Determination of Thiabendazole Residues in Fruits by High-Performance Liquid Chromatography and Their Confirmation after *p*-Nitrobenzyl Derivative Formation

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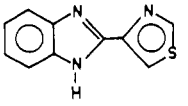
The determination of thiabendazole (TBZ) residues in fruits and the confirmation of their presence have been carried out by two sensitive and simple techniques. When high-performance liquid chromatography with a UV detector is used for the analysis of TBZ residues, it is essential that the interfering substances be eliminated by a combined partitioning scheme and silica gel column chromatography technique. The conversion of TBZ to the *p*-nitrobenzyl derivative gives quantitative results with the procedure adopted. The recoveries from fortified fruits varied from 78.6 to 103.2% in the range 0.02–1.0 mg/kg. The sensitivity was such that TBZ could be detected down to a level of 0.002 mg/kg for pears and bananas and 0.02 mg/kg for oranges. The presence of TBZ can be quantitatively and qualitatively confirmed by a method that involves the formation of a *p*-nitrobenzyl derivative.

Thiabendazole, 2-(4-thiazolyl) benzimidazole (TBZ), is an anthelmintic and antifungal agent extensively used for the post harvest protection of citrus fruits and bananas.



Several methods for the determination of TBZ residues have been reported. Most of the precedures involve rigorous cleanup of sample extracts and final measurement by spectrophotometry or gas–liquid chromatography (Norman et al., 1972; Mihara et al., 1973; Aharonson and Ben-Aziz, 1973; Ebel and Herold, 1974; Miller et al., 1974; Association of Official Analytical Chemists, 1975; Ott, 1975; Otteneder and Hezel, 1975; Tanaka and Fujimoto, 1976). A large amount of work has been done on the conversion of polar TBZ to stable, volatile derivatives and on ensuring successful gas-chromatographic analysis. The derivatives formed from the reaction of TBZ with trimethylsilyl reagents (Jacob et al., 1975), methylation agents (Tanaka and Fujimoto, 1976; Van den Heuvel et al., 1977), pentafluorobenzoyl chloride (Nose et al., 1977), and pentafluorobenzyl bromide (Tjan and Jansen, 1979) have been studied. More recently, high-performance liquid chromatography (LC) using UV or fluorescence detectors (Maeda and Tsuji, 1976; Farrow et al., 1977) has been proposed as an alternative method.

This paper describes a variety of modifications which lead to significant improvements on previously reported high-performance LC methods. Recourse has been made to the formation of a chemical derivative for confirming the identity of fungicide residues, as well as quantifying them. Derivatization was carried out prior to introduction of TBZ into the high-performance LC column.

EXPERIMENTAL SECTION

Apparatus. High-performance liquid chromatography was carried out with a Perkin-Elmer Model Series 3 chromatograph, equipped with a Model LC-55 B variable-wavelength UV detector and a Rheodyne 7105 injection valve with a 175-μL injection loop. Two columns were used: (1) HC-ODS/Sil-X reversed-phase column (Perkin-Elmer 089-0716; 0.26 × 25 cm) with methanol–water (1:1) as the mobile phase; (2) Silica-A/10 column (Perkin-Elmer 089-0702; 0.26 × 25 cm) with tetrahydrofuran–chloroform (1:9) or chloroform as the mobile phase.

General operating conditions were as follows: solvent flow, 0.5–1.0 mL/min; detection, 305 nm; sample size, 5–10 μL depending on the instrument response; sensitivity, 0.5–0.05 AUFS. All chromatograms were recorded by using a Perkin-Elmer Model 56 recorder. Chart speed was 5 mm/min. Standard solution of TBZ was prepared from a 100 μg/mL stock solution in ethanol. Appropriate aliquots were evaporated to dryness under N_2 and then reconstituted in suitable volumes of ethanol or chloroform to match the concentrations expected in the sample. The concentrations of these solutions were usually 10–5 ng/μL. The concentations of TBZ in the samples were calculated from the peak height response of the samples and standard injected onto the column.

Mass spectra (MS) were measured at 70-eV ionization on a Varian 112 S spectrometer.

