Microcoulometric Gas Chromatographic Analysis of Grapes and Cottonseed for Chlorobenzilate Residues

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Recent entomological investigations into the use of the acaricide chlorobenzilate have created the need for residue data at a high level of sensitivity. Gas chromatography employing a microcoulometer detector was found to be sufficiently sensitive to detect chlorobenzilate residues. Extraction and cleanup techniques for grapes and cottonseed are reported. Conditions of operation of the gas chromatograph are also reported. The application of the cleanup procedures to other crops has been investigated. Other cleanup techniques involving gas chromatography are also discussed and evaluated. Confirmation of pesticide integrity during chromatography has been shown by infrared analysis.

THE ACARICIDE ethyl 4,4′-dichlorobenzilate, commonly known as chlorobenzilate, has been investigated in an effort to provide an effective means of mite control on grapes and cotton. The authors' laboratory, cooperating with other departments of the university, was called on to provide the necessary data for possible recommendation for the use of this compound on these crops in California.

A survey of the literature disclosed two analytical procedures for chlorobenzilate. Blinn *et al.* (*3*) developed a method for determining residues of this compound in citrus products. Their method consisted of hydrolyzing chlorobenzilate to 4,4′-dichlorobenzilic acid followed by oxidation of the acid to 4,4′-dichlorobenzophenone. The ketone was measured by ultraviolet absorption at 264 mμ, or, alternatively, the ketone was converted to the 2,4-dinitrophenylhydrazone and the absorption measured at 510 mμ. However, this procedure gives erratic results with some crop materials.

Harris (*6*) proposed a method based on the Schechter-Haller procedure for DDT (*7*) in which the chlorobenzilate was nitrated and the resulting compound reacted with sodium methylate to produce a red color measured at 538 mμ. This method was applied to the analysis of residues in apples and strawberries.

The introduction of several detectors specifically designed for measuring an organohalogen compound that emerges from the gas chromatograph simplified the analysis of chlorobenzilate. The application of a gas chromatographic procedure using a microcoulometer detector for the analysis of residues of chlorobenzilate in grapes and cottonseed is reported here.

Reagents

Nitromethane (Commercial Solvents Corp., New York, N. Y.).

Chlorobenzilate (technical product 90% pure, Geigy Agricultural Chemicals, Yonkers, N. Y.).

Florisil, 60 to 100 mesh (Floridin Co., Tallahassee, Fla.).

Carbon, Nuchar C-190N (West Virginia Pulp and Paper Co., Covington, Va.).

Apparatus

Wiley Mill, Model No. 3, equipped with a 2-mm. screen.

Gyrotory Shaker (New Brunswick Scientific Co., New Brunswick, N. J.).

Flash rotary evaporator, batch model (Buchler Instruments, New York, N. Y.).

Dohrmann Model 100 gas chromatograph (Dohrmann Instruments Co., San Carlos, Calif.) (*5*).

F & M Model 500 gas chromatograph (F & M Scientific Corp., Avondale, Pa.).

Experimental

Preliminary Examination of Chlorobenzilate by Gas Chromatography. Chlorobenzilate solutions (50-μg. aliquots) were applied to the F & M gas chromatograph with a thermal conductivity detector and temperature programmed at 15° C. per minute with a starting temperature of 100° C. Helium flow rate was 60 ml. per minute. When a 2-foot stainless steel column containing 2½% SE-30 silicone rubber on Analabs ABS 60- to 70-mesh Chromosorb W was used, the peak emerged at 210° C. The authenticity of the peak area was verified by infrared spectra of collected fractions compared to the spectrum of known chlorobenzilate (Figure 1).

