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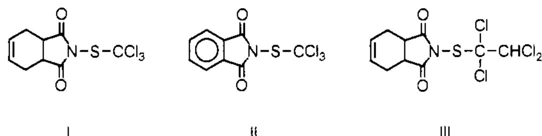
High-Pressure Liquid Chromatographic Determination of Captan, Captafol, and Folpet Residues in Plant Material

Bruno Büttler* and Wolf D. Hörmann

A specific method is described for quantitative determination of captan, captafol, and folpet residues in deciduous fruits, grapes, and cereals. The procedure involves the extraction of the samples with acetone and cleanup by partition between acetone-water and *n*-hexane. Individual cleanup procedures by column chromatography on silica are described for the separate determination of each of these fungicides. The simultaneous determination of captan, captafol, and folpet involves cleanup by gel chromatography. The fungicides are separated by high-pressure liquid chromatography on a cyano bonded phase and detected by conductivity measurement with a photoconductivity detector operated at 254 nm. Recoveries in the range of 75-120% indicate that these procedures are suitable for the residue analysis of these fungicides with detection limits of 0.02 mg/kg in fruit and grain and of 0.05 mg/kg in other plant materials.

Captan [*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, I], folpet [N-(trichloromethylthio)phthal-



imide, II], and captafol [*N*-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide, III] are three fungitoxic chemicals which have in common the phthalimide-type skeleton.

Numerous gas and thin-layer chromatographic (GC; TLC) residue methods exist. Only a few newer methods are referred to here; the publications contain further references. Pomerantz et al. (1970) described the simultaneous determination of captan, folpet, and captafol in various crops using electron capture GLC detection. Either QF-1 or XE-60 columns were used after acetonitrile extraction from fortified crops, partitioning into dichloromethane-petroleum ether, and cleanup on Florisil. With this procedure, the limit of detection for captan and folpet was 0.1 mg/kg and for captafol was 0.8 mg/kg. Barker and Flaherty (1972) improved the analytical method of Pomerantz. It was claimed that residues of folpet and captan at the 0.05 mg/kg level and of captafol at the 0.1 mg/kg level could be determined. Carlstrom (1971) reported the degradation of captan under certain gas chromatographic conditions. The possibility of decomposition of folpet under the conditions of gas chromatography made Carlstrom (1977) describe a high-pressure liquid chromatography method, using UV detection for the determination of this fungicide in formulations. Lemperle and Strecker (1971) reported the determination of folpet and captafol in grapes. They recommended that the chromatograms should not be evaluated via a standard curve because of the changing sensitivity of the electron capture detector. We too noted that the changing sensitivity of detection caused problems when determining captan, captafol, and folpet by gas chromatography, using electron capture or microcoulometric detection.

Various conditioning procedures were reported in the literature. No one gave a significant and lasting improvement in routine determination of these fungicides at the residue level in different crops. As a result of the changing sensitivity, the evaluation of the chromatograms by standard curves was not possible. The required limits of determination were often not reached. The reliable and rapid determination of residues of captafol, captan, and folpet to be an unresolved problem was also mentioned by Greve (1979).

Changing sensitivity of the electron capture detector, probably due to absorption and/or decomposition during the gas chromatographic process, was the major problem we encountered when we decided to develop a residue method based on high-pressure LC. We first developed a residue method for the determination of folpet by high-pressure LC using UV detection. Poor extinction coefficients of captan and captafol did not allow the analogous determination of captafol and captan.

At that time Tracor introduced a new selective detector for liquid chromatography on the market. This photo-

Ciba-Geigy Ltd., Agricultural Division, CH-4002 Basel, Switzerland.

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conductivity detector was claimed to be very sensitive for certain halogen- and nitrogen-containing compounds. A chromatogram of a 25-ng standard of captan, detected with the photoconductivity detector of Tracor after separation on a ZORBAX CN column with isooctane–methanol–2-propanol (85:10:5) was presented in the operation manual (Tracor Instruments, 1978) supplied with the high-pressure LC detector. As captafol and folpet were also separated on this column and detected with the same good sensitivity as captan by the photoconductivity detector, we began to develop a residue method based on these high-pressure LC conditions. Our work resulted in a rapid, reproducible, and specific method for the determination of these fungicides at the residue level.

