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Note

Multiresidue method for fungicide residues in fruit and vegetables

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The efficiency and cost-effectiveness of multiresidue methods have prompted their application to a number of pesticides in food. Luke *et al.*[1] have developed a procedure involving acetone extraction followed by partitioning into hexane–dichloromethane which is capable of determining at least 112 compounds in a variety of foods. This method does not require clean-up other than partitioning and relies on gas–liquid chromatography (GLC) using specific detectors to avoid interference by co-extractives. Similarly, Martindale[2] developed a multiresidue method for 21 pesticides on potatoes which employed only a partitioning step for clean-up and a combination of six different chromatographic systems for determination. In a modification of the Luke method, Blaha and Jackson[3] used solvents of increasing polarity in the partitioning step, followed by gel permeation chromatography and GLC to determine 17 organophosphorous pesticides in 41 foods. Andersson and Ohlin[4] also employed the Luke extraction and gel permeation chromatography to determine 126 pesticides in several foods. Quantitative recoveries were obtained using GLC and selective thermionic and flame photometric detection. A different extraction procedure[5] involving the use of dichloromethane and methanol followed by water removal with Extrelut cartridges was used for some compounds such as carbendazim and thiophanate methyl, which were poorly recovered by acetone extraction.

These multiresidue methods involve the use of relatively large volumes of organic solvents in partitioning and clean-up steps and require associated glassware or automated apparatus in the case of gel permeation chromatography. Solid-phase extraction cartridges containing normal- or reversed-phase supports have become available commercially and offer the potential of simplifying the purification of the initial extract as well as reducing the amount of solvent consumed. Successful applications have been found in the determination of N-methyl carbamates in fruits and vegetables[6] and for a variety of pesticides in water[7]. The present study was conducted to examine the use of solid-phase extraction in a multiresidue method for fungicide residues in foods.

EXPERIMENTAL

Instrumentation

GLC was carried out on a Varian 3500 apparatus equipped with a ^{63}Ni elec-

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tron-capture detector, a Model 8035 autosampler, an on-column injector, a 30 m × 0.32 mm I.D. column coated with DB-5 to a film thickness of 0.25 μm, and a Model 650 data system. Helium carrier gas was supplied to the column at a linear velocity of 40 cm/s, and the nitrogen make-up gas flow-rate to the detector was 22 ml/min. The detector was maintained at 300°C. The initial injector temperature was 100°C and after a 0.5-min delay programmed at 100°C/min to 250°C. The initial column temperature was 100°C and after a 0.5-min delay programmed first at 50°C/min to 190°C and then, after 9 min at this temperature, at 30°C/min to 25°C where it was maintained for 5 min to elute high-boiling co-extractives.

High-performance liquid chromatographic (HPLC) separations were performed on a 30 cm × 3.9 mm I.D. column packed with 10-μm μBondapak C_{18} stationary phase. The mobile phase was delivered to the column isocratically at 1.0 ml/min with a Beckman 110B pump connected through a 100-μl injection loop and a Hamilton C_{18} guard column. The effluent was monitored by a Waters Model 440 absorbance detector with a 254-nm filter connected in series to a Schoeffel FS 970 fluorometer set at an excitation wavelength of 302 nm with a KV 370 emission filter.

Reagents

Solvents were purchased from Caledon Labs. (Georgetown, Canada) and were HPLC grade. Standards of the twelve respective fungicides were obtained from the repository of pesticide standards maintained in the Food Research Division and were at least 98% pure. Each compound was weighed accurately and dissolved in acetonitrile to give a stock solution containing approximately 2 mg/ml. A composite spiking solution containing 48 μg/ml was used to spike samples at the 5-ppm level and was prepared by adding 0.6 ml of each stock solution to a 25-ml volumetric flask and making to the mark with acetonitrile. A 1:10 dilution of this composite was used to prepare samples spiked at 0.5 ppm.

Solid-phase extraction was carried out using a cartridge containing 0.5 g of C_{18} stationary phase (Supelco, Bellefonte, PA, U.S.A.). The cartridge was washed before use by rinsing sequentially with 60 ml each of methanol, dichloromethane, again with methanol, and then 30 ml of water. This procedure removed substances which produced background peaks on the gas chromatogram. The extraction cartridges were eluted using a vacuum manifold supplied by the manufacturer and were fitted with detachable reservoirs to contain the sample and elution solvents.

The mobile phase for HPLC separations contained 60% methanol and 40% aqueous buffer prepared by adjusting 0.01 *M* H_3PO_4 to pH 7.0 with aqueous trimethylamine. The mobile phase was supplied to the column at a flow-rate of 1 ml/min.

Analytical procedure

The respective commodities were purchased at a local food store and 500-g samples homogenized in a Waring blender. Subsamples (10 g) were spiked with the composite fungicide standard in acetonitrile (1 ml) to give 0, 0.5 or 5.0 ppm of each compound, mixed, and extracted with acetone (35 ml) by blending on a Polytron homogenizer. The extract was filtered through Whatman No. 1 paper on a Buchner funnel using gentle vacuum and the filtrate transferred to a volumetric flask and brought to 50 ml with acetone. A 10-ml aliquot of extract was diluted to 50 ml with water and added to the reservoir of a pre-washed extraction cartridge.

