Capillary Gas Chromatographic Determination of Captafol in Vegetables, Fruits, and Grains

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Captafol is a fungicide that has been used because of its low acute mammalian toxicity. Recently it was found that the pesticide causes several kinds of cancer. To control the levels in foods, it is necessary to establish a reliable, efficient, and sensitive method for monitoring captafol in domestic and imported products consumed in Japan. The method presented describes the determination of captafol in citrus fruits, vegetables, soybeans, and wheat. The captafol is extracted with acetonitrile or acetone, and, if necessary, partitioned with hexane. The residual pesticide is purified by Florisil column chromatography and by optional charcoal column chromatography. The eluate is concentrated and injected into a gas chromatograph equipped with a fused silica capillary column and an electron capture detector. Recoveries of captafol from market samples fortified at levels of 0.005 and 0.01 ppm ranged from 64 to 88%.

INTRODUCTION

Captafol (Figure 1) has been widely used as a fungicide because it inhibits mycelial growth of germinating fungal spores. Early studies indicated that this compound was not mutagenic in the mouse dominant lethal mutagenicity study in mice and in the host mediate assay in rats (Bridges, 1975; Kennedy et al., 1975; FAO/WHO, 1977). However, captafol has been found to increase the incidence of neoplastic lesions in the kidneys of rats of both sexes and the incidence of neoplastic nodules in the liver of female rats (FAO/WHO, 1985). A 2-year carcinogenicity study with B6C3F1 mice revealed that captafol induced hemangioendotheliomas, papillomas, sequamous cell carcinomas, hyperplastic nodules, and hepatocellular carcinoma (Ito et al., 1988). These studies suggest a broad spectrum of carcinogenicity. Recently, the carcinogenicity of captafol was confirmed by a 2-year test in the F344 rat (Tamano et al., 1990). In light of these studies, the Committee on Scientific and Regulatory Issues Underlaying Pesticide Use Patterns and Agricultural Innovation (Thornton et al., 1987) warned that the estimated dietary oncogenic risk was 5.94×10^{-4}.

The analysis of captafol in foods is a very challenging problem. It is unstable in foods because it reacts with thiol groups in cysteine and glutathione and degrades into thiophosgen or phthalimide derivatives. It disappears rapidly in homogenates of cucumber or radish (Nutahara et al., 1978). Also, the standard procedure, which utilizes a packed column for gas chromatography, does not eliminate interference peaks that have been identified as organochlorine pesticides and phthalates (FDA, 1978). Those problems have led to difficulties in obtaining reliable and reproducible analytical results.

Many analytical methods for captafol have been reported. Since captafol had been thought to be one of safest pesticides (FAO/WHO, 1977) in the world, quantitative levels of the standard analytical methods range from 0.1 to 2 ppm (FDA) and from 1 to 5 ppm (JEPA). To monitor lower levels of captafol contamination in foods, it was necessary to establish a new method. Initial efforts led to those methods that employ packed columns for gas chromatographic separation and electron capture detector (ECD) for detection (Gilvydis and Walters, 1983, 1984). This combination is not satisfactory because chlorinated pesticides, PCBs, phthalates, and many other electron captive materials interfere with the identification and determination. Multiresidue methods utilize a minimal cleanup with a selective detection system (Hall detector) (Luke et al., 1975, 1981, 1988; Luke and Doose, 1983), gel chromatography with a electron capture detector (Steinwandter, 1988), and a standard cleanup with AED (Miyahara et al., 1992). Those methods yield analyses of many pesticides rapidly (Steinwandter, 1989). However, these procedures provide solutions for gas chromatographic detection that contain coextractives, electron captive materials, and nonvolatile materials along with polar pesticides such as organophosphorus compounds. These extracts caused contamination of the gas chromatograph; therefore, reproducible results are difficult to obtain (Szelewski, 1989; FDA, 1991; OVR, 1988; Miyahara et al., 1991). These methods are not adequate for low-level analysis with a capillary gas chromatograph equipped with an electron capture detector. This paper describes a sensitive and reliable procedure that uses capillary gas chromatography and electron capture detection for captafol in fruits, vegetables, and grains.

