

CAPTAN-02

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COMPONENT

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Determination by Gas Chromatography of Captan and
Tetrahydrophthalimide Residues in Raw and Processed Agricultural Crops

Report No. WRC 89-51

June 1, 1989

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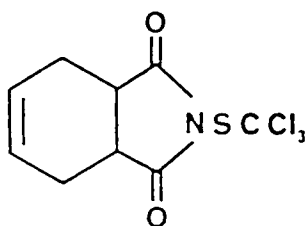
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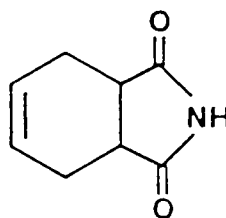
I. SUMMARY/INTRODUCTION

This method is intended for determining residues in raw and processed agricultural crops of captan at levels of 0.05 to 100 ppm and of tetrahydrophthalimide (THPI) at levels of 0.05 to 10 ppm. This method is an updated version of the Chevron Chemical Company method "Determination of Captan Residues and its Primary Metabolite Tetrahydrophthalimide in Crops" (reference 1). Therefore, only data obtained for the analysis of apple fruits are presented herein to illustrate the use of this method.

Captan is the active fungicidal ingredient in the various formulated products marketed by ICI Americas Inc. under the trademark "CAPTAN". The chemical name assigned to captan by Chemical Abstracts Service (9th CI, CAS no. 133-06-6) is 3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione. Tetrahydrophthalimide (THPI) is the principal degradation product of captan. The chemical name for THPI (CAS no. 27813-21-4) is 1,2,3,6-tetrahydrophthalimide. The chemical structures are given below.



Captan



Tetrahydrophthalimide
(THPI)

Captan and THPI are extracted from the sample matrix by macerating the matrix with ethyl acetate in the presence of phosphoric acid and anhydrous sodium sulfate. The extract is filtered. The filtrate is washed with 1% aqueous phosphoric acid solution, and the solvent is removed. The residual material is placed on a nuchar/silica gel column. Column elution with various eluents accomplishes extract cleanup and analyte separation. The analytes are quantified by using gas chromatography, in-line reductive pyrolysis, and electrolytic conductivity detection. Halogen (HCl) detection is used for captan analyses, and nitrogen (ammonia) detection is used for THPI analyses.

II. MATERIALS/METHODS

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment with equivalent performance specifications and reagents of comparable purity can be used. Avoid the use of plastic equipment.

A. Apparatus

1. Gas Chromatograph. Varian model 2100 designed for use with packed columns. The gas chromatograph is used with manual injections.
2. Coulson Electrolytic Conductivity Detector. Operate in the halogen and nitrogen modes for the analysis of captan and THPI, respectively.
3. Gas-Chromatographic Column. Captan: 4.0 ft x 0.25 inch o.d. borosilicate-glass column packed with 3% SP2401 on 100-120 mesh Supelcoport. THPI: 2.5 ft x 0.25 inch o.d. borosilicate-glass column packed with 5% EGSS-X on 100-120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA).
4. Evaporator. Equipped with a temperature-controlled water bath.
5. Omni-Mixer. With adapter for use with 1-pt Mason jars.
6. Chromatographic Columns. 400 x 25 (o.d.) mm borosilicate-glass column equipped with Teflon stopcock.
7. Separatory Funnels. 125- and 500-mL capacity with Teflon stopcock.
8. Filter Paper. Whatman no. 541.
9. Syringes. 25-uL capacity (Hamilton 702N) for injection of solutions into the gas chromatograph.

B. Reagents

1. Solvents. Acetone, acetonitrile, benzene, dichloromethane, ethyl acetate, and hexane. All solvents must be of high purity and suitable for use in trace organic analyses by gas chromatography.
2. Captan and Tetrahydrophthalimide (THPI). Analytical reference-standards were supplied by Chevron Chemical Company, 15049 San Pablo Avenue, Richmond, CA 94804-0010. Captan (lot no. 5722-43) was 99% in purity. THPI (lot no. 9197-19) was 99.9% in purity.