After passage of the diluted sample extract through the cartridge, the adsorbent was washed with 10 ml of 40% methanol in water which was discarded and the fungicides were eluted with 5 ml of methanol. Care was taken during the washing and elution steps to prevent the column from going dry. For analysis by GLC, an aliquot of the eluate (4 ml) was transferred to a 15-ml centrifuge tube, diluted to 10 ml with water, and extracted with 5 ml of toluene. A 1:5 and 1:50 dilution of the toluene extract were placed in the autosampler rack for injection. Quantitation was carried out by reference to a linear calibration curve defined by the injection of a standard mixture of all compounds at five concentrations ranging from 20 to 80 ng/ml.

For determination by HPLC, a 0.5-ml aliquot of eluate was diluted to 2.5 ml with mobile phase and 100 μl were injected into the chromatograph. Biphenyl was determined by its absorbance at 254 nm and *o*-phenylphenol by flurorescence. Quantitation was accomplished by reference to a 40 ng/ml standard of each compound injected in duplicate with each set of six samples. Samples containing 5 ppm of fungicide were determined after dilution 1:25 with mobile phase.

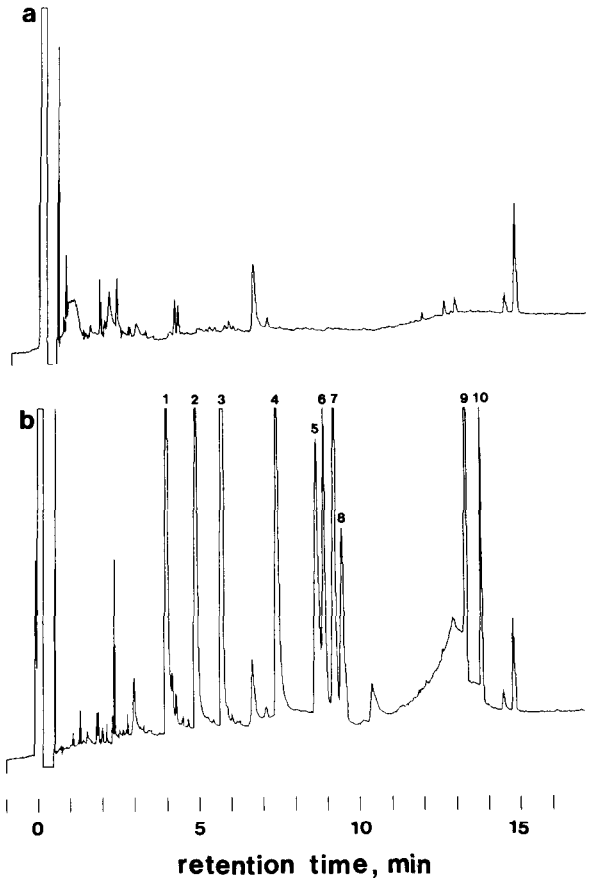

Fig. 1. GLC profiles of (a) unspiked apple and (b) apple spiked with 0.5 ppm of various fungicides. Peaks: 1 = dichloran; 2 = chlorothalonil; 3 = vinclozolin; 4 = triadimefon; 5 = anilazine; 6 = captan; 7 = folpet; 8 = procymidone; 9 = captafol; 10 = iprodione.

RESULTS AND DISCUSSION

Acetone was selected as the solvent for initial extraction because it has been shown[1,3,8] to be effective in the extraction of a large number of pesticides of diverse nature from a range of matrices with good recovery. Dilution of the extracts to a 20% acetone content was necessary to effect adsorption of all the fungicides onto the stationary phase. They were then recovered quantitatively from the cartridge by elution with methanol. Prewashing of the C_{18} cartridge before use with several column volumes of solvent was necessary to remove substances which interfered with the GLC and HPLC determinations. Contamination of both the packing material and the plastic container have been noted previously and the identity of several of these compounds reported[9].

GLC profiles of purified apple extract are shown in Fig. 1 and are typical of all commodities examined. The rise in baseline before the captafol peak (9) is due to decomposition on the GLC column and limited the quantitation limit of it to 1 ppm, since the peak area of captafol was linear with concentration injected above this level but decreased in a non-linear manner below it. Thus, the spiking levels for captafol were 1 and 10 ppm whereas the remainder of the fungicides were spiked at 0.5 and 5 ppm.