Two cleanup procedures were developed: column chromatography on silica for the individual compounds and gel chromatography for a mixture of the three.

EXPERIMENTAL SECTION

Sample Preparation. A representative 800–1000-g subsample of green plant material (green cereal plants) or fruit (apples; pears; grape berries) was chopped in a food cutter. Dry plant materials (straw; grain) were entirely ground together with dry ice in a cross-beater mill. The dry ice was allowed to evaporate.

Extraction. A 50-g subsample of *fruit* was placed in a 250 cm^3 wide mouth jar. Acetone (100 cm^3) was added and the sample macerated with a high-speed homogenizer. After being shaken for 1 h on a mechanical shaker, the slurry was filtered through a Büchner funnel using suction. The filter cake was washed twice with acetone (20 cm^3). Filtrate and washings were combined and collected.

A 40-g subsample of *green plant material* or *straw* or a 50-g subsample of *grain* was placed in a 500 cm^3 wide mouthed jar. One hundred grams of grain was taken if the sample was to be cleaned up by gel chromatography. Acetone (300 cm^3) and anhydrous sodium sulfate (40 g) for green plant materials were added. The green plant material was also macerated with the high-speed homogenizer. After extraction of the sample for 1 h on a mechanical shaker, the slurry was filtered through a fluted filter paper and an aliquot of 150 cm^3 was taken.

Cleanup and Partition. The acetonic plant or fruit extract was transferred to a 1000-cm^3 separatory funnel and diluted with 500 cm^3 of deionized water and 20 cm^3 of saturated brine. The aqueous acetone solution was shaken 3 times with 75 cm^3 of *n*-hexane each. The combined *n*-hexane extracts (225 cm^3) were washed once with a mixture of water (100 cm^3) and brine (20 cm^3). The *n*-hexane extract was filtered through a plug of cotton and evaporated to dryness. The water phases were discarded.

Two alternative column cleanup procedures were used. The silica column cleanup is suitable for the individual cleanup and determination of each of the fungicides, whereas the gel chromatography allows the simultaneous elution, followed by the simultaneous determination of all three fungicides. Individual determination is simpler and is adequate in cases where the sample history is known e.g., in supervised trials. The simultaneous determination, although somewhat more complicated, is useful for samples with unknown history, e.g., for enforcement purposes.

Silica Column. Individual Cleanup for Each Compound. A suspension of 30 cm^3 of silica (silica gel 60, 0.063–0.2 mm, E. Merck AG., Darmstadt, West Germany) in *n*-hexane was poured into a chromatographic tube of 23-mm i.d. and 30-cm length to yield a column bed of 70 ± 5 mm height. The residue from the partition step was dissolved in 2 cm^3 of *n*-hexane by swirling the flask and transferred to the column. The solvent in the column was drained to the top of the silica. The flask was rinsed twice with 2 cm^3 of *n*-hexane each, which were also transferred to the column. For each compound a separate column had to be used.

Captan. The column was washed with 50 cm^3 of *n*-hexane–ether (2:1); this eluate was discarded as a forecut. Captan was eluted with 75 cm^3 of *n*-hexane–ether (1:2).

Folpet. The column was washed with 50 cm^3 of *n*-hexane–ether (4:1); this eluate was discarded as a forecut. Folpet was eluted with 75 cm^3 of *n*-hexane–ether (3:1).

Captafol. The column was washed with 100 cm^3 of *n*-hexane–ether (2:1); this eluate was discarded as a forecut. Captafol was eluted with 75 cm^3 of *n*-hexane–ether (1:1).

The eluates were collected and evaporated to dryness.

Gel Column: Simultaneous Cleanup of the Three Fungicides. An AutoPrep 1001 gel permeation chromatograph (GPC) (Analytical Biochemistry Laboratories, Inc., Columbia, MO) equipped with a 60.0 × 2.5 cm i.d. column, packed with 50 g of Bio-Bead SX3 resin, 200–400 mesh (Bio-Rad Laboratories, Richmond, CA), compressed to a bed length of ~30 cm, was used. The eluting solvent was cyclohexane–ethyl acetate (1:1) pumped at a constant flow rate of 2.5 cm^3/min, with an operating pressure of ~20 kPa. Before the samples could be processed with the gel permeation system, it was necessary to determine the elution volume of the three fungicides. The AutoPrep 1001 GPC autofractionates a sample into 23 10-cm^3 fractions for elution profile determination. This is accomplished by collecting the gel column eluant for 4 min from each of the 23 sample collection tubes at a constant flow rate of 2.5 cm^3/min. The fractions were analyzed by injection into the liquid chromatograph equipped with the photoconductivity detector. Under these conditions captan, captafol, and folpet eluted in the 120–190-cm^3 fraction.

