Simultaneous Determination of Carbaryl, Malathion, Fenitrothion, and Diazinon Residues in Sesame Seeds (*Sesamum indicum* L.)

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A method is described for the simultaneous determination of carbaryl (1-naphthyl methylcarbamate), malathion [diethyl (dimethoxythiophosphorylthio) succinate], fenitrothion (O,O-dimethyl O-4-nitro-*m*-tolyl phosphorothioate), and diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) in sesame (*Sesamum indicum* L.) seeds. Sesame seeds were Soxhlet extracted with *n*-hexane, and the extract was subjected to a liquid−liquid partitioning and column cleanup to remove the oily coextractives prior to analysis by high performance liquid chromatography (HPLC). The mean percent recoveries (± standard deviations) from sesame seeds fortified with carbaryl (0.004 to 0.035 μg/g), malathion (0.53 to 4.25 μg/g), fenitrothion (0.22 to 1.78 μg/g), and diazinon (0.54 to 4.35 μg/g) were 83.3 ± 5.7, 85.5 ± 6.6, 85.6 ± 7.2, and 88.4 ± 4.8, respectively. The method was used for the simultaneous analysis of carbaryl, malathion, fenitrothion, and diazinon residues in sesame seeds obtained from an Ethiopian field crop that had been treated with the pesticides during its growing period.

Keywords: *Carbaryl; malathion; fenitrothion; diazinon; Sesame seeds; HPLC analysis; cleanup of oily coextractives*

INTRODUCTION

Since ancient times, sesame (*Sesamum indicum* L.) has been considered an important seed crop because of its high oil content, resistance to oxidative deterioration, and medicinal value (Fukuda et al., 1994). Sesame remains an important agricultural commodity in Ethiopia where it is used primarily as an oil crop by the local population. In addition, substantial quantities of sesame seeds are exported from Ethiopia, totaling nearly 100 000 tons in 1999. During the growing season several pesticides, including carbaryl (1-naphthyl methylcarbamate), malathion [diethyl (dimethoxythiophosphorylthio) succinate], fenitrothion (O,O-dimethyl O-4-nitro-*m*-tolyl phosphorothioate), and diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate), are routinely applied to sesame crops for insect control. Pesticides are applied on sesame up to three weeks prior to harvesting in Ethiopia, raising concern regarding human exposure to pesticide residues. Therefore, monitoring of pesticide residues in sesame seeds, the principal agricultural product of sesame, is clearly needed to protect public health.

Although several multi-residue methods have been reported for the determination of carbaryl, malathion, fenitrothion, and diazinon residues in sweet cherries (Bicchi et al., 1997), fruits (Dorea et al., 1996), and water (Hernandez et al., 1993; Mallet et al., 1990), there is as yet no published method available for the simultaneous determination of these pesticides in oily crops. The oil content in some sesame seeds may be as high as 60 wt % (Fukuda et al., 1994). Matrixes high in lipid content often require several separation steps in sample preparation to provide extracts suitable for quantitation by using high performance liquid chromatography (HPLC). The goal of this study was to develop an HPLC method for the simultaneous determination of sub-part-per-million (e.g., μg/g) residues of carbaryl, malathion, fenitrothion, and diazinon for applications involving monitoring of routinely applied pesticides in sesame seeds

MATERIALS AND METHODS

All solvents used were high purity Burdick and Jackson (McGraw Park, IL) brand and were used as received. Carbaryl, malathion, fenitrothion, and diazinon reference standards were obtained in high purity (>97%; PolyScience Corporation, Niles, IL), and known amounts of the pesticides were mixed in 100 mL of *n*-hexane to give a working standard containing 0.088, 10.64, 4.45, and 10.88 μg/mL of each, respectively. The working standard was used for sample spiking and in the preparation of calibration standards. The concentration of carbaryl in the working standard mixture was considerably lower because it was much more responsive to ultraviolet detection with the HPLC. Having similar peak responses for the four pesticides in HPLC chromatograms made it easier to interpret recovery results.

A representative sample (~1 kg) of sesame seeds was collected from a sesame field in Ethiopia that had received the four above-noted pesticides during the growing season. The sample was collected three weeks after the last pesticide application. A second representative sample (also ~1 kg) was obtained from the control field plot at a different location that did not receive any of the pesticides during the growing season. About 250 g of sesame seeds were sub-sampled and ground using a mortar and pestle. The ground material contained 6% (wt/wt) moisture as determined by heating 2-g samples at 100

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Table 1. Spiking Levels of Sesame Seeds with the Four Pesticides

	pesticide (μg/g)			
sample no.	carbaryl	malathion	fenitrothion	diazinon
1	0.004	0.53	0.22	0.54
2	0.009	1.06	0.44	1.08
3	0.018	2.13	0.89	2.17
4	0.035	4.25	1.78	4.35

°C in an oven for 24 h. The material was mixed thoroughly and retained for further study.