Chlorobenzilate was also applied to the Dohrmann instrument, which utilizes a microcoulometer detector (*5*). The microcoulometer detector gave greater sensitivity and selectivity and was therefore used for the analysis of this com-

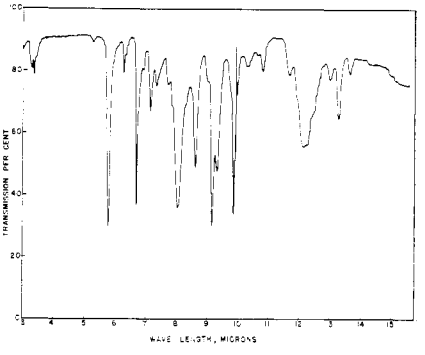

Figure 1. Infrared spectrum of chlorobenzilate collected from F & M gas chromatograph

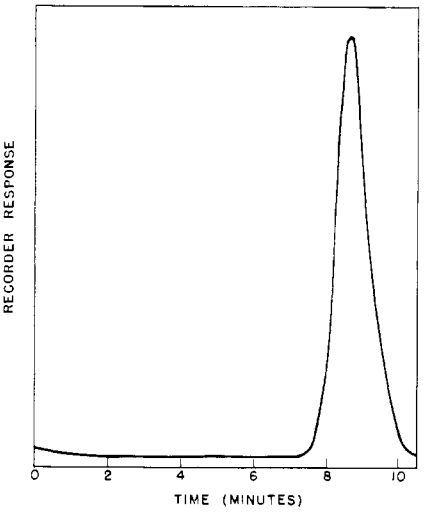

Figure 2. Gas chromatogram of chlorobenzilate using a microcoulometer detector

pound (Figure 2). Operating conditions for the Dohrmann apparatus included an injection temperature of 270° C., column temperature 260° C., nitrogen and oxygen flow rates each 100 ml. per minute, and sample size 12 μg. Initially, the chromatographic column consisted of 6-foot stainless steel tubing containing 20% Dow-11 silicone grease on acid-washed Chromosorb P. The silicone grease had been previously fractionated to remove inorganic silica and other impurities. Replacing the stainless steel tubing with quartz tubing resulted in an improvement in column efficiency (*1*). Reports reaching this laboratory indicate that others have replaced metal columns with glass columns in other types of equipment and obtained improved recovery.

Subsequent to the work reported here the authors made an extensive investigation of the microcoulometer gas chromatograph system, replacing the aluminum injection block and the metal chromatographic column with an all-glass system (*2*). This change was first suggested by a study of comparison of column efficiencies of copper, stainless steel, aluminum, and quartz columns which showed that quartz columns resolved the pesticides more efficiently than metal columns (*1*). Compounds of the DDT-type showed marked improvement in terms of per cent recovery. However, the data indicated a consistent loss of a portion of such compounds that appeared to be directly related to the halogen content of the compound. The structural similarity of chlorobenzilate to DDT suggests that an all-glass, or preferably, an all-quartz, gas chromatograph system would be more desirable than the metal block and metal column.

Extraction. Grapes were macerated in a Waring Blendor, and cottonseed was passed through a Wiley mill with a 2-mm. screen. The plant materials were then tumbled for 1 hour with redistilled benzene. The plant material–solvent ratio was 1:2 for the grapes and 1:4 for the cottonseed meal. The extracts were separated from the solids by filtration through a fluted filter paper, dried with sodium sulfate, and bottled for subsequent analysis. Control samples were fortified with 1 p.p.m. of chlorobenzilate prior to the extraction step.

Cleanup. COTTONSEED. An aliquot of the cottonseed extract equivalent to 25 grams of cottonseed was evaporated to near dryness using a rotary flash evaporator. The residue was transferred to a separatory funnel with 50 ml. of hexane, which was extracted with three 50-ml. portions of nitromethane. The nitromethane fractions were evaporated to near dryness on the flash evaporator and the concentrates transferred to 1- × 15-cm. columns with ca. 5 ml. of benzene. (The Florisil had been heated at 260° C. for 3 hours and stored in wax-sealed bottles.) The column was washed with three 10-ml. portions of benzene, followed by 50 ml. of 20% diethyl ether in benzene. The chlorobenzilate was eluted from the column with 50 ml. of a 1:2 (v./v.) acetone-benzene mixture. The eluate was concentrated to 250 μl. for subsequent analysis on the Dohrmann gas chromatograph. Eluates are concentrated by removing the bulk of the solvent in a round-bottomed flask using a rotary vacuum evaporator with a built-in solvent trap. The concentrate is transferred to a sedimentation tube with 5- or 10-μl. divisions in the calibrated portion. Further concentration is made with a gentle stream of air directed at the surface of the liquid. The final solution is stirred with an off-center stopper on the shaft of a small electric motor.

