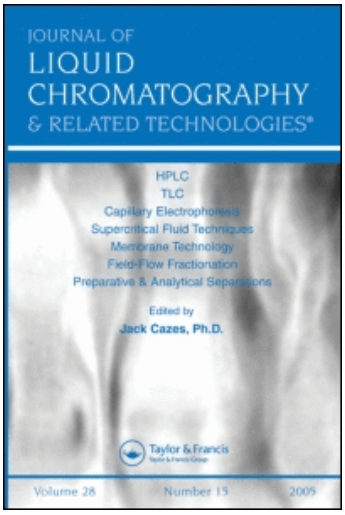

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DETERMINATION OF CAPTAN, FOLPET, AND CAPTAFOL IN WATER, APPLES, AND LETTUCE BY QUANTITATIVE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Captan, folpet, and captafol were determined in water, lettuce, and apples by TLC of extracts on preadsorbent silica gel layers, detection with silver nitrate reagent, and densitometric scanning. The fungicides were extracted from water on Chromosorb 102 microcolumns. Cleanup on a Florisil column was required for the food extracts. Recoveries from distilled and tap water ranged from 76-98% at 0.02 ppm and 81-94% at 0.007 ppm. Recoveries from lettuce ranged from 88-94% and from apples 84-90%, both at 0.25 ppm. The selectivity, sensitivity, and precision of the method are adequate for routine residue analysis.

INTRODUCTION

The phthalimide compounds captan [N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide], folpet [N-(trichloromethylthio)-

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phthalimide], and captafol [N-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide] are widely used, singly or in combination, as broad-spectrum protective fungicides for the control of diseases on various fruit and vegetable crops, such as apples, lettuce, tomatoes, strawberries, potatoes, and grapes, and in wheat. Tolerance levels specified by the U. S. Environmental Protection Agency range from 0.25 ppm of captafol in apples to 100 ppm of captan in lettuce and are generally in the range of 15 to 50 ppm for most fruits and vegetables. Analyzed samples typically contain less than 1-5 ppm, however (1). (The U. S. EPA has now proposed a ban on the use of captan on foods).

Gas chromatography (GC) is the most widely used procedure for determination of captan, folpet, and captafol residues. A comparative study (2) indicated that a 5% SP-2401 packed column was most suitable, and the Mills and Luke et al. multiresidue methods can be used for sample preparation prior to GC (1). Column HPLC has been used for the separation and determination of the three fungicides in plant material using photoconductivity detection and a cyano bonded column (3), and photoconductivity detection in tandem with ultraviolet detection has also been reported (4).

Thin layer chromatography (TLC) utilizes an "open" system that involves multiple application of samples and standards that are developed concurrently and detected statically. This leads to recognized advantages compared to "closed" column methods (GC and HPLC) in which single, sequential samples are eluted and detected dynamically. These advantages include high sample throughput; the

flexibility to optimize separation, detection, and quantification for the particular analyte(s) of interest; simplicity; and good accuracy and precision (because samples and standards are processed together under essentially identical conditions) (5-7). In addition, samples may require less cleanup because each layer is used only once and then discarded. TLC with densitometry has been applied to a wide variety of analyses including pesticides, the most recent example being the determination of chlorpyrifos insecticide residues (8).

Captan and captafol were determined simultaneously in apples and potatoes at 0.2 ppm by TLC of cleaned-up extracts on homemade layers prepared from silica gel H plus 0.1 M aluminum chloride. Spraying the developed plate with 0.1 M sodium chlorate and heating produced fluorescent zones that were scanned with a densitometer (9). Attempts by us to reproduce these results and extend the method to folpet residues were unsuccessful using commercial precoated TLC and HPTLC plates impregnated with $AlCl_3$ by spraying or dipping. Therefore, we developed a quantitative TLC method for the three fungicides based on detection of the chlorine atoms with silver nitrate chromogenic reagent on preadsorbent silica gel G layers. With preadsorbent plates, sample application can be carried out rapidly, and the spotting area automatically produces sharp, narrow bar- or streak-shaped zones of constant size, even though different sample volumes are used. Accurate, precise, and sensitive densitometry requires that initial zones of samples and standards have small, uniform dimensions (10). The method and its

successful application to determinations of residues in fortified water (0.02 and 0.007 ppm) and apples and lettuce (0.25 ppm), the latter chosen as representative fruit and vegetable samples containing pesticides at the lowest tolerance level, are described below.