HPLC profiles from the same apple extract are given in Fig. 2. The fluorometric detector tracing is free of interferences while that of the UV detector indicates the presence of several UV-absorbing materials in the earlier part of the chromatogram. However, no interferences were observed in any commodities at the retention time of

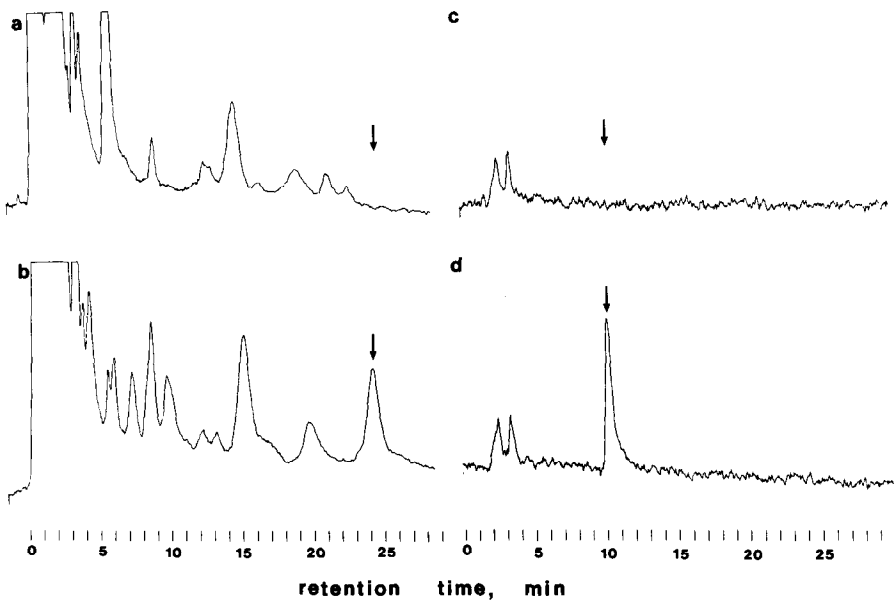

Fig. 2. HPLC profiles of unspiked apple (a and c) and of apple spiked with 0.5 ppm of biphenyl (b) and *o*-phenylphenol (d). Tracings a and b show absorbance at 254 nm while c and d record fluorescence emission. Retention time of biphenyl is 24 min and that of *o*-phenylphenol is 10.5.

TABLE I

RECOVERIES (%) OF FUNGICIDES ADDED TO FRUIT AND VEGETABLES AT 0.5 AND 5.0 ppm

Compound	*Grape*	*Apple*	*Tomato*	*Pear*	*Cucumber*	*Strawberry*	*Orange*	*Potato*
0.5 ppm spike								
Dichloran	83	87	93	94	100	85	81	76
Chlorothalonil	93	86	83	92	97	90	83	82
Vinclozolin	96	87	91	97	101	100	89	90
Triadimefon	96	84	98	94	98	102	91	94
Anilazine	97	82	93	89	102	102	91	101
Captan	92	85	88	103	91	119	76	88
Folpet	96	84	85	98	77	102	86	91
Procymidone	92	80	96	94	96	100	87	82
Captafol[a]	96	82	84	92	88	102	85	85
Iprodione	105	81	90	96	102	96	93	106
o-Phenylphenol	92	99	92	101	99	92	92	90
Biphenyl	91	88	80	83	97	82	82	90
5.0 ppm spike								
Dichloran	74	88	89	88	95	77	77	76
Chlorothalonil	82	84	87	89	97	85	90	89
Vinclozolin	83	85	88	88	99	88	89	88
Triadimefon	83	85	91	88	98	89	95	92
Anilazine	86	87	96	87	98	91	96	101
Captan	79	86	83	92	90	84	80	86
Folpet	83	85	85	90	91	87	90	90
Procymidone	81	85	90	86	97	88	88	84
Captafol[b]	83	84	84	86	95	88	91	88
Iprodione	85	84	92	92	106	87	100	97
o-Phenylphenol	96	102	91	104	101	95	95	99
Biphenyl	82	78	98	95	94	87	87	90

[a] 1.0 ppm.
[b] 10.0 ppm.

biphenyl. Although anilazine and dichloran were separated from the solvent front and are detectable by UV absorption, interfering co-extractives prevented their accurate quantitation, particularly at 0.5 ppm.

Satisfactory recoveries of the twelve fungicides were obtained from the eight commodities spiked in duplicate at 0.5 and 5 ppm as shown by the data in Table I. To obtain an indication of the repeatability of the procedure, apple was spiked at 0.5 ppm and analyzed in triplicate on two separate days. A mean coefficient of variation of 4.0% (range 2.3–8.0%) was obtained for recoveries of the twelve compounds.

The present data indicate that solid-phase extraction using a C_{18} cartridge is capable of effecting rapid isolation and clean-up of several fungicides from acetone extracts of fruit and vegetables while reducing the amount of organic solvent required for the clean-up phase of the analytical procedure. This reduction in solvent volume could be further improved if manufacturers provided materials free of platicizers and other monomeric materials which interfere in the determinative step. Although we did not examine the products of different suppliers, it has been shown[9] that some contain materials of a higher degree of purity and may be capable of providing

satisfactory blanks without extensive washing. The extension of this approach to other classes of pesticides and to compounds of greater polarity remains to be addressed in other studies.

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