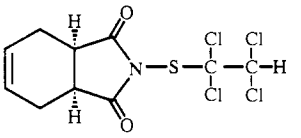

Figure 1. Structure of captafol.

EXPERIMENTAL PROCEDURES

Apparatus. (*a*) *Gas Chromatograph with Electron Capture Detector (GC-ECD).* A Shimadzu GC9A with an electron capture detector in combination with a Shimadzu CR4A data processor was used for measurement of peak areas. Injector, detector, and oven temperatures, as well as oven programs, and columns are listed in Table I.

(*b*) *Mill.* The mill used was a Shibata Laboratory minimill.

(*c*) *Blender.* The blender used was a Nisseiki blender.

Reagents. (*a*) All organic solvents used were of pesticide grade (Wako Pure Chemical Co., Osaka). Mutually saturated hexane and acetonitrile were used in acetonitrile and hexane partition.

(*b*) The capatafol standard was of analytical grade (97–100%, Wako).

(*c*) Stock standard solution was prepared as follows: 100 mg of captafol was dissolved in 10 mL of acetone and diluted to

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Table I. Gas Chromatographic Operating Conditions

column				temp,[a] °C		
liquid phase	i.d., mm	length, m	film thickness, μm	C	I	D
5% SE30	3	1.5[b]	0.0	200	250	250
CBP10	0.53	10	1[c]	200	250	250
CBP1	0.53	12	1[c]	180	250	250
MSP5	0.53	15	5[c]	240	280	280
DB-1	0.32	30	0.25[d]	230	280	280
DB-5	0.25	30	0.25[e]	210	270	280
HP-1	0.20	12	0.33[f]	g	270	280

[a] C, I, and D denote column temperature, injection temperature, and detector temperature, respectively. [b] Chromosorb W (AW-DMCS, 80–100 mesh). Gas flow: carrier gas (N_2), 40 mL/min. [c] Gas flow: carrier gas (He), 16 mL/min; makeup gas (N_2), 40 mL/min. [d] Gas flow: carrier gas (He), 9 mL/min; makeup gas (N_2), 50 mL/min. [e] Gas flow: carrier gas (He), 2 mL/min; makeup gas (N_2), 60 mL/min. [f] These are the conditions for GC–mass. Splitless: waiting time (valve time), 1 min. Gas flow: carrier gas, 2 mL/min. [g] Oven temperature was programmed as follows: isothermal at 50 °C for 1 min and then programmed to 200 °C at the rate of 20 °C/min.

volume with hexane. Working standard solutions were prepared by diluting the stock standard solution with hexane.

(*d*) Samples (wheat, soybeans, cucumber, Hassaku, apple, radish, radish leaf, and potato) were purchased from retail markets in Tokyo.

(*e*) Florisil PR (Floridin Co., Pittsburgh, PA) was activated at 130 °C for 12 h.

(*f*) Charcoal was of pesticide grade (Darco G60, manufactured by Atlas Powder Co.).

(*g*) Microcrystalline cellulose powder was supplied by Asahi Kasei with particle size of 100–200 μm for chromatography.

Extraction Procedure for Fruits and Vegetables. Edible portions (100 g) of sample were homogenized for 5 min by blender. For the recovery studies, captafol in acetone was added at this step to the sample (20 g) at the levels of 0.005 and 0.01 μg/g. (The solution was sprinkled onto the homogenate, and the mixture was stirred.) Acetone (100 mL) was added to a 20-g test portion of the homogenate, and the mixture was shaken vigorously for 10 min and then filtered with the aid of vacuum through a suction funnel packed with a 5-mm layer of Celite 545. To the filtrate was added 300 mL of 2% sodium chloride aqueous solution. The mixture was extracted with two 100-mL portions of methylene chloride, and the aqueous layer was discarded. The extract was dried by passage through a layer of anhydrous sodium sulfate held in a filter funnel with filter paper. After filtration, the sodium sulfate was washed with a small portion of methylene chloride. The organic solution was evaporated to near dryness, and a stream of nitrogen was used to complete the evaporation. Hexane (5 mL) was added to dissolve the residue, and the solution was subjected to Florisil column chromatography.