The residue from the partition step was dissolved in 10 cm^3 of cyclohexane–ethyl acetate (1:1). A 5-cm^3 aliquot was injected onto the GPC and processed according to the conditions indicated above. The 120–190-cm^3 fraction was collected and evaporated to dryness.

If no automatic gel chromatograph is available, manual execution of the above steps is possible, although time consuming.

High-Pressure Liquid Chromatography. Two stainless steel columns (250-mm length; 4.6-mm i.d.) were connected in series. They were filled with Zorbax CN, a cyano bonded phase (Du Pont, Wilmington, DE). The assembled columns were attached to an Orlita SK 15-3 (Orlita KG, Giessen, West Germany) reciprocating membrane pump and to a Model 965 photoconductivity detector (Tracor Instruments, Austin, Tx) operated with the mercury lamp at 254 nm (4.4 mW/cm^2). For separation of captan, captafol, and folpet, a column performance of ~12 000 theoretical plates (K' = 2.07) for each column was necessary.

The column was operated at ambient temperature with a flow rate of 1.7 cm^3/min, resulting in a pressure of ~13 MPa. The mobile system consisted of a mixture of isooctane–methanol–2-propanol (85:10:5). All solvents were of analytical grade. The mobile phase had to be conditioned overnight by recirculation through the ion-exchange cartridge of the detector.

Standard solutions of 0.2 $\mu g/cm^3$ up to 2 $\mu g/cm^3$ containing one or all three fungicides in the conditioned mobile phase were prepared. Fifty cubic millimeters of four different standard solutions were introduced into the high-pressure LC system via a sample loop. A linear plot of the fungicide concentrations vs. the recorder responses (peak heights) yielded a straight line.

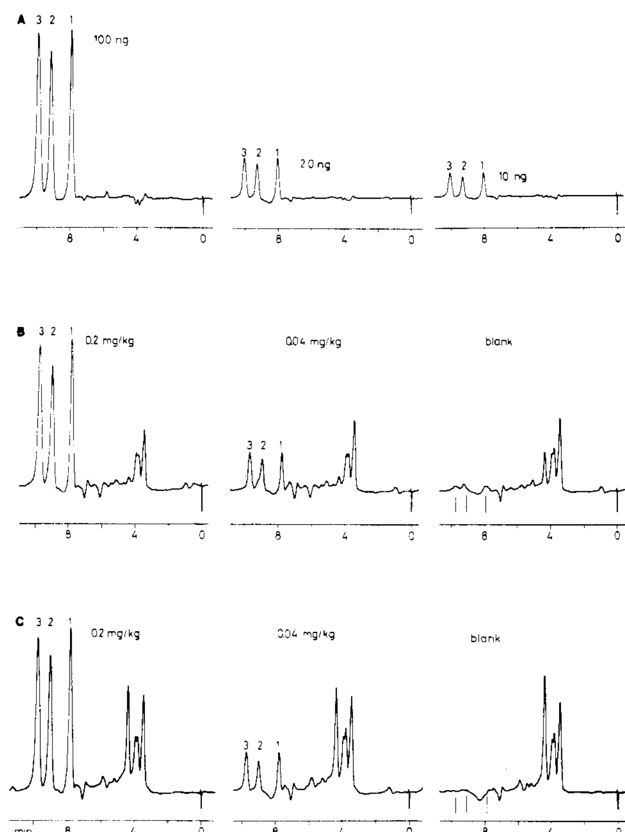

Figure 1. (A) Chromatograms of standard: folpet (1), captan (2), and captafol (3). (B) Chromatograms of apples spiked with folpet (1), captan (2), and captafol (3). (C) Chromatograms of grain spiked with folpet (1), captan (2), and captafol (3). Equivalent of 500 mg of apple or grain sample injected. Flow rate: 1.7 cm^3/min; 965 photoconductivity detector (254 nm). Sensitivity: 1 × 20.