Reagents. All organic solvents were of "pure" grade and were distilled before use. Celite 545 was obtained from BDH; Kieselgel 60, 70–230 mesh ASTM, was from Merck; *p*-nitrobenzyl bromide was from EGA-chemie.

Extraction. Samples (from 100 to 500 g) of chopped fruit peel or pulp were transferred to a blender jar and homogenized with ethyl acetate (from 200 to 700 mL) for 15 min. The mixture was centrifuged and filtered into a suitable volume flask. The procedure was repeated with additional (from 100 to 300 mL) ethyl acetate. The combined ethyl acetate extracts, after adding 2 g of Celite 545 (Thornburg, 1963), were evaporated until just dry in a rotary evaporator at 50 °C.

Cleanup Procedures. The extraction residue was taken up in three (35-mL) portions of H_2SO_4 (0.5 N) and filtered through a G3 fritted glass filter. The filtrate was collected in a 200-mL separating funnel and extracted with chloroform (3 × 20 mL). After phase separation, the chloroform layers were discarded. When the aqueous solution was not clear, it was filtered through a cotton plug. pH was adjusted to 8.5–9.0 by adding 60% NaOH solution, and then the aqueous solution was extracted with ethyl acetate (3 × 50 mL). The combined ethyl acetate extracts were dried with anhydrous Na_2SO_4 and, after adding 0.2 g of Celite, evaporated until just dry by using a rotary evaporator at 50 °C.

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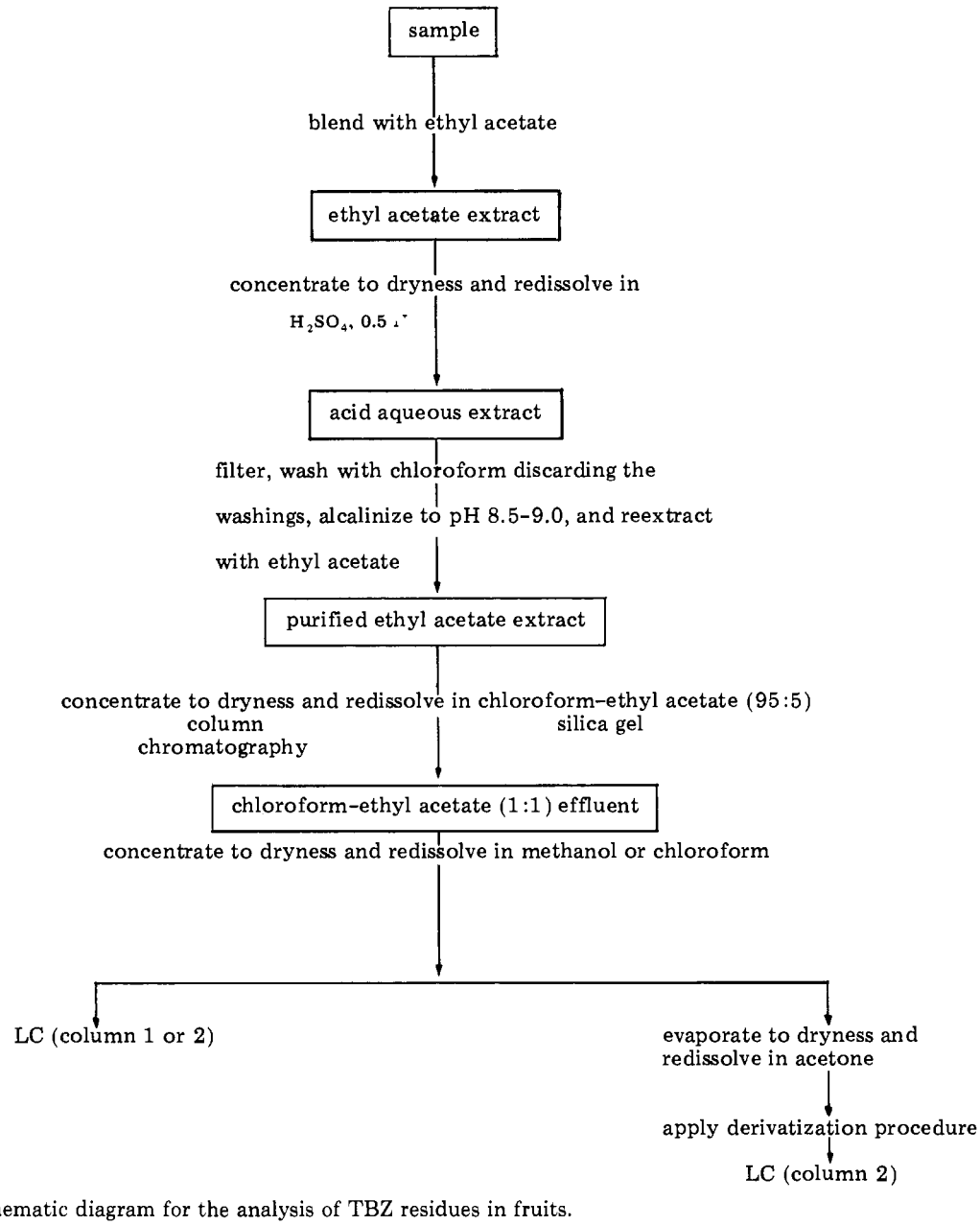

