RESIDUES AND TRACE ELEMENTS

Determination of Benomyl, Diphenyl, *o*-Phenylphenol, Thiabendazole, Chlorpyrifos, Methidathion, and Methyl Parathion in Oranges by Solid-Phase Extraction, Liquid Chromatography, and Gas Chromatography

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A simple and rapid method was developed for determination of benomyl, diphenyl (DP), *o*-phenylphenol (OPP), thiabendazole (TBZ), chlorpyrifos, methidathion, and methyl parathion in whole oranges. These compounds were extracted from a mixture of samples and anhydrous sodium acetate with ethyl acetate. The ethyl acetate extract was concentrated and cleaned up by passing through tandem solid-phase extraction columns consisting of anion-exchange and primary/secondary amine bonded silica. The eluate was concentrated and volume was adjusted with methanol for subsequent liquid chromatography (LC) and gas chromatography (GC). Benomyl (as methyl-2-benzimidazole carbamate, MBC), DP, OPP, and TBZ residues were determined by LC with fluorescence detection. Recoveries at 3 fortified levels (0.1, 1, and 10 μg/g) ranged from 63.9 to 97.4%, with coefficients of variation (CVs) of 1.6 to 15.5%. Limits of detection (LODs) were 0.01 μg/g for DP, OPP, TBZ and 0.05 μg/g for benomyl. Chlorpyrifos, methidathion, and methyl parathion residues were determined by GC with flame photometric detection. Recoveries ranged from 90.4 to 97.0%, with CVs of 2.1 to 5.9%. LODs were 0.005 μg/g for chlorpyrifos and methyl parathion, and 0.01 μg/g for methidathion.

Benomyl, diphenyl (DP), *o*-phenylphenol (OPP), and thiabendazole (TBZ) are fungicides widely used on citrus fruits for pre- and postharvest protection. Benomyl (as methyl-2-benzimidazole carbamate, MBC) and TBZ residues are frequently found in citrus fruits (1). In Japan, the tolerance levels of DP, OPP, and TBZ residues in whole oranges are 70, 10, and 10 ppm, respectively. At present there is no established tolerance level for benomyl.

Nakazato et al. (2) have reported a method for DP, OPP, and TBZ in citrus fruits. This method, which consists of extraction with ethyl acetate, concentration of extract, and liquid chromatography (LC) with fluorescence detection, has been used routinely. However, naturally occurring fluorescence from citrus fruits often interferes with detection of these fungicides. In addition, the limits of detection (LODs) are high (≤0.1 ppm). Some methods recently reported for fungicide residues in citrus fruits (3, 4) include laborious liquid–liquid partition with pH adjustment.

Organophosphorus pesticides, chlorpyrifos, methidathion, and methyl parathion are widely used as insecticides and their residues are frequently found in citrus fruits (1, 5, 6). In Japan, the tolerance levels of chlorpyrifos and methyl parathion residues in whole oranges are 0.3 and 0.2 ppm, respectively. At present, methidathion does not have an established tolerance level. A recently reported multiresidue method for organophosphorus and organochloride pesticide residues in oranges (6) is based on matrix solid-phase dispersion and determination by gas chromatography (GC) with electron capture detection. A multiresidue method for fungicides, organophosphorus, and organochloride pesticides residues in citrus fruits also has been reported (7). This method is based on liquid–liquid partition, gel permeation chromatography cleanup, and determination by GC/mass spectrometry (MS) with mass-selective detection.

A rapid and simple method was developed for determination of fungicides and organophosphorus pesticides residues such as benomyl, DP, OPP, TBZ, chlorpyrifos, methidathion, and methyl parathion in whole oranges. The method is based on a simple solvent extraction, solid-phase extraction (SPE) cleanup replacing laborious liquid–liquid partition, and determination by LC with fluorescence detection and GC with flame photometric detection (FPD).

METHOD

Apparatus

(a) *Speed cutter.*—Model MK-K74 (Matushita, Osaka, Japan).

(b) *Homogenizer.*—Physcotron (Nichion, Tokyo, Japan).

(c) *Kiriyama rohto.*—60 mm id (Sibata Scientific Technology, Tokyo, Japan).