The dry residue of the eluate from the silica column or from the gel filtration column was dissolved in an appropriate volume of the mobile phase in such a way that with a 50-mm^3 sample loop a maximum of 500 mg of sample was dosed for fruit and grain and a maximum of 200 mg of sample for green plant material and straw. The mobile phase used for dissolving the samples had to be conditioned overnight through the ion-exchange cartridge of the detector.

Representative chromatograms are shown in Figure 1. Retention times of folpet, captan, and captafol were 470, 550, and 580 s, respectively.

RESULTS AND DISCUSSION

A flow diagram of the extraction and cleanup procedure for folpet, captan, and captafol is presented in Figure 2. Two cleanup procedures were outlined: a silica gel column cleanup for the individual determination of each fungicide and a gel permeation chromatography cleanup for the simultaneous determination of all three fungicides.

The silica gel column cleanup allowed the quantitation of each fungicide with limits of determination of 0.02 mg/kg in fruit and grain and 0.05 mg/kg in other plant materials. Application of elution conditions to the silica gel column chromatography, which eluted the three fungicides in the same fraction, resulted in an insufficient cleanup of the samples for the residue determination at the limits of determination indicated above.

In order to present a screening method for the simultaneous determination of all three compounds with the same limit of determination as for the individual determination, we tried gel column chromatography. The three fungicides eluted from a 30-cm Bio-Bead 5X3 column in the 120–190-cm^3 fraction clean enough to be determined simultaneously at the above limits of determination. Although the cleanup is very efficient, this procedure can only be recommended to laboratories equipped with an automatic gel chromatograph, because processing one sample needs at least 80 min.

Typical recoveries for the individual determination of each fungicide at two different fortification levels in major crops are presented in Table I.

Table II presents typical recoveries of folpet, captan, and captafol added at two different fortification levels to each crop. The samples were cleaned up by gel chromatography and the fungicides determined simultaneously.

The applicability of the developed technique was also proven by the analysis of field samples treated with one of these fungicides. They were mainly analyzed by using the silica column cleanup.

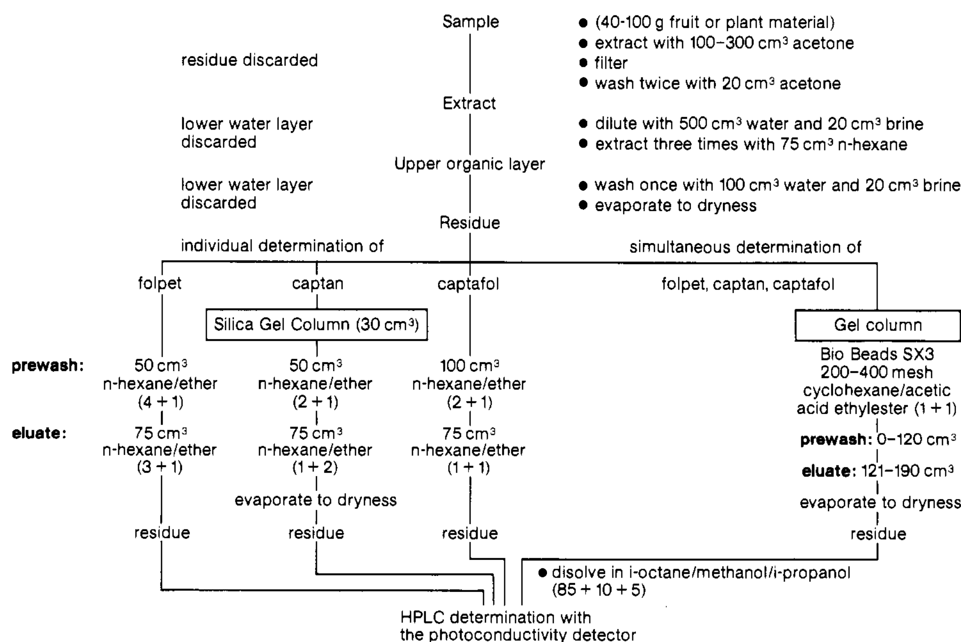

Figure 2. Flow chart of extraction and cleanup procedure for folpet, captan, and captafol.