Figure 1. Schematic diagram for the analysis of TBZ residues in fruits.

A chromatography column (2.5 cm d) with a fritted disk (G1) was filled with a slurry prepared by shaking 10 g of Kieselgel 60 with chloroform–ethyl acetate (95:5) and allowed to settle.

The residue from the solvent partition cleanup procedure was taken up on three (5-mL) portions of chloroform–ethyl acetate (95:5) and transferred to the top of the column. The first portion was left to be absorbed almost thoroughly before adding the other. The same solvent mixture (100 mL) was added to the column. All eluates were discarded. The column was then eluted with 120 mL of chloroform–ethyl acetate (1:1). This eluate was collected and evaporated until just dry in vacuo at 50 °C.

High-Performance Liquid Chromatography. The residue was dissolved in methanol or chloroform (0.1–0.5 mL), and aliquots of these solutions were injected on to the liquid-chromatographic column. The methanol solution is suitable for injection onto column 1; the chloroform solution is suitable for injection onto column 2 by using tetrahydrofuran–chloroform (1:9) as the mobile phase.

Derivatization Procedure. After TBZ was determined, the remaining methanol or chloroform solution was evaporated until just dry in a rotary evaporator at 50 °C. This residue or the residue from the Cleanup Procedures section, dissolved in 2.5 mL of acetone, was placed in a 10-mL test tube. Three-tenths milliliter of 30% K_2CO_3 solution and 3 mg of *p*-nitrobenzyl bromide (*p*-NB-Br) in 0.5 mL of acetone were added. The tube was closed with a tightly fitting ground-glass joint and the joint was sealed with parafilm. The tube was then immersed in a paraffin oil bath at 110 °C. Three hours after the beginning of the reaction, the test tube contents were evaporated in a gentle stream of dry nitrogen and the residue was dissolved in an appropriate volume of chloroform (0.1–0.5 mL). Aliquots of this solution were injected onto liquid chromatographic column 2 by using chloroform as the mobile phase.

Figure 1 is a schematic diagram of the method described.

RESULTS AND DISCUSSION

Control experiments demonstrated that the conversion of TBZ to the *p*-NB derivative was quantitative under the operating conditions. The mass spectrum of the *p*-NB derivative of TBZ was characterized by an intense peak