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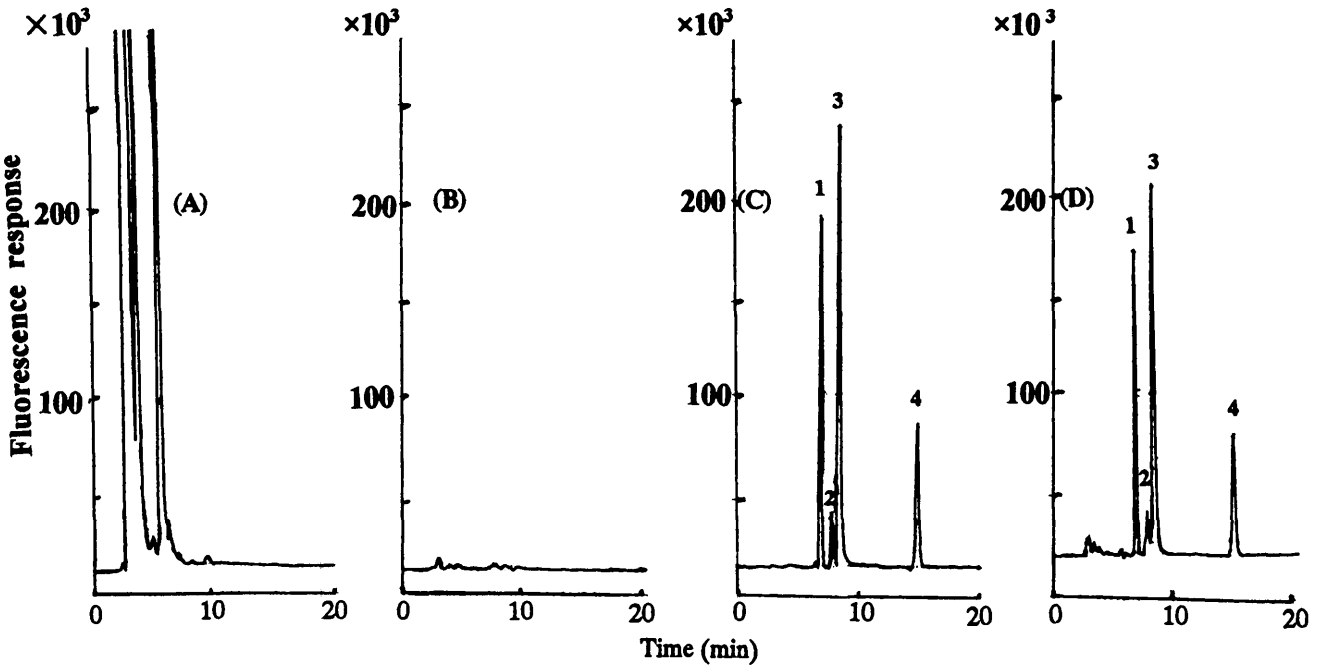

Figure 1. Liquid chromatograms of (A) orange extract without SPE cleanup, 10 g/2 mL; (B) control orange extract, 10 g/2 mL; (C) mixed standard for LC each at 5.0 µg/mL; and (D) fortified orange (10 g/2 mL) with benomyl (as MBC), DP, OPP, and TBZ each at 1 µg/g. Peak 1, OPP; peak 2, MBC; peak 3, TBZ; peak 4, DP.

(d) *SPE vacuum manifold.*—Vac Elut 24 (Varian Sample Preparation Products, Harbor City, CA).

(e) *Rotary vacuum evaporator.*—RE47 (Yamato, Tokyo, Japan).

(f) *SPE cartridges.*—Bond Elut SAX, 500 mg and Bond Elut PSA, 500 mg (Varian). Attach anion exchange (SAX) cartridge below primary/secondary amine (PSA) cartridge by using Bond Elut cartridge adapter (Varian).

(g) *LC column.*—Supelcosil LC-18 (particle size 5 µm, 250 × 4.6 mm id; Supelco, Bellefonte, PA) with TSK gel 80 guard column (particle size 5 µm, 15 × 3.2 mm id; Tosoh, Yokyo, Japan).

(h) *Liquid chromatograph.*—Model PU-980 pump (Jasco, Tokyo, Japan); Model 821-FP fluorescence detector (Jasco); Model 807-IT integrator (Jasco); flow rate, 1.0 mL/min; injection volume, 10 µL; column temperature, 40°C; detector, 285 nm excitation and 315 nm emission.

(i) *Gas chromatograph.*—Shimadzu GC 14A (Shimadzu, Kyoto, Japan) equipped with FPD system operating in P mode and TC-5 wide-bore capillary column, 30 m × 0.53 mm id, 1.5 µm film thickness (GL Sciences, Tokyo, Japan). Gas chromatograph operating conditions: oven temperature program, 1 min at 150°C, 10°C/min to 250°C, hold 10 min; injector, 280°C; detector, 250°C; carrier gas flow, nitrogen 0.6 kg/cm^2 head pressure; air, 0.9 kg/cm^2; hydrogen, 0.75 kg/cm^2; direct injection; injection volume, 2 µL.