Acetonitrile was tried as the partitioning agent, but too much of the cottonseed oil remained in the acetonitrile fraction, and nitromethane was much more efficient in extracting the pesticide from the oil.

Grapes. Grape extracts were subjected to the same cleanup procedure without success. Both activated and nonactivated Florisil were tried, but under the conditions used, the grape pigments eluted from the column with the acaricide.

An aliquot of the anhydrous benzene grape extract equivalent to 25 grams of grapes was agitated with 2.0 grams of Nuchar C-190N carbon for 1 hour. The mixture was filtered through a double thickness of fluted filter paper, and the filtrate was concentrated to 250 μl. for gas chromatographic analysis, as previously described.

Results and Discussion

Recovery data and chlorobenzilate residues found in grapes and cottonseed are illustrated in Table I. Gas chromatographic column efficiency for chlorobenzilate was similar to that noted previously (*1*) for DDT-type compounds.

Plant material containing high percentages of pigments and/or oils can temporarily ruin the efficiency of the gas chromatograph. In fact, one application of a highly contaminated sample can close down the operation of the instrument for as much as 24 hours. For the Dohrmann instrument, removing the contaminants necessitates flushing the chromatographic system with large amounts (up to 500 μl.) of solvents, such as xylene and benzene, and heating the system at 250° to 300° C. for several hours or an overnight period. If this treatment does not correct the problem, the chromatographic system must be dismantled, cleaned with basic and acidic solvents, and the column itself must be refilled with new packing material. Therefore, whether the chromatographic system is of all-metal or all-glass construction, a rigorous cleanup procedure must precede application of the sample to the gas chromatograph. A contaminated sample may also temporarily poison an electron capture detector causing it to be dismantled and cleaned. Grape pigments or cottonseed oil are especially disastrous to a gas chromatograph column.

Table I. Chlorobenzilate Residue Data

Days after Treatment	P.P.M. Found[a]	Recovery from Fortified Controls, %	
	GRAPES		
0 (Control)	<0.05	84[b]	
1	5.4	83	
8	5.0	78	
14	3.9	77	
21	2.6	96	
	COTTONSEED		
0 (Control)	<0.05	87[b]	60[c]
15	0.12	87	...
70	<0.05	88	60
93	<0.05	88	60

[a] Sensitivity, 0.05 p.p.m.
[b] Controls fortified at 1.0 p.p.m.
[c] Fortified at 0.10 p.p.m.

Cleanup problems continue to consume a large portion of the analyst's time. Unfortunately, there is no universal type of alumina, Florisil, or charcoal mixture which is a panacea for the separation of pesticides from the many natural and processed food products with which the analyst is confronted. This was evidenced in the analysis of grapes and cottonseed for chlorobenzilate residues. Florisil clarification could be applied to cottonseed but not to grapes because of pigment problems; carbon clarification could be applied to grapes but not to cottonseed because of oil problems. It would be advantageous to have available a standardized grade of Florisil that would be applicable for the removal of all foreign materials; however, this seems to be improbable. Published procedures on the standardization of Florisil by subjecting it to high temperature treatments and subsequent rigid storage conditions are not universally applicable.

Different commercial carbons vary greatly in their adsorptive properties. However, after screening many available carbons, the authors found that Nuchar C-190N was quite consistent in character and could be used as a clarification agent for many highly pigmented plant extracts containing certain pesticides. With this carbon, it was not necessary to water-saturate the benzene. When no prior data are available, it is always advisable to make a preliminary check of the adsorption

properties of the carbon and to determine the recovery of a given pesticide.

Other problems potentially present in the cleanup step are the loss of the pesticide because of multiple handling steps and volatility. Chlorobenzilate was not volatile under the conditions used. Also, because of the development of detectors with increased sensitivity, the organic reagents that are used in the isolation of the pesticide must be free of impurities, such as halogens, which will be detected in microgram or picogram amounts on the gas chromatograph.

Crafts (*4*) has demonstrated that the isopropyl ester of 2,4-D undergoes hydrolysis on the leaf surface of a plant whereby the acid is adsorbed and translocated. The authors also have evidence that esters of 2,4-D revert to the parent acid in plant materials obtained from experimental plots. Therefore, the question arises whether or not chlorobenzilate may partially hydrolyze to the free acid during weathering of the chemical on the plant in the field. No studies were made on this phase of the subject. The free acid would not chromatograph under the conditions of column operation used in this study.