Table I. Mean Recoveries of TBZ Added to Various Samples before Extraction

	recovery from fruits, %[a]					
	as TBZ (column 1 LC)[b]			as *p*-NB derivative (column 2 LC)		
fruit fortified	1 ppm	0.5 ppm	0.02 ppm	1 ppm	0.5 ppm	0.02 ppm
pear peel	98.3 ± 2.1 (4)	90.2 ± 2.4 (4)	94.9 ± 1.6 (4)	94.8 ± 3.1 (4)	87.5 ± 1.5 (4)	89.8 ± 2.7 (4)
pear pulp	98.9 ± 1.7 (3)	85.7 ± 1.9 (3)	94.6 ± 2.3 (3)	97.3 ± 1.7 (3)	78.4 ± 2.2 (3)	92.3 ± 1.3 (3)
banana peel	95.7 ± 1.9 (4)	89.6 ± 2.8 (4)	81.4 ± 2.4 (3)	95.0 ± 2.4 (4)	92.1 ± 3.0 (4)	78.6 ± 1.2 (3)
banana pulp	103.2 ± 2.9 (3)		85.3 ± 1.8 (4)	94.2 ± 2.1 (3)		88.1 ± 2.4 (4)
orange peel	89.6 ± 1.9 (4)	87.1 ± 1.5 (4)		84.3 ± 2.9 (4)	88.1 ± 2.2 (4)	
orange pulp	83.4 ± 2.3 (3)	85.2 ± 2.0 (5)		93.2 ± 1.8 (3)	81.4 ± 2.3 (5)	

[a] ±standard error (number of determinations). [b] LC = high-performance liquid chromatography.

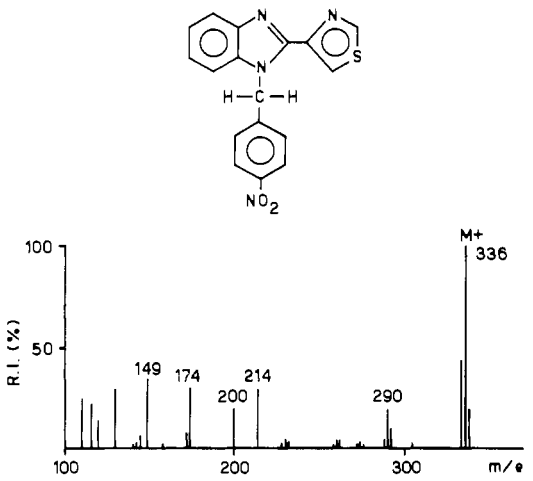

Figure 2. Mass spectra and structure of *p*-NB derivative of TBZ.

corresponding to the expected molecular ion M^+ at *m/e* 336 and weak peaks at *m/e* 290 ($-NO_2$), 214 ($-C_6H_4NO_2$), 200 ($-CH_2C_6H_4NO_2$), 174 ($-CH_2C_6H_4NO_2$, $-NCH$), 149 ($-H$, $-C_6H_4NC_4H_2NS$). Hence, the mass spectral data clearly establish the structure for the *p*-NB derivative of TBZ shown in Figure 2.

Recovery and control determinations were conducted on peel or pulp of different fruits. The samples were fortified with ethyl acetate solutions of TBZ at varying concentrations. The solvent was evaporated and samples were processed as outlined in Figure 1. The mean recovery values are summarized in Table I and are satisfactory for the concentrations tested. No substantial differences can be observed between recoveries of TBZ and those of the *p*-NB derivative. The combination of a partitioning scheme, also used by Maeda and Tsuji (1976), and silica gel column chromatography was essential to eliminate interfering substances when TBZ is isolated and analyzed by high-performance LC with a UV detector. On the basis of the chromatograms of the numerous blanks tested, 0.002 mg/kg was fixed as the lower determination limit for pears and bananas, and 0.02 mg/kg was fixed for oranges. These values are of about the same order of magnitude as those reported when a fluorescence detector was used (Maeda and Tsuji, 1976). It was not possible to measure less than 0.02 mg of TBZ residues/kg, owing to interference from the orange constituents not removed by the cleanup procedure. This is in agreement with Mestres et al. (1970), who experienced greater difficulties in eliminating interference with oranges than other fruits when they used a UV monitor for the determination of the residues.

Some of the chromatograms obtained by injecting purified extracts of different fortified samples are reported in Figure 3 and this also shows the effect of the TBZ/*p*-NB-Br reaction.

Our results suggest that derivatization can be successfully applied to the detection of TBZ residues in fruits. It can, also, be used as an alternative to the method without conversion for the confirmation of the presence of TBZ, as well as for the quantification of the TBZ residues.