3. Sodium Sulfate, Anhydrous. Of high purity and suitable for use in trace organic analyses by gas chromatography.
4. Phosphoric Acid, 85%. Reagent grade.
5. Nuchar. S-N (Westvaco; Covington, VA).
6. Silica Gel. 100-200 mesh, grade 923 (Davison Chemical). Heat at 125°C for 18 hours, and allow to cool to ambient temperature in a sealed container prior to use.
7. Keeper Solution. 10% (w/v) 1-decanol in acetone.
8. Calibration and Fortification Solutions.

To prepare a 1.00 mg/mL (= 1000 ug/mL) stock solution of an analyte, place 25 ± 0.1 mg of analyte in a 25-mL volumetric flask. If the primary standard is not 100% pure, the weight of primary standard used must be adjusted for the purity as follows:

$$W = \frac{25}{P}$$

where W = weight of standard used (mg),
P = purity of the standard (100% = 1.00).

Add 10 to 20 mL of acetone to the 25-mL volumetric flask, and swirl the flask to dissolve the analyte. Then, slowly add more acetone until the bottom of the solvent meniscus aligns with the volumetric mark. Close the flask with a glass stopper, and mix thoroughly.

To prepare working calibration solutions, serially dilute the acetone stock-solution with benzene to give 10, 1.0, 0.10, and 0.010 ug/mL solutions or other concentrations as required. To prepare working fortification solutions, serially dilute the acetone stock-solution with ethyl acetate to give 100, 10, and 1.0 ug/mL solutions, or other concentrations as required.

NOTE: Captan has a very low solubility in organic solvents. Be aware that for 1000 and 100 ug/mL concentrations, captan could crystallize out of solution, especially if the solution is chilled in a freezer. The freezing point of benzene is -11°C (12°F).

9. pH 11 Buffer.

C. Analytical Procedure

Chop a representative one-third subsample of the frozen commodity in a food chopper. Add pulverized dry ice to the sample during chopping to ensure that the sample remains frozen. Place the chopped sample in a freezer (about 30 min) to allow the dry ice to sublime before removing an aliquot for extraction.

1. Extraction

Place 20 g of sample in a 1-pt jar. Add 3 mL of 85% phosphoric acid to ensure that a strongly acidic environment is maintained. Add sufficient anhydrous sodium sulfate to absorb the moisture liberated from the sample (about 175 g). Add 100 - 120 mL of ethyl acetate to ensure a thorough blending, and blend the sample for 3 min with an Omni-Mixer. Decant the ethyl acetate extract into a glass filter lined with a Whatman no. 541 filter that contains about 40 g of anhydrous sodium sulfate. Collect the filtrate in a 500-mL flask. Repeat the sample extraction two additional times with sufficient ethyl acetate to allow a thorough blending. Collect each filtrate in the same flask. The combined volume of ethyl acetate used in the three extractions should be 300 mL. Continue with either (a) or (b).

a. Non-oily Crops

Transfer the combined ethyl acetate extract to a 500-mL separatory funnel. Wash the extract three times by using 50 mL of 1% aqueous phosphoric acid each time. Discard the aqueous wash solutions. Pass the ethyl acetate through anhydrous sodium sulfate and into a 500-mL flask. Place the flask on a rotary evaporator, and remove almost all of the solvent with the aid of a water bath at 40 C. Remove the last traces of solvent with a gentle stream of nitrogen. Dissolve the residual material in 10 mL of dichloromethane and perform a column cleanup and separation.

b. Oily Crops

Evaporate the ethyl acetate from the combined extract. Transfer the residual material to a separatory funnel by using a total of 25 mL of acetonitrile and 100 mL of hexane. If necessary, add sodium sulfate to the round-bottom flask to aid in removal of the residue from the wall 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, and evaporate the acetonitrile. Dissolve the residual material in 10 mL of dichloromethane and perform a column cleanup and separation.