EXPERIMENTAL

Analytical standards of captan, folpet, and captafol were obtained from Ortho (Chevron Chemical Co.). Stock standard solutions were prepared in toluene at a concentration of 1.00 mg/ml and TLC standards by quantitative 10.0 to 100 and 1.00 to 100 dilutions to give 100 ng/µl and 10.0 ng/µl solutions, respectively.

Analyses were performed on 20x20 cm Analtech silica gel GF plates that contained a preadsorbent spotting area and 19 channels 0.9 cm in width. Plates were prewashed with methylene chloride-methanol (1:1) and dried in a hood before use. Fungicides were applied at 50-2000 ng levels by spotting 4.00 to 20.0 µl of the appropriate TLC standard vertically down the center of the pre-adsorbent areas of the lanes using a 25 µl Drummond Dialamatic dispenser. Water and food samples were applied in the same manner from acetone or toluene solvent to plates containing standard zones for comparison.

Plates were developed for a distance of 10 cm beyond the layer junction in a paper lined, saturated rectangular glass TLC chamber with methylene chloride-hexane (9:1). Plates were air dried, and fungicides were detected by dipping into silver nitrate reagent and

exposure to UV light as previously described (8). Zones were scanned with a Kontes Model 800 fiber optics scanner in the single beam transmission mode using the 8 mm light beam and white phosphor (440 nm peak wavelength). Chromatograms were drawn and peak areas reported by a Hewlett-Packard Model 390A recorder/integrator coupled to the scanner. All zones were scanned twice, and calibration curves were calculated by a linear regression program run on a Commodore 64 minicomputer.

Actual analyses were demonstrated using fortified pesticide-free distilled and tap water and lettuce and apple samples purchased in a local market. Spiking solutions at the 4.00 µg/ml level were prepared by dilution of 2.00 ml of the 100 µg/ml standard solution of each fungicide to 50.0 ml with acetone. Fortified water samples were prepared in Erlenmeyer flasks by adding 0.25 ml of the spiking solution to 60.0 ml of distilled or tap water and shaking vigorously for several minutes. The resultant sample contained 0.0167 ppm of each pesticide added. Extraction columns containing 0.2g of Chromosorb 102 were prepared in "large volume" Pasteur pipets by addition of 1 ml of water slurry as described earlier (11). After pre-washing with acetone and distilled water (all the acetone must be removed), the fortified water and an unfortified blank were passed through separate columns. The flask, funnel on top of the column, and the inner column walls were washed well with distilled water, the column was blown dry with a gentle stream of nitrogen gas (ca. 20 minutes), and the trapped fungicide(s) eluted with 2 ml of acetone

into a 4 ml vial. A rubber bulb was used to force all of the eluent out of the column into the vial. The solution was concentrated to ca. 10-20 µl and the entire sample was spotted for TLC, including two 20 µl acetone rinses of the vial. Standards were applied to adjacent lanes of the same plate. After chromatography, detection, and scanning, the amount of pesticide(s) in the sample was calculated by comparison of the sample and standard peak areas (1000 ng theoretical for 100% recovery).

Fresh lettuce and apple samples (100g) were homogenized by blending and fortified by adding 1.00 ml of 25 µg/ml spiking solutions of the pesticides in acetone (0.25 ppm). The samples were processed by the Luke et al. procedure with optional Florisil cleanup using the modified elution system (15% and 50% ethyl ether in petroleum ether eluents) (1). The procedures followed are published in the FDA Pesticide Analytical Manual, volume I, sections 232.4 and 212.24. In summary, the sample was blended with acetone and filtered, pesticides were extracted from the aqueous filtrate with petroleum ether-methylene chloride (1:1), the extract was concentrated and diluted with acetone and petroleum ether, and the extract solution was chromatographed on a fully-activated Florisil column with elution by 200 ml of 15% ethyl ether in petroleum ether and 200 ml of 50% ethyl ether in petroleum ether. The combined eluates were evaporated to a final volume of 500 µl, measured in a small graduated centrifuge tube. Twenty µl of sample was spotted in duplicate along with duplicate comparative standards (1000 ng theoretical for 100% recovery).