Extraction Procedure and Partition for Soybeans and Wheat. The edible portion (100 g) of sample was ground sufficiently to pass a 0.5-mm mesh sieve by minimill. (Caution: keep from overheating the sample by slowly feeding the sample into mill.) For the recovery studies, captafol in acetone was added at this step to the sample (20 g) at levels of 0.005 and 0.01 μg/g. (The solution was sprinkled onto the ground sample.) To a 20-g test portion of the ground and sieved sample was added 100 mL of acetonitrile, and the mixture was shaken vigorously for 10 min. The mixture was filtered with the aid of vacuum through a 5-mm layer of Celite 545 in a funnel. The organic solvent was evaporated to near dryness, and a stream of nitrogen was used to complete the evaporation. The residue was dissolved in 15 mL of hexane and extracted with three portions of 30 mL of acetonitrile. The combined acetonitrile extracts were evaporated to dryness under reduced pressure. The residue was dissolved in 5 mL of hexane, and the solution was subjected to Florisil column chromatography.

Florisil Column Chromatography. A slurry of 20 g of Florisil in hexane was poured into a glass column for chromatography (21 mm i.d. × 300 mm). Sodium sulfate (5 g) was added on top of the column. The extract in hexane was applied to the top of the column. The column was then eluted with 200 mL of 6% ether in hexane and the eluate discarded. Captafol was then eluted with 150 mL of 50% ethyl acetate in hexane. After removal of the solvent under reduced pressure, the residue was dissolved in 5 mL of hexane for charcoal column chromatography.

Table II. Recovery of Captafol from Fortified Crops

	fortification level			
	0.005 μg/g		0.01 μg/g	
crop	recovery,[a] %	CV%	recovery,[a] %	CV%
cucumber	64	7.8	66	9.0
Hassaku	74	4.7	80	4.0
apple	70	5.5	76	4.1
radish	79	5.9	84	6.4
radish leaf	64	8.2	68	9.3
potato	72	5.2	75	5.8
wheat	71	4.6	79	4.4
soybean	83	3.9	88	3.5

[a] The values are means of triplicate examinations.

Charcoal Column Chromatography. Adsorbent mixture was prepared as follows: 0.5 g of charcoal was mixed with 4.5 g of microcrystalline cellulose. The charcoal column (10 mm i.d. × 300 mm) was packed with 5 g of adsorbent mixture slurried in 20 mL of ether. Sodium sulfate (5 g) was placed on top of the column. The extract in hexane was applied to the top of the column. The column was then eluted with 200 mL of ether. After removal of the solvent, the residue was dissolved in 5 mL of hexane for gas chromatographic determination.

RESULTS AND DISCUSSION

To evaluate the method described, recovery studies were carried out with several types of samples spiked at levels of 0.005 and 0.01 ppm. The recoveries thus obtained are shown in Table II. Overall recoveries averaged 64–88%. All CV % ranged from 3.5 to 9.3. Control extracts did not contain detectable levels of captafol. From these results, the limit of quantitation (LOQ) is estimated to be 0.01 ppm (ACS Committee on Environmental Improvement, 1980).

The limit of detection (LOD) is defined as a response that is 3 times greater than the width of the baseline. The LOD for captafol was found to be 0.005 ng for all samples. Consequently, the same subsample weight and solution volume were satisfactory for all matrixes. For the most part, the chromatograms of samples tested were free of interfering peaks unless the sample contained a high level of bis(2-ethylhexyl) phthalate or other phthalates. Several samples of each commodity were analyzed, but no significant differences were noticed in the chromatograms. Chromatograms for a standard solution (Figure 2a), extracts from the control (Figure 2b,c; Figure 3a–f), and spiked (Figure 2d,e; Figure 3g–l) samples (wheat, soybeans, fruits, and vegetables) are shown in Figures 2 and 3.

The elution profiles of captafol and related organochlorine pesticides from Florisil column and gas chromatographic column are illustrated in Figure 4. A mixture of captafol and several organochlorine pesticides was deposited on the Florisil column according to the procedure described under Florisil Column Chromatography. To establish the procedure, 50-mL fractions were collected. The first eluent was 7% ether in hexane (fractions 1–6). The second eluent was 50% ethyl acetate in hexane (fractions 7–9). From these results, the elution volumes for the procedure were established (first fraction, 200 mL; second fraction, 150 mL). Captafol was separated from organochlorine pesticides (BHCs, dieldrin, aldrin, DDE, DDD, DDT, captan) that were found in the extracts by the chromatographic procedure. Those pesticides were selected because retention times indicated the potential for overlap or interference with captafol.

