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A Gas–Liquid Chromatographic Method for the Determination of Dodine Residues on Foods

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A gas–liquid chromatographic method was developed for the determination of dodine residues on fruit crops. The procedure involves extraction with methanol, partitioning with chloroform, and derivatization with hexafluoroacetyl acetone. The method yields mean recoveries of 84% or greater from 0.05 to 10.0 ppm of residue and has a lower detectable limit of 8 ppb. Confirmation of the derivative by GLC–mass spectrometry is described.

Dodine (*n*-dodecylguanidine acetate) is an agricultural fungicide introduced in 1957 and used in the control of certain diseases of fruit trees such as scab on apple or pear, leaf spot on cherry, and foliar diseases on strawberry. The presently available method for the determination of dodine residues involves a colorimetric procedure (Steller et al., 1960) and is unsuitable for regulatory purposes. Thus, it was considered desirable to develop a gas–liquid chromatographic method which would detect low levels and permit confirmation by mass spectrometry.

Because of their polar nature, guanidines require structural modification to render them sufficiently volatile for gas chromatography. Some guanido-containing compounds have been chromatographed as the corresponding trifluoroacetylated amine formed after alkaline hydrolysis (Hengstmann et al., 1974). A simpler and more sensitive method has been described which involves condensation with hexafluoroacetyl acetone to form a substituted pyrimidine (Erdtmansky and Goehl, 1975). Application of this approach to dodine provided a derivative which was amenable to gas–liquid chromatography and which could be detected at low concentration.

EXPERIMENTAL SECTION

Materials. Dodine was supplied by American Cyanamid and was labeled as assaying 100%. Solutions of dodine used for the fortification of samples were prepared in reagent grade methanol and were added to samples in volumes of 0.5 ml or less prior to initial extraction.

A reference standard of 2-dodecylamino-4,6-bis(trifluoromethyl)pyrimidine was prepared by refluxing dodine (500 mg; 1.74 mmol) for 4 h with hexafluoroacetyl acetone (2.0 g; 9.6 mmol) in benzene (10 ml). The resulting yellow solution was taken to dryness on a rotary evaporator and the amber oil taken up in warm hexane (10 ml). Upon cooling, a small quantity of dodine precipitated and was removed by filtration. The solvent was evaporated and the residual liquid permitted to crystallize at room temperature. The yield was 630 mg (91%), mp 34–35 °C. A 60-MHz NMR spectrum in deuteriochloroform gave the following resonances: δ (ppm) 7.08, 3.50, 1.30, 0.92. The ratio of their intensities was 1:2:20:3, respectively. The mass spectrum gave a molecular ion at m/e 399 as shown in Figure 1.

Analytical Procedure. The sample (5.0 g) was homogenized with methanol (50 ml) at high speed for 1 min in a Sorvall Omni-Mixer. The homogenate was filtered through Whatman No. 1 paper on a Buchner funnel using gentle vacuum. An aliquot (5.0 ml) of the filtrate was added to 0.1 M NaOH (30 ml) in a 125-ml separatory funnel. The dodecylguanidine was extracted with chloroform (10 ml) and the extract evaporated to dryness in a 15-ml centrifuge tube using a stream of nitrogen. A solution of redistilled hexafluoroacetyl acetone (10 μl) in benzene (400 μl) was added to the dried sample. A 10 × 150 mm air condenser was attached and the bottom 1 cm of the tube heated by immersion in a sand bath at 85 °C for 1 h.

After heating, the tube was cooled and the solvent removed by evaporation under a stream of nitrogen. The sample was then dissolved in hexane (1.0 ml) and applied to a small column of silicic acid. The column was prepared by placing a 5 × 25 mm bed of silicic acid (Mallinckrodt, AR, 2847, 100 mesh) on a plug of glass wool in a Pasteur pipet, slurrying it with hexane, and packing under nitrogen pressure. The sample was driven onto the column with nitrogen and the eluate discarded. The derivative was then eluted with 30% benzene in hexane (1.0 ml) and an aliquot (2 μl) analyzed by gas–liquid chromatography.

Gas–Liquid Chromatography. Analyses were carried out on a Hewlett Packard 5700 A gas chromatograph fitted with a 6 ft × 4 mm i.d. glass column and ^{63}Ni electron capture detector. The column was packed with 5% butanediol succinate on 100–120 mesh Chromosorb W, HP and preconditioned for 48 h at 200 °C under a flow of argon–methane (95:5) carrier gas. The column oven was maintained at 170 °C and detector at 300 °C. The carrier gas flow rate was 24 ml min^{-1}. Under these conditions and with a routine working attenuation, 100 pg of standard produced a peak with 40% full scale deflection. Samples