Reagents

(a) *Anhydrous sodium acetate.*—Reagent grade (Wako Pure Chemical Industries, Osaka, Japan).

(b) *Anhydrous sodium sulfate and ethyl acetate.*—For pesticide residue analysis (Wako Pure Chemical Industries).

(c) *Sodium n-dodecyl sulfate.*—Kanto Chemical, Tokyo, Japan.

(d) *Acetonitrile and methanol.*—LC grade (Wako Pure Chemical Industries).

Table 1. Recoveries of benomyl, DP, OPP, and TBZ from fortified oranges

Compound	Fortification level, µg/g	Recovery, %[a]	CV, %
Benomyl (as MBC)	0.1	97.4	7.4
	1	86.0	2.3
	10	81.4	5.4
DP	0.1	84.5	8.7
	1	86.1	1.6
	10	89.1	3.5
	70[b]	92.1	2.7
OPP	0.1	79.9	8.7
	1	83.6	2.2
	10[b]	91.6	2.7
TBZ	0.1	63.9	15.5
	1	84.1	5.0
	10[b]	90.6	2.2

[a] $n = 5$ at each fortification level.
[b] Japanese tolerance level.

Table 2. Recoveries of chlorpyrifos, methidathion, and methyl parathion from fortified oranges

Compound	Fortification level, µg/g	Recovery, %[a]	CV, %
Chlorpyrifos	0.03	90.4	5.9
	0.1	97.0	2.1
	0.3	96.2	3.2
Methidathion	0.1	92.3	5.6
Methyl parathion	0.1	94.0	5.9

[a] $n = 5$ at each fortification level.

(e) *Mobile phase.*—Dissolve 2.88 g sodium *n*-dodecyl sulfate in 400 mL water. Add 400 mL acetonitrile and 200 mL methanol. Adjust pH to 2.5 with phosphoric acid.

(f) *Pesticide standards.*—DP, MBC, TBZ, and methyl parathion (Wako Pure Chemical Industries); OPP (Tokyo Kasei Kogyo, Tokyo, Japan); chlorpyrifos and methidathion (Riedel-de-Haen, Seelze, Germany).

Standard Solutions

(a) *Stock mixed standard solution for LC (DP, MBC, OPP, and TBZ, each at 100 µg/mL).*—Dissolve each 10 mg standard in 100 mL methanol. Store at 4°C.

(b) *Working mixed standard solution for LC (0.5, 1, 5, and 10 µg/mL).*—Transfer 0.5, 1.0, 5.0, and 10.0 mL stock solution to individual 100 mL volumetric flasks. Dilute to 100 mL with methanol.

(c) *Stock mixed standard solution for GC (chlorpyrifos, methidathion, and methyl parathion, each at 100 µg/mL).*—Dissolve each 10 mg standard in 100 mL methanol. Store at 4°C.

(d) *Intermediate mixed standard solution for GC (10 µg/mL).*—Dilute 10 mL stock solution to 100 mL with methanol.

(e) *Working mixed standard solution for GC (0.05, 0.15, 0.5, and 1.5 µg/mL).*—Transfer 0.5, 1.5, 5.0, and 15.0 mL intermediate solution to individual 100 mL volumetric flasks. Dilute to 100 mL with methanol.

Sample Preparation

Cut whole oranges into small pieces and chop well in speed cutter. Store at –18°C in polyethylene bags if not used immediately.

Sample Extraction and Cleanup

Weigh 10 g chopped oranges in 200 mL glass beaker. Add 1 g anhydrous sodium acetate and mix thoroughly. Add 50 mL ethyl acetate and 30 g anhydrous sodium sulfate to beaker. Homogenize mixture for 3 min. Pass homogenate through filter paper (60 mm id) fitted in Kiriyama rohto under vacuum. Transfer solid residue to 200 mL glass beaker and repeat homogenization with 50 mL ethyl acetate and filtration step. Rinse solid residue on filter paper with 5 mL ethyl acetate. Combine ethyl acetate extracts and evaporate to ca 1 mL in 200 mL round-bottom flask with rotary vacuum evaporator at 40°C. Precondition tandem SPE cartridges with 3 mL ethyl acetate. With a Pasteur pipet, apply concentrated ethyl acetate extracts to tandem SPE cartridges on SPE vacuum manifold. Rinse flask with 3 mL ethyl acetate. Add rinses to cartridges. Pass through cartridges additional 6 mL ethyl acetate (let each solution pass through cartridges at ca 2 mL/min by vacuum). Collect ethyl acetate eluate in 10 mL calibrated centrifuge tube with rim. Evaporate eluate carefully to ca 0.5 mL in centrifuge tube with rotary vacuum evaporator at 40°C. Adjust volume to 2.0 mL or appropriate volume with methanol for subsequent LC and GC determination.