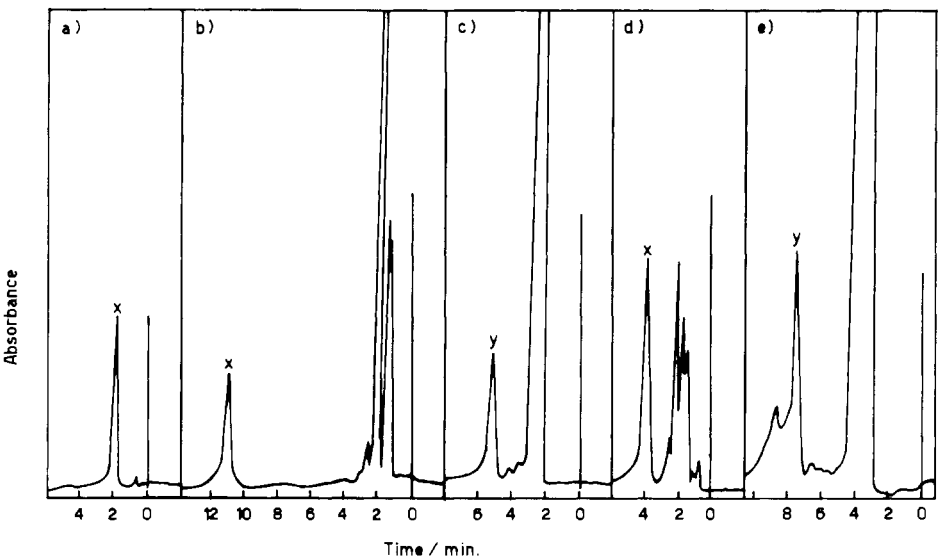

Figure 3. High-performance LC of extracts of fruit peels (500 g). 5-μL samples were injected. Pear peels were fortified at 0.05 mg/kg, determined as TBZ (a, column 1; b, column 2) and as the *p*-NB derivative (c, column 2) ; final extract volume was 0.2 mL; solvent flow was 1.0 mL/min; sensitivity was 0.5 AUFS. orange peels were fortified at 0.1 mg/kg, determined as TBZ (d, column 1) and as the *p*-NB derivative (e, column 2); final extract volume was 0.5 mL; solvent flow was 0.5 mL/min; sensitivity was 0.2 AUFS. Peak X is TBZ; peak Y is the *p*-NB derivative of TBZ.

LITERATURE CITED
Aharonson, N.; Ben-Aziz, A. *J. Assoc. Off. Anal. Chem.* **1973**, *56*, 1330.
Association of Official Analytical Chemists "Official Methods of Analysis", 12th ed.; AOAC: Washington, DC, 1975; p 801.
Ebel, S.; Herold, G. *Dtsch. Lebensm. Rundsch.* **1974**, *70*, 133.
Farrow, J. E.; Hoodless, R. A.; Sargent, M.; Sidwell, J. A. *Analyst (London)* **1977**, *102*, 752.
Jacob, T. A.; Carlin, J. R.; Walker, R. W.; Wolf, F. J.; VandenHeuvel, W. J. A. *J. Agric. Food Chem.* **1975**, *23*, 704.
Maeda, M.; Tsuji, A. *J. Chromatogr.* **1976**, *120*, 449.
Mestress, R.; Campo, M.; Tourte, J. *Ann. Falsif. Expert. Chim.* **1970**, *No. 691*, 160.
Mihara, M.; Kondo, T.; Tanabe, H. *Shokuhin Eiseigaka Zasshi* **1973**, *14*, 179.
Miller, V. L.; Gould, C. J.; Csonka, E. *J. Agric. Food Chem.* **1974**, *22*, 90.
Norman, S. M.; Fouse, D. C.; Craft, C. C. *J. Agric. Food Chem.* **1972**, *20*, 1227.
Nose, N.; Kobayashi, S.; Tanaka, A.; Hirose, A.; Watanabe, A. *J. Chromatogr.* **1977**, *130*, 410.
Ott, D. E. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 160.
Otteneder, H.; Hezel, U. *J. Chromatogr.* **1975**, *109*, 181.
Tanaka, A.; Fujimoto, Y. *J. Chromatogr.* **1976**, *117*, 149.
Thornburg, W. W. "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives"; Zweig, C., Ed.; Academic Press: New York, 1963; Vol. I, 99.
Tjan, G. H.; Jansen, J. T. A. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 769.
VandenHeuvel, W. J. A.; Wood, J. S.; Di Giovanni, M.; Walker, R. W. *J. Agric. Food Chem.* **1977**, *25*, 386.