2. Column Cleanup and Separation

Place a thick glass wool plug in the bottom of the column. Place 15 g of nuchar:silica gel (5:95 w/w) mixture in the column. Place a glass wool plug on top of the mixture. Wash the column sequentially with 100 mL of 5% ethyl acetate in dichloromethane and two 25-mL portions of dichloromethane. Add the 10-mL dichloromethane extract to the column, and use two 10-mL portions of dichloromethane to complete a quantitative transfer. Allow the dichloromethane to drain until the liquid level drops to the top of the glass wool plug. Elute the column with 20 mL of dichloromethane; discard the eluate. Elute the captan with 150 mL of 5% ethyl acetate in dichloromethane. Change the collection flask, and elute the THPI with 150 mL of 20% acetone in dichloromethane. Add three drops of keeper to each eluate. Remove the solvent from each eluate with a rotary evaporator and a water bath at 40 C. Proceed with gas chromatographic analyses. If the gas chromatographic analysis of THPI sample extracts indicates that additional cleanup is required for quantitation, follow the procedure given in the next paragraph.

Transfer the sample extract to a 50-mL round-bottom flask and evaporate the solvent just to dryness. Add 25 mL of alkaline water (pH 11), and transfer the sample to a 125-mL separatory funnel. Wash the aqueous phase twice with 25 mL of dichloromethane each time; discard the dichloromethane washes. Acidify the aqueous phase by adding 3 mL of concentrated phosphoric acid. Extract the aqueous phase twice with 25 mL of dichloromethane each time. Filter each dichloromethane extract through anhydrous sodium sulfate and into a round-bottom flask. Evaporate the solvent just to dryness. Proceed with gas chromatographic analysis.

Note: 1) Preservatives such as ethanol in the dichloromethane will affect the elution profile of the nuchar-silica gel column. 2) THPI can be lost if it continues to be subjected to heat and reduced pressure after the solvent has been evaporated.

3. Fortification

Analyze unfortified and fortified control samples with each sample set to demonstrate method recovery. For example, for 20-g samples, place 20 g of untreated

control sample into a 1-pt Mason jar; add 0.20 mL of the 1.0, 10, 100, or 1000 ug/mL fortification solution to produce a fortification level of 0.01, 0.10, 1.00 or 10.0 ppm (Use of similar volumes and concentrations are appropriate). Perform the sample extraction and extract cleanup as detailed in sections C.1 and C.2 above.

D. Instrumentation

1. Operating Conditions

Follow the manufacturer's instructions for operation of the gas chromatograph and Coulson electrolytic conductivity detector. The specific conditions listed below were used to generate the data and chromatograms presented in this report.

Gas Chromatograph:

	<u>captan analysis</u>	<u>THPI analysis</u>
Column liquid-phase:	SP 2401	EGSS-X
Carrier gas:	hydrogen	hydrogen
Column flow-rate:	110 mL/min	90 mL/min
Inlet temperature:	240°C	240°C
Oven temperature:	166°C	180°C
Volume injected:	20 uL	20 uL
Total run time:	4 min	5 min

Coulson Electrolytic Conductivity Detector:

Pyrolysis temperature: 810°C
 Pyrolysis gas: hydrogen
 Catalyst: Ni for nitrogen mode, none
 for halogen mode

Using the above conditions, the elution times of captan and THPI were 2.4 and 2.4 min, respectively. See Figures 1 and 2 for typical chromatograms for captan and THPI analyses, respectively.

2. Calibration

Calibrate the gas chromatograph by using the analyte calibration solutions specified in section II.B.8. Calibrate the instrument by using 0.15, 0.80, 1.6 and 2.0 ug/mL solutions or similar concentrations as appropriate.

3. Analysis of Extracts

Dissolve the final residue in a volume of solvent sufficient to give a concentration of 5.0 g of original sample weight/mL of benzene (e.g., 4.0 mL for a 20-g sample). Inject suitable amounts of calibration

solutions (0.15, 0.80, 1.6, and 2.0 ug analyte/mL benzene) into the gas chromatograph to construct a standard curve. Because the captan response is nonlinear to the amount injected, a "Shipman's Curve" is used to draw a curve through the data points. Inject the same volume of the sample extracts and calibration solutions. Determine the concentration of the analyte in the sample extract by comparing the obtained peak height to the standard curve.

