used to draw line between points. Inject the same volume of each sample and standards. Determine the concentration of the sample solution by comparing the peak height of the sample with standard curve.

THPI

Dissolve residue in volume of solvent to make a concentration of 10 g/ml. Inject a suitable volume of the reference standard solution (1.0 µg/ml THPI in benzene) to obtain a 10 - 15 cm peak. Inject the same volume of sample solution. Determine the concentration of the sample solution by comparing the peak height of the sample with that of the standard.

G. O. BREAULT

Reviewed by: 

GOB:vll

cc: J. B. Leary
Residue Lab (2)
R&D Files