A precise amount (~25 g) of ground sesame seed material obtained from the control plot was fortified with an aliquot of the four pesticide mixture made up in *n*-hexane to obtain the desired fortification levels (Table 1). Four sets of samples were fortified in triplicate. Samples 1, 2, 3, and 4 (Table 1) received 1.25, 2.5, 5.0, and 10.0 mL additions, respectively, of the working standard. The solvent (hexane) was allowed to evaporate overnight, and the sample was mixed thoroughly and transferred to a cellulose Soxhlet-extraction thimble. The sample was Soxhlet extracted with 200 mL of *n*-hexane for 4 h. The hexane extract was concentrated to 10 mL using rotary flash evaporation and subsequently partitioned twice into 40-mL portions of acetonitrile (presaturated with *n*-hexane) in a separatory funnel. The acetonitrile extracts were combined, diluted with 200 mL of 2% aqueous sodium sulfate, and back-extracted into two separate 40-mL portions of *n*-hexane in a separatory funnel. The hexane extracts were combined and passed through a 5-cm column of granular anhydrous sodium sulfate to remove residual water. The sodium sulfate was subsequently washed with 20 mL of fresh *n*-hexane, which was combined with the hexane extracts. The hexane extract was reduced to a final volume of 5 mL by using rotary flash evaporation followed by nitrogen gas blowdown.

The hexane extract obtained as described above was further subjected to column chromatography. Activated (135 °C) silica gel (60−200 mesh, Mallinckrodt, Phillipsburg, NJ) was packed in a 22-cm-long glass pipet (6.4 mm id), plugged with glass wool, and topped with 1.0 cm of anhydrous sodium sulfate, leaving 4 cm of unfilled column space for sample loading. The column was conditioned using 10 mL of *n*-hexane. An aliquot (4.0 mL) of the extract was transferred to the column and eluted sequentially with 10 mL of *n*-hexane followed by 20 mL of acetonitrile. The elutes were evaporated separately under a gentle stream of nitrogen gas to a final volume of 0.25 mL.

The HPLC (Hewlett-Packard model 1100 binary pump) was equipped with a variable-wavelength UV−Vis detector (HP model 1100) tuned to 225 nm. A C18 stainless steel analytical column (Phenomenex Luna, 150 mm × 4.6 mm, 3 μm dia.; Phenomenex, Torrance, CA) was used at ambient temperature. A stainless steel guard column containing pelicular C18 (Whatman Inc., Clifton, NJ) preceded the analytical column. An isocratic mobile phase (50% acetonitrile and 0.1% acetic acid and 50% double-distilled water) was used at a flow rate of 1.5 mL/min. Injections of 20 μL were performed in HPLC quantitative analysis. Injections of the sample extracts were alternated with calibration standards to account for any possible changes in retention times and peak areas of the pesticides because of the continued operation of the HPLC. Four-point calibration curves for each pesticide were constructed from the peak areas of the calibration runs. The HPLC detector responses were linear ($r^2 > 0.99$) for carbaryl, malathion, fenitrothion, and diazinon in the range of 0.002−0.035 μg/mL, 0.25−4.5 μg/mL, 0.1−2.0 μg/mL, and 0.25−4.5 μg/mL, respectively. The concentrations of the pesticides in the extracts were determined by comparing the peak areas with those of the reference standards via calibration curves. Peak identity in the samples was determined by comparing the retention times with those of the reference standards.

RESULTS AND DISCUSSION

The HPLC retention times under the experimental conditions described in this study for carbaryl, malathion, fenitrothion, and diazinon were 3.4, 11.6, 12.7, and 20.1 min, respectively (Figure 1). No change in the retention times and/or peak shapes of the pesticides was observed within a set of analyses, which normally included ~20 samples between calibrations. The limits of detection (LODs) were estimated (Table 2) as 3 times the standard deviations (sd) of triplicate recoveries from sesame seeds spiked at 0.002, 0.27, 0.11, and 0.27 μg/g with carbaryl, malathion, fenitrothion, and diazinon, respectively, using an approach reported by Wigfield et al. (1996). Figure 2 (curve d) illustrates an HPLC chromatogram for the four pesticides at the above spike levels in sesame seeds. It should be noted that the LODs reported in this study were based on spike concentrations thought to be approaching the least measurable peak areas in the HPLC chromatograms under the experimental conditions described. Samples having pesticide concentrations in sesame seeds lower that the LODs were considered nondetectable.