Table I. Typical Recoveries of Folpet, Captan, and Captafol: Individual Cleanup and Determination of Each Compound

	sample	fortification level, mg/kg	% recovery ± SD
folpet	grapes	0.04	96.7 ± 12.7 (n = 7)
		0.2	88.7 ± 11.2 (n = 8)
captan	apples	0.04	108.2 ± 13.7 (n = 5)
		0.5	81.6 ± 3.2 (n = 5)
captafol	wheat grain	0.04	96.4 ± 15 (n = 8)
		0.2	89.3 ± 7.6 (n = 6)
	wheat straw	0.1	82.8 ± 5.4 (n = 7)
		0.5	81.7 ± 8.5 (n = 14)
	wheat ears	0.1	82.6 ± 7.5 (n = 8)
		0.5	82.2 ± 8.5 (n = 8)

Table II. Recoveries of Folpet, Captan, and Captafol: Simultaneous Determination

sample	fortification level, mg/kg	% recovery		
		folpet	captan	captafol
grapes	0.04	105	113	92
	0.2	105	95	95
apples	0.04	120	90	113
	0.2	100	85	90
wheat grain	0.04	95	75	92
	0.2	95	90	95
wheat straw	0.1	94	97	94
	0.5	84	94	94

We generally noted that storage of the samples for hours in polar solvents (methanol; ethanol; acetonitrile) resulted in lower recovery values. We therefore took acetone as the extraction solvent, processed the samples rapidly, and only interrupted analysis when samples were dried or dissolved in apolar solvents.

The detector is very sensitive to even minor changes in the mobile phase composition. The samples were therefore evaporated to dryness after the column cleanup and dissolved in the conditioned mobile phase for the final high-pressure LC determination.

No variation in the sensitivity of the detector could be observed for months, once the mobile phase was conditioned through the ion-exchange cartridge of the detector. Absorption and/or decomposition of the compounds on the column have never been observed. As the decomposition of these compounds under gas chromatographic conditions may not be excluded, the high-pressure LC determination combined with the specific and sensitive photoconductivity detection is the analytical procedure to be preferred.

The new method outlined is suitable for the residue determination of folpet, captan, and captafol with limits of determination of 0.02 mg/kg in fruit and of 0.05 mg/kg in other plant materials.

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Gas–Liquid Chromatographic Determination of Residues of Methiocarb and Its Toxic Metabolites with the Flame Photometric Detector after Derivatization with Methanesulfonyl Chloride

Jay C. Maitlen

Residues of methiocarb, methiocarb sulfoxide, and methiocarb sulfone were determined by oxidation of the compounds to methiocarb sulfone, hydrolysis of the sulfone to its phenolic form, and then derivatization of the phenol to its mesylate with methanesulfonyl chloride. Residues of the methiocarb sulfone mesylate were then determined with a gas chromatograph equipped with a flame photometric detector operated in the sulfur mode. The procedure was applied to crops of spinach, celery, rhubarb, raspberries, and peas. Average residues in methiocarb-treated crops of spinach and celery were 3.67 and 0.43 ppm, respectively. No detectable residues were found in rhubarb, raspberries, or peas; the lower limit of detection was 0.05 ppm.

Methiocarb [4-(methylthio)-3,5-xylyl methylcarbamate, also known as mesurol] is a carbamate pesticide used on a variety of field, vegetable, and fruit crops. Abdel-Wahab et al. (1966) demonstrated that methiocarb is readily oxidized to its sulfoxide and sulfone metabolites, and these compounds have been shown by Metcalf et al. (1967) to be cholinesterase inhibitors.

Several methods, such as the high-pressure liquid chromatography procedure of Lawrence (1977), the thin-layer–gas chromatographic procedure of Ernst et al. (1975), the microwave emission procedure of Bache and Lisk (1968), and the gas chromatographic procedures of Van Middelem et al. (1965) and Lorah and Hemphill (1974), have demonstrated the detection of methiocarb residues, but none of these methods was applied to the determi-

Yakima Agricultural Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Yakima, Washington 98902.

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