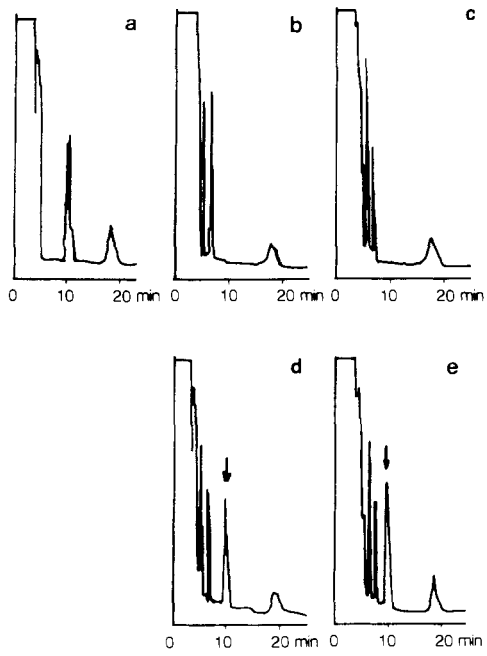

Figure 2. Chromatograms obtained with DB-1 column: a, captafol standard (0.01 ng); b and c, control wheat and soybeans; d and e, 0.01 ppm spiked wheat and soybeans, respectively. Arrow indicates captafol peak.

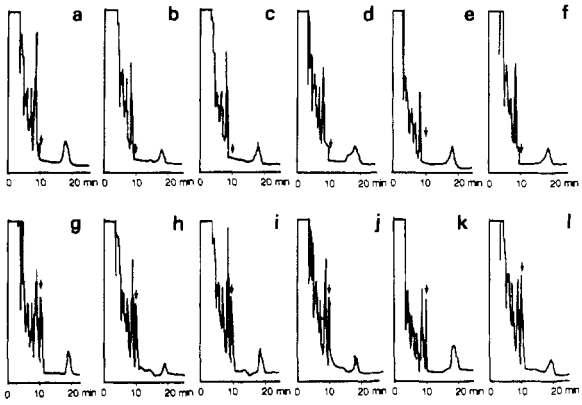

Figure 3. Chromatograms obtained with DB-1 column: a–f, control cucumber, Hassaku, apple, radish, radish leaf, and potato, respectively; g–l, 0.01 ppm spiked cucumber, Hassaku, apple, radish, radish leaf, and potato, respectively. Arrow indicates captafol peak.

Several types of gas chromatographic columns were tested for the determination of captafol. Table III shows the effects of column liquid phase on the calibration curves of captafol. Relative sensitivities may be compared by examining the slopes (a) of calibration curves. The sensitivity for captafol depended on the polarity of the column used. Nonpolar columns (DB-1 and CBP-1) gave good responses, whereas polar columns (CBP-10 and MSP-5) yielded poor responses.

The calibration curve determined for the detection system appeared to be linear from 0.012 to 0.1 ng with a DB-1 column. For several kinds of capillary columns, r^2, a, and the intercepts (b) of calibration curves are shown in Table III. The data illustrate that the zero-response level for DB-1 is only 7×10^{-3} ng of captafol, whereas the equivalent of 1.2 ng is irreversibly adsorbed on the CBP-10 column.

For confirmation, gas chromatography with a mass spectrometric detector was utilized. As shown in Table III, the calibration curve was linear in the range 0.1–100 ng with HP-1 (Table I). The mass spectrum and total ion chromatogram are shown in Figure 5. The captafol peak is readily identified by the spectrum.