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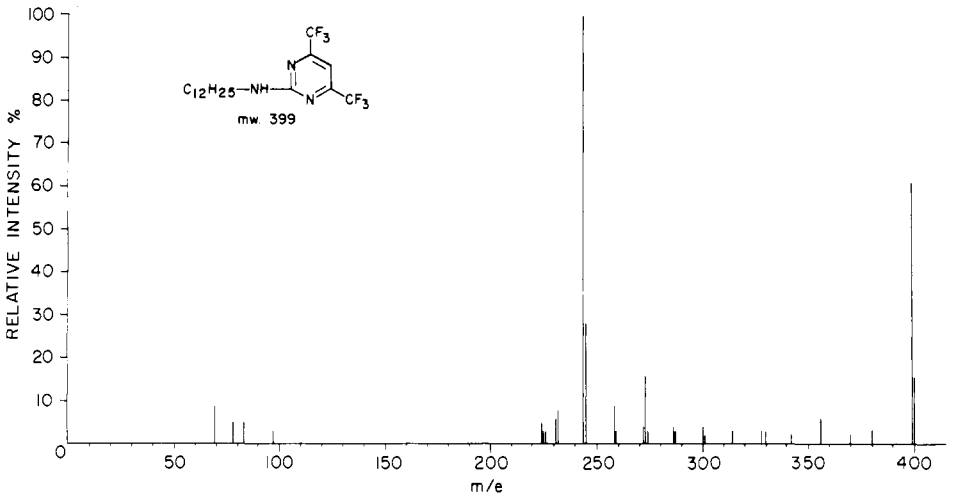

Figure 1. Mass spectrum of 2-dodecylamino-4,6-bis(trifluoromethyl)pyrimidine. Sample introduced by probe at 20 °C; ionization voltage, 70 eV.

Table I. Recovery of Dodine from Various Commodities

Dodine added, ppm	Dodine recovered,[a] %			
	Pear	Cherry	Strawberry	Apple
0.050	84.6	80.0	79.4	89.1
0.100	82.3	86.5	85.6	88.1
1.00	83.3	79.1	106	85.0
5.00	84.4	83.1	114	86.9
10.0	86.3	91.5	113	88.4
Mean recovery (% ± SE)	84.2 ± 0.7	84.0 ± 2.3	99.8 ± 7.1	87.5 ± 0.7

[a] Values are the average of duplicate determinations.

were quantitated by comparison of the peak height to that of a 2-dodecylamino-4,6-bis(trifluoromethyl)pyrimidine standard.

GLC–Mass Spectrometry. Analyses were performed using a Varian MAT 311A double-focusing high-resolution spectrometer coupled to a Varian 1440 GLC. The GLC contained a 6 ft × 4 mm i.d. glass column packed with 5% OV-25 on 100–125 mesh Chromosorb W, HP. The column was maintained at 200 °C and was purged with helium carrier gas at a flow of 30 ml min^{-1}. Using these parameters, 2-dodecylamino-4,6-bis(trifluoromethyl)pyrimidine had a retention time of 2.75 min. The mass spectrometer was operated with the interface at 230 °C, source at 250 °C, and an ionizing voltage of 70 eV. The M^+ ion was monitored at m/e 399 with a resolution of 2000. Under these conditions, 100 pg of derivative produced a peak with 30% of full scale deflection.

RESULTS AND DISCUSSIONS

The derivatization of dodine was found to be quantitative when carried out as described. The presence of methanol (20%) or a shorter reaction time (30 min) reduced the yield to approximately 30%.

Typical gas–liquid chromatograms of extracts from various commodities carried through the analytical procedure are shown in Figure 2. The minimum detectable limit, defined as twice background, was found to be 8 ppb.

The recoveries obtained from fruit fortified with dodine are shown in Table I. A linear relationship existed between the amount added to the sample and that found after extraction, derivatization, and cleanup between 0.05 and 10.0 ppm. Mean recoveries for the range of concentrations examined were 84% or greater.

Confirmation of the residues was possible by injection of an aliquot (2 μl) of the column eluate into the GLC–mass spectrometer. The sensitivity of confirmation by single ion monitoring was similar to that of electron capture detection. Presumably, confirmation could be achieved at lower levels by concentrating the column eluate.

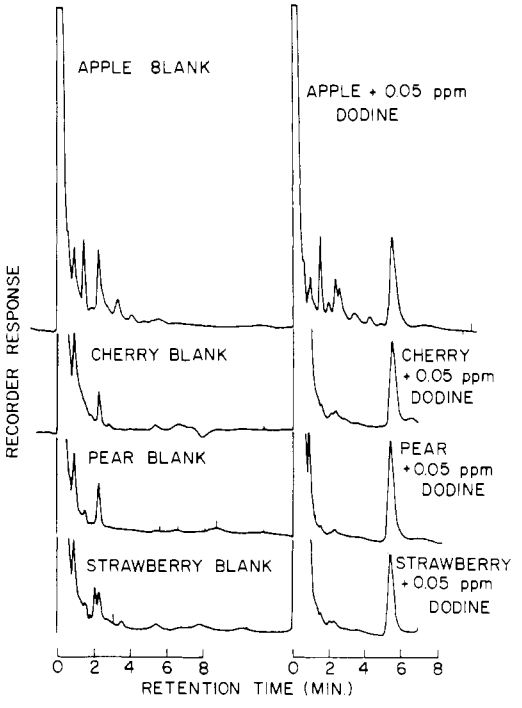

Figure 2. Gas-liquid chromatograms of dodine determinations performed on four fruits with and without the addition of 0.05 ppm of dodine. Each injection represents the equivalent of 1 mg of sample.