Standards for analyses of samples fortified with more than one fungicide were applied to the layer by a single application of a mixed solution, or individual solutions could be applied in a vertical row to the preadsorbent area of a single lane.

RESULTS AND DISCUSSION

Captan, captafol, and folpet had respective R_F values of 0.36, 0.42, and 0.57 on the preadsorbent silica gel layer developed with methylene chloride-hexane (9:1). All of these values were within the optimum range of 0.3 to 0.7 for accurate and precise quantification. The zones appeared as sharp, narrow dark brown streaks on a white background. Figure 1 illustrates the separation of the three compounds. The optimum UV irradiation time was 30 minutes for maximum contrast between the zones and background. Zones were scanned immediately after the irradiation period but could be stored for at least one hour in the dark without significant darkening of the background. Silica gel G layers must be used because polymer-bound, hard layers give a dark background with silver nitrate detection reagent (8).

Calibration curves for all three fungicides typically had linearity (R) values of 0.98 or greater in the 100-2000 ng range. The visual detection limit was about 50 ng, but 100 ng was the lowest amount that could be scanned consistently with precision. The calibration curves for the three compounds had similar slopes and intercepts, but the exact values of these parameters differed

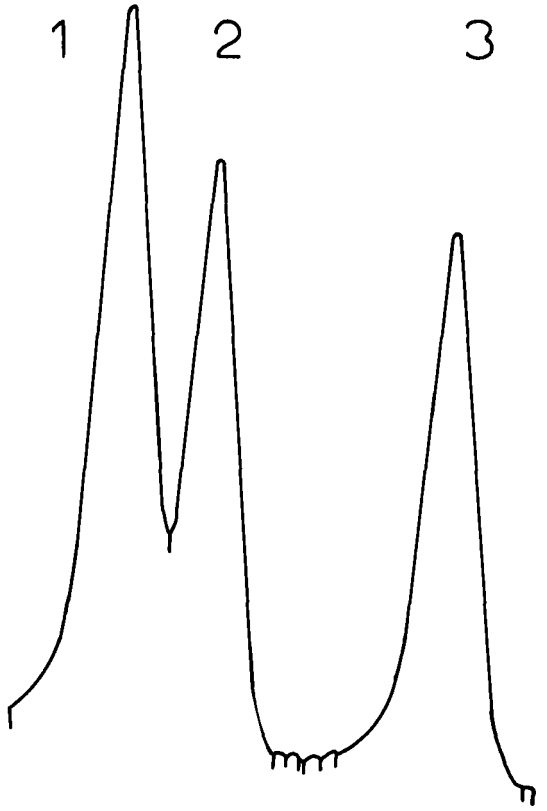

Figure 1. Densitometer scan with attenuation X6 of a standard mixture containing 1500 ng each of captan (1), captafol (2), and folpet (3) after development on preadsorbent silica gel and detection with $AgNO_3$ reagent.

from plate to plate. To correct for these variations, standards and samples were always run together on the same plate. Figure 2 shows typicsl peaks and areas for a series of captan standards. The calibration curve calculated from the area data of these scans had an R value of 0.999.

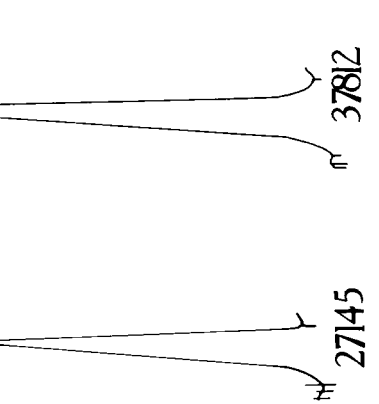

Figure 2. Scans and peak areas of 100-2000 ng of captan using the Kontes Model 800 scanner and HP Model 3390A recorder/integrator with attenuation X7 (X3 for the 100 ng zone).