Inject the sample extracts using the same conditions used for calibration. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of retention times (within 0.03 min) with those of the calibration chromatograms. Reinject the calibration solution after injection of every two to four sample extracts and at the end of the chromatographic run. Calculate the concentration of the analyte(s) in the sample extract by comparing it to the closest standard (peak height) or by use of a standard curve.

E. Interferences

Extractives from crops could potentially contribute peaks with retention times coincident with or near that of the analyte(s). Satisfactory resolution can usually be achieved with appropriate oven temperature manipulations or column selection (length, phase). Alternate chromatographic columns and conditions for their use are given below.

Gas-Chromatographic Column. Captan: 4.0 ft x 0.25 inch o.d. borosilicate-glass column packed with 10% DC-200 on 80-100 mesh Chromosorb W HP. THPI: 2.0 ft x 0.25 inch o.d. borosilicate-glass column packed with 20% OV-11 on 100-120 mesh Chromosorb W HP. THPI: 2.0 ft x 0.25 inch o.d. borosilicate-glass column packed with 1% HiEff 8BP on 100-120 mesh Gas-Chrom Q.

	<u>captan analysis</u>
Column liquid-phase:	DC-200
Carrier gas:	hydrogen
Column flow-rate:	90 mL/min
Inlet temperature:	240°C
Oven temperature:	105°C
Furnace temperature:	810°C

	<u>THPI analysis</u>	<u>THPI analysis</u>
Column liquid-phase:	OV-11	HiEff 8BP
Carrier gas:	hydrogen	hydrogen
Column flow-rate:	120 mL/min	145 mL/min
Inlet temperature:	240°C	240°C
Oven temperature:	160°C	180°C
Furnace temperature:	810°C	810°C

Figures 1 and 2 show typical chromatograms. Analyze extracts of samples from untreated plots to demonstrate the absence of interferences from sample matrices, solvents, and labware. Captan is chemically similar to the fungicides captafol and folpet. Similar to captan, the degradation of captafol can produce THPI, whereas folpet produces phthalimide. Phthalimide will not interfere in the analysis of THPI. Similar to captan, folpet contains the (trichlormethyl)thio moiety, whereas captafol contains a (tetrachlorethyl)thio moiety.

F. Confirmatory Techniques

Unexpected positive results, as in untreated control or pre-application samples, should be confirmed by other means. Confirmation can be achieved by quantitation by using a different detector type, such as a nitrogen-phosphorus or mass-selective (selective-ion-monitoring) detector.

G. Calculations

The concentration of the analyte in the original sample is calculated by using the external standard method, i.e., the response obtained for the analyte in the sample extract is compared to the response obtained from a separate injection of a known amount of analyte (calibration solution). It is assumed for the calculations outlined below that the injection volumes for all calibration solutions and sample extracts are fixed at the same volume.

1. Linear Detector-Response

a. Calibration Factor

Calculate the response factor, F, for injection of a calibration solution as follows:

$$F \text{ ((ng/uL)/response unit)} = \frac{C_{\text{std}}}{R_{\text{std}} \times S}$$

where C_{std} = concentration of calibration solution, ng/uL
 R_{std} = response units (e.g., peak height, peak area, electronic units) from detector for calibration solution
 S = ratio of amount (g) of sample extracted to volume (mL) of extraction solvent used.

If the extract has been concentrated or diluted, S can be calculated as follows:

$$S = \frac{W_{\text{sample}}}{V_{\text{solvent}}} \times \frac{V_{\text{initial}}}{V_{\text{final}}}$$

where W_{sample} = total weight of sample extracted, g

V_{solvent} = total volume of solvent used in extraction, mL

V_{initial} = volume of initial extract taken for analysis, mL

V_{final} = final volume of extract after concentration or dilution, mL

b. Analyte in Sample

Calculate the analyte concentration, R, in the original sample as follows:

$$R \text{ (ug/g or ppm)} = F \times R_{\text{sample}}$$

where F = response factor, (ng/uL)/response unit

R_{sample} = response units from detector for analyte in the sample extract

Averaged response factors obtained from injections of calibration solution before and after injection of sample extracts may be used for calculation of the analyte concentration in the sample.