The application of the gas chromatographic procedure to the analysis of grapes and cottonseed for chlorobenzilate residues eliminated several tedious and painstaking fractionation and chemical conversion steps and gave more precise and specific residue data.

Acknowledgment

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Literature Cited

(1) Beckman, H. F., Bevenue, A., *J. Chromatog.* **10,** 231 (1963).
(2) *Ibid.*, **12,** 109 (1963).
(3) Blinn, R. A., Gunther, F. A., Kolbezen, J., J. AGR. FOOD CHEM. **2,** 1080 (1954).
(4) Crafts, A. S., *Weeds* **8,** 19 (1960).
(5) "Dohrmann Operational Manual," Dohrmann Instruments Co., San Carlos, Calif., 1962.
(6) Harris, H. J., J. AGR. FOOD CHEM. **3,** 939 (1955).
(7) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, H. L., *Ind. Eng. Chem., Anal. Ed.* **17,** 704 (1945).

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HERBICIDE RESIDUES

Determination of Amiben in Tomatoes by Electron Affinity Gas Chromatography

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A method is described for the determination of 3-amino-2,5-dichlorobenzoic acid (amiben) in tomatoes. It involves extraction of amiben at pH 2 into benzene from an alkali-hydrolyzed tomato extract. The acid is then methylated with BF_3-methanol, and the ester is determined by electron affinity gas chromatography. Recovery of the herbicide from 0.05 to 1.25 p.p.m. ranged from 70 to 123%. About 0.02 p.p.m. of the herbicide may be detected. Residues observed in field-treated samples are reported.

THE HERBICIDE 3-amino-2,5-dichlorobenzoic acid (amiben) may be used for weed control in vegetables and certain other crops. In this paper, a method is described for the determination of residues of this compound in tomatoes based on electron affinity gas chromatography of the methyl ester.

Equipment

A Barber-Colman Model 10 gas chromatograph was used with a battery-operated (*4, 5*) Barber-Colman No. A-4071, 6-cc. detector containing 56 μc. of radium-226. The detector was operated at 3.5 volts, which was found to be its optimum for electron capture by chlorinated compounds. A 90,000-megohm resistor was added to the electrometer to give additional gains of 3,000; 10,000; and 30,000. The 3,000 and 10,000 settings were used in this study. The recorder was a Wheelco, 0 to 50 mv. equipped with 10-inch chart paper, running 10 inches per hour.

The column was borosilicate glass, U-shaped, 9-mm. o.d., and 6 feet long. The packing was 5% Dow Corning high-vacuum silicone grease (ethyl acetate–fractionated) on 80- to 100-mesh acid-washed Chromosorb W. Connections between the column and detector were made with metal hypodermic tubing and glass elbows, using silicone rubber through septums. The operating temperatures for the column, flash heater, and detector were 200°, 265°, and 235° C., respectively, and nitrogen (35 cc. per minute) was the carrier gas. The column was conditioned for 16 hours at 230° C. before use.

Procedure

Weigh 10 grams of well-blended tomatoes in a 100-ml. beaker. Add 10 ml. of 2*N* sodium hydroxide, cover with a watch glass, and boil the mixture for 30 minutes. Transfer the digest quantitatively to a 50-ml., glass-stoppered centrifuge bottle with about 10 ml. of 0.1*N* sodium hydroxide and centrifuge at 2000 r.p.m. Filter the supernatant liquid through No. 41 Whatman paper into a 200-ml. beaker. Shake the residue in the bottle with 25 ml. of 0.1*N* base and centrifuge as above. Decant the supernatant liquid and use this liquid to rinse the filter. Repeat this step using 25 ml. of the 0.1*N* base and again centrifuge and filter the solution. Combine the alkali filtrates.

Adjust the pH of the solution to 7.5 with 5*N* hydrochloric acid using a pH meter and transfer the solution to a 250-ml. separatory funnel. Extract the solution four times with 50 ml. of diethyl ether and once with 50 ml. of benzene. Discard the organic solvents. Adjust the pH of the aqueous solution to 2.0 with 5*N* hydrochloric acid and extract