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Residues of Four Ethylenebis(dithiocarbamates) and Their Decomposition Products on Field-Sprayed Tomatoes

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Ethylenebis(dithiocarbamates) and their decomposition products ethylenethiuram monosulfide, ethylenebis(isothiocyanate), and ethylenethiourea were determined on tomatoes after field spraying with four different formulations. The highest residues were found on crops treated with the manganese-containing products. After 14 days, 24–37% of the initial ethylenebis(dithiocarbamate) remained. The heating of homogenates of the treated crop material resulted in a 38–48% conversion to ethylenethiourea.

In a previous study of the dissipation of maneb and its decomposition products on two crops (Newsome et al., 1975), it was found that significant levels of all compounds remained 2 weeks after application. It was also observed that the residues of maneb were excellent precursors of ethylenethiourea (ETU) when subjected to heat.

The present investigation was initiated to provide residue data for other ethylenebis(dithiocarbamates) (EBDC's) as compared to maneb and to determine the extent to which these residues were converted to ETU under conditions of cooking. In addition, data were sought on the levels of ethylenebis(isothiocyanate) (EBI) present after treatment with various EBDC's, since there was previous chromatographic evidence that it existed on tomatoes treated with maneb (Engst et al., 1968).

EXPERIMENTAL SECTION

Tomato Plots. Tomato plants (Ottawa 78 variety) were grown at the Ottawa Research Station, Agriculture Canada during the summer of 1975. Five plots were planted, four of which were treated with different EBDC's and one of which was sprayed with water as a control. A plot consisted of two 30-ft rows, each containing 10 plants. Each treated area was separated from the others by a 4 ft high polyethylene vapor barrier to prevent contamination by drift. Total rainfall during the test interval was 5.82 in.

Treatment with EBDC's. The EBDC's used in this experiment were purchased from a local supplier and consisted of: Manzate D, an 80% maneb formulation; Dithane M-45, a product containing 80% mancozeb; Polyram 80-W, containing 80% metiram; and Zineb 75W, containing 75% zineb. All products were applied at the maximum rates recommended by the manufacturers which were as follows: Manzate D, 3 lb (2.4 lb of active ingredient (AI))/acre; Dithane M-45, 3 lb (2.4 lb of AI)/acre; Polyram 80-W, 2 lb (1.6 lb of AI)/acre; and Zineb 75W, 3.25 lb (2.4 lb of AI)/acre. Seven treatments of each fungicide were applied as aqueous suspensions at intervals of 7 days.

The sprayer was an E.R.S. self-propelled plot sprayer developed by Gary Hergert, Engineering Research Service, Agriculture Canada for the application of spray solutions to small areas in a manner simulating commercial low-pressure sprayers. The apparatus consisted of a chassis containing a 5-gal reservoir, pump, pressure regulator, and drop tubes for three Spraying Systems no. 6508 nozzles mounted on a spray boom. The sprayer was operated at a constant forward speed of 2 mph and a pump pressure of 30 psi, resulting in a 103 gal/acre output. The EBDC's were maintained in suspension during spraying by agitation provided by by-pass liquid from the pressure regulator.

Sampling. Samples were collected immediately after the final application of fungicide and at intervals up to 14 days thereafter. Each plot was divided into 6 sampling areas from which approximately 500 g of tomatoes was removed after each interval. Immediately upon receipt at the laboratory each sample was homogenized without prior washing or peeling and subsamples removed for ethylenethiuram monosulfide (ETM) and EBI analysis. The remaining homogenate was frozen at −18 °C for later analysis of the parent EBDC's and ETU.

Heat Treatment. Tomato homogenate (5 g) was heated to boiling in a 125-ml flask connected to a reflux condenser. The elapsed heating time was 10 min. After cooking, the samples were cooled and extracted with ethanol for the determination of ETU.

Analytical Methods. EBI (Newsome, 1976) and ETM (Newsome, 1975) were determined by gas–liquid chromatography exactly as described. ETU was determined by gas–liquid chromatography of the trifluoroacetylated *S*-benzyl derivative (Pecka et al., 1975) on a 6-ft column of 2% butanediol succinate on Chromosorb W, HP. The EBDC's were analyzed by hydrolysis and gas–liquid chromatography of the resulting ethylenediamine (Newsome, 1974).

RESULTS AND DISCUSSION

The dissipation of the various EBDC's with time is given in Table I. Residues left by the manganese-containing

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