2. Nonlinear Detector-Response

a. Analyte in Sample

Generate a standard curve by plotting the concentration of the calibration solution (C_{std}) as the x-axis and the corresponding response units from the detector (R_{std}) as the y-axis for a range of analyte concentrations as shown in Figures 3 and 4. Take the response units from the detector for the analyte in the sample extract (R_{sample}) and determine the concentration of the analyte in the sample extract (C_{sample}) by using the standard curve.

Calculate the analyte concentration, R, in the original sample as follows:

$$R \text{ (ug/g or ppm)} = C_{\text{sample}}/S$$

b. Calibration Factor

Calculate the response factor, F, for the theoretical injection of an appropriate calibration solution as follows:

$$F((\text{ng}/\mu\text{L})/\text{response unit}) = R/R_{\text{sample}}$$

III. DISCUSSION

A. Precision and Accuracy

Fortified apple samples were prepared as described under section II.C.2 and analyzed according to this method to establish accuracy. Table 1 shows recoveries from apple samples fortified from 0.05 to 100 ppm of captan ranged from 82 to 111 % with a mean recovery of 88% (n=18) and

coefficient of variation (CV) of 6.7%; $CV = 100 \times (\text{standard deviation}/\text{mean})$. Recoveries from apple samples fortified from 0.05 to 10 ppm of THPI ranged from 71 to 115% with a mean recovery of 90% (n=18) and CV of 9.9%. The precision of the method depends on variations in extraction and instrumental analysis. The variations in extraction and instrumental analyses can be evaluated from the data obtained during analyses of fortified samples. The coefficient of variations given in Table 1 are a measure of precision.

B. Detection Limit

The detection limit of the method is 0.05 ppm for each of the two analytes as determined by fortifications at the 0.05-ppm level with at least a 5-mm peak height.

C. Dry-Weight Basis

This method determines the residues on an as-received basis, i.e., fresh raw or processed agricultural crop.

D. Safety Precautions

Personnel untrained in the routine safe-handling of chemicals and good laboratory practices must not attempt to use this procedure. Information on any specific chemical regarding physical properties, hazards, toxicity, and first-aid procedures can be found on the Material Safety Data Sheets accompanying the chemical or available from the chemical supplier. In general, always wear safety glasses with sideshields, work in a well ventilated area, avoid inhaling vapors, and avoid contact of the chemical with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

1. Flammable Solvents. Acetone, acetonitrile, benzene (CANCER SUSPECT AGENT), dichloromethane (CANCER SUSPECT AGENT), ethyl acetate, and hexane.
2. Captan and THPI. Remove contaminated clothing and wash affected skin area with soap and water after any accidental contact. Wash eyes with plenty of water after any accidental contact.

IV. CONCLUSIONS

The method is selective for the analysis of captan and THPI in raw and processed agricultural crops. Although only data for apple fruits are presented herein, this method has been used extensively with a variety of raw and processed agricultural crops. Only commercially available laboratory equipment and reagents are required. The analysis can be completed by one person in an 8-hr period. Untreated and fortified untreated samples should be extracted and analyzed with each set of samples to demonstrate absence of interferences and adequate recovery. If determination of captan at a concentration other than 0.05 to 100 ppm and of THPI at a concentration other than 0.05 to 10 ppm are required, suitably fortified samples must be analyzed to validate the method at that concentration.

V. CERTIFICATION

This is to certify that this is a complete and unaltered report prepared by the Environmental Sciences Department of ICI Americas Inc., Western Research Center.

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VI. REFERENCES

1. "Determination of Captan Residues and its Primary Metabolite Tetrahydrophthalimide (THPI) in Crops", Chevron method no. RM-1K-2 (12-29-82).
2. Morse Laboratories, Inc. worksheet 50022.