Fortification

Fortify 10 g finely chopped control orange samples with DP, MBC, OPP, and TBZ, each at 0.1, 1, and 10 µg/g, and DP at 70 µg/g. Fortify chlorpyrifos at 0.03, 0.1, and 0.3 µg/g, methidathion at 0.1 µg/g, and methyl parathion at 0.1 µg/g. Follow procedure described above.

Results and Discussion

An attempt was made to develop an LC method for benomyl, DP, OPP, TBZ, and chlorpyrifos to address the following problems: (*1*) elimination of liquid–liquid partitioning steps during sample extraction to reduce analysis time and solvent usage; (*2*) elimination of the naturally occurring fluorescent peaks in the orange matrix to prevent deterioration of the LC column and chromatographic interferences; and (*3*) analysis of compounds by LC isocratically within 30 min.

Sample extraction with ethyl acetate under neutralized conditions and filtration was rapid. Cleanup was performed by using anion-exchange (SAX) and primary/secondary amine (PSA) cartridges to replace laborious liquid–liquid partitioning. These cartridges were used for multiresidue pesticides analysis (8, 9). Cairns et al. (8) described successful use of C_{18}, anion-exchange (QMA), and aminopropyl bonded silica (NH_2) Sep Pak cartridges for cleanup of pesticide residues in fruits and vegetables. The C_{18} Sep Pak cartridge removed

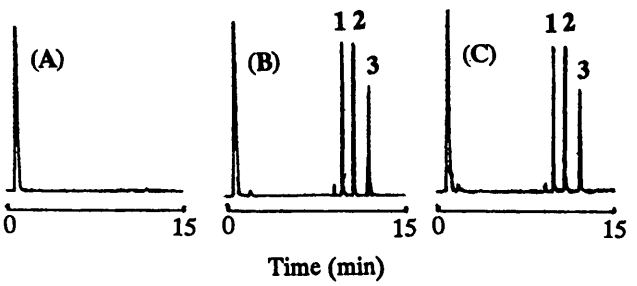

Figure 2. GC–FPD chromatograms of (A) control orange extract, 10 g/2 mL; (B) mixed standard for GC each at 0.5 µg/mL; and (C) fortified orange (10 g/2 mL) with chlorpyrifos, methidathion, and methyl parathion each at 0.1 µg/g. Peak 1, methyl parathion; peak 2, chlorpyrifos; peak 3, methidathion.

long-chain fats and waxes, the QMA Sep Pak cartridge removed colored compounds and flavors, and the NH_2 Sep Pak cartridge removed sugars. We substituted SAX and PSA cartridges for the QMA and NH_2 cartridges. The compounds of analytes were eluted through these SPE cartridges with ethyl acetate. The C_{18} cartridge was not used.

After cleanup, the sample was concentrated and reconstituted with methanol. A small amount of precipitate appeared in the solution. Pipetting of the clear supernatant eliminated the need for a filtration step before injection into the LC and GC systems.

Benomyl (as MBC), DP, OPP, and TBZ were well separated under the LC conditions. The LC mobile phase with ion pairing reagent was used to modify the common LC method for DP, OPP, and TBZ (2). The excitation wavelength (285 nm) and the emission wavelength (315 nm) were adopted from the multiresidue method, using a gradient step LC (9). Under the LC conditions used, chlorpyrifos required a long analysis time (about 36 min) and lacked sensitivity compared with other compounds. This low sensitivity of chlorpyrifos is probably due to its high capacity to be absorbed onto the LC system or its weak fluorescence. Therefore, the analysis of chlorpyrifos was switched to a GC–FPD system.

Benomyl was analyzed as its degradation product, MBC (3, 4, 10, 11). Benomyl residues in samples were converted to MBC under acidic conditions (10) and in ethyl acetate extract during the concentration step (3).