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Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide

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Vegetable oils can be extracted from crushed seeds with liquid or supercritical carbon dioxide. The yields obtained depend upon the pressure and the temperature employed during extraction as well as the size and shape of the seed particles. Oil fractions differing in color, taste, and odor can be recovered at various pressures and temperatures. Parameters influencing the extraction and fractionation of soybean, sunflower seed, and rapeseed oils are described.

Pressing as well as extraction with organic solvents is used widely in the production of vegetable fats and oils. The yields obtained by pressing are not as high as those achieved by extracting oil seeds. Therefore, pressing of intact or ground seeds, a most convenient process, is often followed by extracting the resulting press cake with hot organic solvents, such as petroleum hydrocarbons, for nearly quantitative recovery of the seed oils. Solvent extraction alone is used, e.g., in the commercial production of soybean oil.

The present communication describes the results of studies aimed at substituting organic solvents by liquid or supercritical gases, particularly carbon dioxide, for the extraction of oils from soybeans, sunflower seeds, and rapeseeds at fairly low temperatures.

The complete removal of organic solvents used for extracting seed oils is mandatory, if the oil is to be used for human consumption. Liquid and supercritical carbon dioxide offer the advantage of being easily removable from the extracted oil. In contrast to organic solvents and some of their contaminating components, carbon dioxide is nontoxic, and it cannot easily lead to environmental pollution. Moreover, this inexpensive gas is available on an unlimited scale both from renewable organic resources and from inorganic material including various minerals.

As in the extraction with organic solvents, the efficiency of extraction with liquid and supercritical carbon dioxide is dependent upon its amount and the time it is in contact with the ground seeds. The yield of oil is also influenced by the size and physical structure of the seed particles. In working with liquid and supercritical gases, pressure and temperature during extraction and recovery of the oil are parameters that should receive special attention.

The principle of the equipment used for the extraction of seed oils with liquid and supercritical carbon dioxide, as shown in Figure 1, is simple. Gaseous carbon dioxide is condensed in a diaphragm compressor, C, to a pressure of 350 bar, p1; even higher pressure, up to 700 bar, can be obtained by employing a second compressor. The liquid or supercritical carbon dioxide flows through an extraction vessel, E, containing crushed seeds. The extracted oil is recovered from its solution by lowering the pressure in two stages, in a first trap, S1, to ~200 bar, p2, and in a second trap, S2, to 30–65 bar, p3, that is, below the critical pressure of carbon dioxide. The gas released is again condensed in the compressor, C, thus closing the cycle. Further details of the construction and operation of the equipment used are described under Experimental Section.

EXPERIMENTAL SECTION

Materials. Seeds of soya, *Glycine max* var. Corsoy, were obtained from the American Soybean Council, St. Louis, MO, those of sunflower, *Helianthus annuus* var. Fransol, from the International Sunflower Association, Zevenaar, The Netherlands, and those of rape, *Brassica napus* var. Rapora, from Norddeutsche Pflanzenzucht Hans-Georg Lembke K.G., Hohenlieth, Germany.

Analytical Procedures. Oils were extracted by treating the ground seeds (60–100 mesh) in a Soxhlet apparatus with hexane for 5 h. After evaporation of the solvent, the oil content of the seeds was determined gravimetrically.

The oils extracted with hexane as well as those obtained by extraction with liquid or supercritical carbon dioxide were analyzed by thin-layer chromatography on silica gel

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