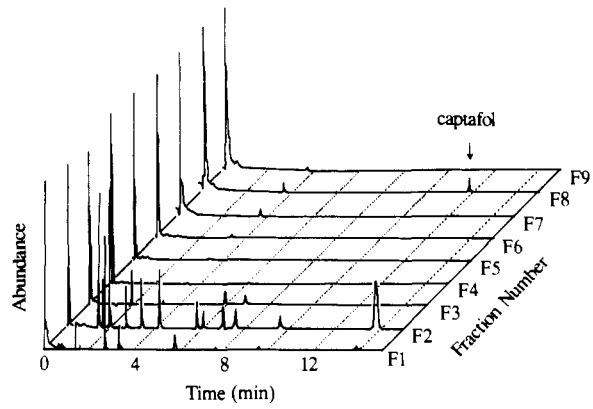

Figure 4. DB-1 chromatograms of 50-mL fractions taken from a Florisil column with captafol (100 ng) and several organochlorine pesticides. The solvents consisted of 7% ether in hexane (fractions 1–6) and 50% ethyl acetate in hexane (fractions 7–9). See text for identities of organochlorine pesticides.

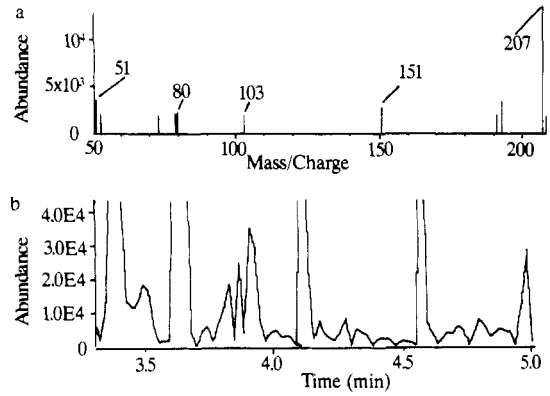

Figure 5. GC–mass results of captafol: a, total ion chromatogram of captafol standard (1 ng); b, mass spectrum of the peak at 3.9 min.

Table III. Effect of Gas Chromatographic Column on Calibration Curve

		calibration curve[b]		
column[a]	range, ng	slope ($\times 10^{-4}$)	intercept	r^2
DB-1[c]	0.012–0.1	111	−8100	0.98
CBP-10	0.5–5.0	10	−117500	0.90
CBP-1	0.01–1.0	44	11600	0.98
MSP-5	0.1–1.0	21	−9000	0.98
HP-1[c,d]	0.1–100	2	2300	0.99

[a] Operating conditions are listed in Table I. [b] $Y = aX + b$; a, slope; b, intercept. [c] Splitless mode. [d] Peak height of total ion mass chromatogram at 3.9 min was measured.

The efficiencies of various steps in the procedure were studied. Extraction of captafol from homogenates with acetone or acetonitrile provided good recoveries (98–100%), and partitioning between acetonitrile and hexane yielded 95% of the captafol in the acetonitrile. A recovery of 98% (CV% 4.8) from the Florisil column was realized when the column was eluted with 200 mL of 50% ethyl acetate in hexane, but no detectable captafol was found when 7% ethyl ether in hexane was used (200 mL). From the charcoal column, 89 and 98% (CV% 5.7 and 6.0, respectively) of the captafol was eluted with 50% ethyl ether in hexane and ethyl ether, respectively. From the results described above, these solvents may be substituted

for the more toxic benzene, which is used as a eluting solvent in the standard charcoal column chromatographic procedure.

It has been reported that captafol disappeared rapidly in homogenates of cucumber and radish and could not be recovered, although this loss could be prevented by addition of organic solvents (Nutahara and Yamamoto, 1978). However, as shown in Table II, recoveries from cucumbers and radish leaves, which were in the range 63–68%, were poor, but recoveries from radish were adequate (79 and 84% at 0.005 and 0.01 ppm, respectively). The CV % values for cucumbers, radish leaves, and radishes were in the range 6–9.5 at 0.005 and 0.01 ppm. The deviations are greater than those of other samples. These results suggest that captafol decomposes in these homogenates even in the presence of organic solvents. This procedure is adequate for analysis of captafol, and CV % values are comparable with those of other methods (Miyahara et al., 1991). During our sampling period, no captafol was found in vegetables, fruits, and grains that were examined.

In summary, a sensitive and reliable GC-ECD method has been developed for the separation and quantification of captafol in various crops. Recoveries, reproducibilities, and sensitivities are adequate for the determination of captafol in several vegetables, fruits, and grains.

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