In our extraction procedure, benomyl residues in orange samples were converted to MBC during sample preparation, extraction, and concentration.

Figure 1 shows liquid chromatograms for orange extract prepared by the commonly used extraction procedure (2; without SPE cleanup, 10 g/2 mL), control orange extract cleaned up by SPE cartridges (10 g/2 mL), mixed standard solution (5.0 μg/mL), and fortified orange at 1 μg/g level (10 g/2 mL). Figure 1B clearly indicates that the fluorescent peaks from the orange matrix were diminished effectively by SPE cleanup in comparison with Figure 1A. Retention times of OPP, benomyl, TBZ, and DP were 6.7, 7.7, 8.2, and 14.7 min, respectively.

Table 1 shows recoveries of benomyl (as MBC), DP, OPP, and TBZ from whole oranges each fortified at 0.1, 1, and 10 μg/g; 70 μg/g only for DP. For the recovery experiment, we used blank orange samples previously determined not to contain pesticides of interest. Recoveries were calculated by comparison with the appropriate working mixed standard. Except for the 0.1 μg/g level, recoveries of 4 compounds were adequate with low coefficients of variation (CVs). Recoveries ranged from 81.4 to 92.1%, with CVs ranging from 1.6 to 5.4%. At the 0.1 μg/g level, some problems were seen with benomyl and TBZ. For quantitation at the 0.1 μg/g level, the sensitivity of the detector was increased. Consequently, trace amounts of orange matrix affected benomyl (as MBC) quantitation. Recovery of benomyl at this level was corrected by subtracting the amounts of peak from the orange matrix. Above the 1 μg/g level, a trace peak in the region of benomyl was negligible and gave no problems. TBZ gave a relatively low recovery with high CV. TBZ was probably retained on the tandem SPE cartridges and not eluted completely. Further studies are needed for benomyl and TBZ at low levels, including those near the LOD.

LODs (signal-to-noise ratio, S/N = 3) were 0.01 μg/g for DP, OPP, and TBZ, and 0.05 μg/g for benomyl (as MBC). The standard calibration curves of these compounds were linear over the range of 0.5–10 μg/mL, with a correlation coefficient of >0.99.

Table 2 shows recoveries of chlorpyrifos from whole oranges fortified at 0.03, 0.1, and 0.3 μg/g. Recoveries ranged from 90.4 to 97.0%, with CVs of 2.1–5.9%. Because of favorable results of chlorpyrifos, additional organophosphorus pesticides, methidathion, and methyl parathion were studied for expanding of the method. Methidathion and methyl parathion residues were frequently found in orange samples (5, 6). Recovery of methidathion and methyl parathion at 0.1 μg/g gave 92.3% with CV of 5.0%, and 94.0% with CV of 5.9%, respectively (Table 2). The method provided acceptable recoveries and precision for these organophosphorus pesticides. It would be of interest to study other organophosphorus pesticides, such as fenitrothion and malathion, which have also been found in orange samples (1, 5, 6).

LODs (S/N = 3) were 0.005 μg/g for chlorpyrifos, and methyl parathion, and 0.01 μg/g for methidathion. The standard calibration curves of these compounds were linear over the range of 0.05–1.5 μg/mL, with a correlation coefficient of <0.99.

Figure 2 shows GC–FPD chromatograms for whole orange control, mixed standard, and fortified orange (0.1 μg/g). No chromatographic interference from the control orange was observed at the retention times of methyl parathion (9.84 min), chlorpyrifos (10.75 min), and methidathion (12.06 min). Other organophosphorus pesticides, such as chlorpyrifos-methyl and parathion eluted very close to parathion methyl and chlorpyrifos, with retention times of 9.82 and 10.78 min, respectively. For absolute confirmation of identity of these organophosphorus pesticides, MS must be used. TBZ was also determined under the GC–FPD conditions at the retention time of 11.89 min, but TBZ could not determined at <2 μg/g. Therefore, the presence of TBZ was not expected to interfere significantly.

Conclusion

Although benomyl, DP, OPP, TBZ, and chlorpyrifos could not be determined simultaneously by LC isocratically, the method was both simple and rapid. Cleanup was performed by the tandem SPE cartridges and replaced liquid–liquid partition. Fluorescent peaks from the orange matrix were diminished effectively by the cartridges, and the fungicides (benomyl, DP, OPP, and TBZ) and organophosphorus pesticides (chlorpyrifos, methidathion, and methyl parathion) residues were analyzed using same sample extract. The procedure did not need an individual specific cleanup.

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