Four samples of distilled water were fortified separately with 0.0167 ppm of the three fungicides. Recoveries using the Chromosorb column procedure described above were 80.7, 83.4, 78.5, and 74.2% for captan; 98.4, 97.8, 93.1, and 90.1% for folpet; and 85.0, 84.7, 77.9, and 94.8% for captafol. Blank samples analyzed with each set of fortified samples showed no TLC spots. These results prove that the fungicides are adequately trapped by the Chromosorb column and eluted with acetone. The procedure should allow recovery of the compounds at lower concentration levels because water will not elute them from the column. This was tested by analyzing duplicate 150 ml distilled water samples fortified separately with 1.00 μg of each fungicide (0.0067 ppm). Recoveries were 88.8 and 80.7% for captan, 90.2 and 93.3% for folpet, and 83.8 and 84.4% for captafol.

Duplicate Easton tap water samples were fortified with all three of the fungicides at the 0.0167 ppm level. The compounds were trapped, eluted, and analyzed together along with a blank tap water sample. Recoveries were comparable to those obtained for samples fortified singly with the fungicides: 89.2 and 89.2% for captan, 88.4 and 89.4% for folpet, and 76.7 and 83.9% for captafol. No other interfering zones were detected in the sample lane or in the blank. Presumably the only limit to the sensitivity of the method is the patience one has in passing large volumes of water through the column and possible extraction of impurities on the column from contaminated water, which might be eluted and could interfere with the densitometry of the analytes.

Blank lettuce and apple samples (100g) were fortified at 0.25 ppm and processed by the Luke et al. procedure with supplemental Florisil column cleanup. The Florisil column step was necessary to allow GC determination with an electron capture detector (1), and it was also required in this study to provide a clean TL chromatogram with no zones that interfered with fungicide quantification. Duplicate lettuce samples were fortified with both captan and folpet, and recoveries were 88.3 and 87.8% for captan and 90.3 and 93.7% for folpet. Duplicate apple samples were fortified with captan and captafol, and recoveries were 84.3 and 89.6% for captan and 90.0 and 89.4% for captafol. Blank sample chromatograms had no zones at the positions of the pesticides, and included several other non-interfering zones that were also present in the sample chromatograms. The recovery values cited are the average of the duplicate sample aliquots that were spotted. Agreement of duplicates was almost always within 5% and usually within 2-3%.

The above results for representative fortified samples of water and crops demonstrate the acceptable precision and accuracy (recovery) of the quantitative TLC procedure for determining captan, folpet, and captafol. The use of a Chromosorb polymer column for extracting the compounds from water is much more convenient than solvent extraction in a separatory funnel. The method is applicable to any food sample that can be adequately extracted and cleaned-up prior to TLC.

REFERENCES

1. Gilvydis, D. M. and Walters, S. M., J. Assoc. Off. Anal. Chem., 67, 909 (1984).

2. Gilvydis, D. M. and Walters, S. M., J. Assoc. Off. Anal. Chem., 66, 1365 (1983).

3. Buettler, B and Hoermann, W. D., J. Agric. Food Chem., 29, 257 (1981).

4. Walters, S. M. and Gilvydis, D. M., Liq. Chromatogr. HPLC Magazine, 1, 302 (1983).

5. Coddens, M. E., Butler, H. T., Schuette, S. A., and Poole, C. F., Liquid Chromatogr. HPLC Magazine, 1, 282 (1983).

6. Fenimore, D. C. and Davis, C. M., Anal. Chem., 53, 253A (1981).

7. Borman, S. A., Anal. Chem., 54, 790A (1982).

8. Sherma, J. and Slobodien, R., J. Liq. Chromatogr., 7, 2735 (1984).

9. Francoeur, Y. and Mallet, V. N., J. Assoc. Off. Anal. Chem., 60, 1328 (1977).

10. Touchstone, J.C. and Sherma, J. (eds.), Densitometry in Thin Layer Chromatography - Practice and Applications, Wiley Interscience, NY, NY, 1979, Chapter 4.

11. Sherma, J., Chandler, K., and Ahringer, J., J. Liq. Chromatogr., 7, 2743 (1984).