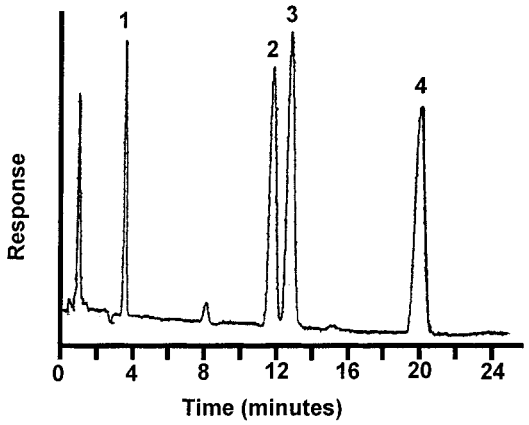

Figure 1. HPLC chromatogram of the reference standards: 1, carbaryl; 2, malathion; 3, fenitrothion; and 4, diaznon.

Table 2. Limits of Detection (LOD) and Recoveries of Pesticides in Spiked Sesame Seeds[a]

pesticide	LOD (μg/g)	% recovery ± sd
carbaryl	0.005	92 ± 7
malathion	0.036	90 ± 5
fenitrothion	0.029	86 ± 2
diazinon	0.049	94 ± 6

[a] Sesame seeds were fortified in triplicate at 0.002 μg/g (carbaryl), 0.27 μg/g (malathion), 0.11 μg/g (fenitrothion), and 0.27 μg/g (diazinon) to estimate limits of detection.

An initial attempt to clean up the extracts obtained from the Soxhlet extraction of the spiked sesame samples was performed by column chromatography using activated silica gel alone. Injections of the hexane and acetonitrile silica gel eluates overloaded the HPLC column with the oily co-extractive material. Attempts to use sulfuric acid treated silica gel (8.0 mL of concentrated sulfuric acid per 20.0 g of silica gel) (Gardinali et al., 1996) proved ineffective because of low pesticide recoveries, although the HPLC baseline was dramatically improved. Furthermore, efforts to use gel permeation chromatography (GPC) to separate lipids from the pesticides were also unsuccessful because the oily co-extractives in the Soxhlet extracts of sesame seeds overloaded a preparatory-scale GPC column (Phenomonex Phenogel, 300 mm × 21.2 mm, 10-μm dia. particles). Successful GPC would have required the extraction of substantially less sample, thus increasing detection limits. Therefore, it was necessary to devise an alternative cleanup procedure that would remove

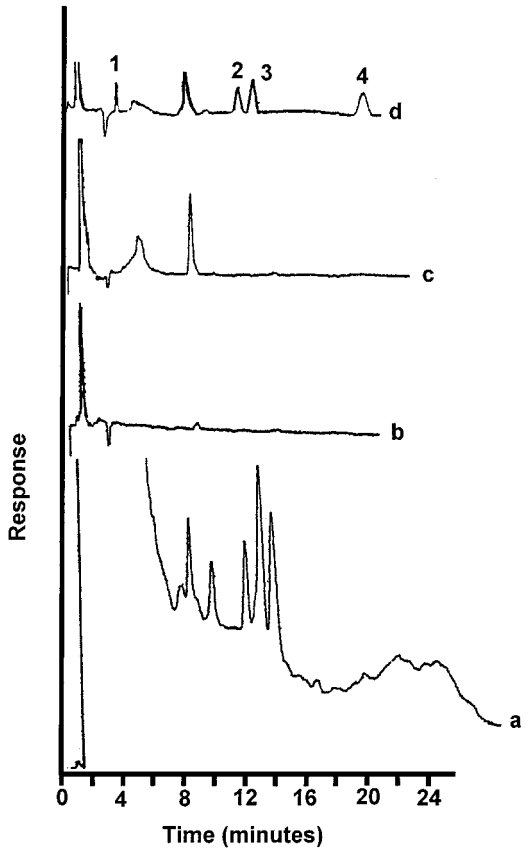

Figure 2. HPLC chromatograms of Soxhlet extracts of unspiked sesame seeds following liquid−liquid partitioning only (a), and after further silica gel cleanup including the hexane fraction (b), and acetonitrile fraction (c). Curve d shows the acetonitrile fraction from silica gel chromatography for spiked sesame seeds at the lowest concentrations shown in Table 2. (Refer to Figure 1 for peak labels.)

most of the oily co-extractives from the extracts prior to silica gel chromatography and HPLC analysis without affecting detection limits.

The material obtained from the Soxhlet extraction of sesame seeds was subjected to hexane−acetonitrile (liquid−liquid) partitioning followed by silica gel column-chromatography. Figure 2 illustrates the signal background obtained by injecting the extract from the unspiked (control) sesame sample following the liquid−liquid partitioning step (curve 2a). It appeared that liquid−liquid partitioning removed some of the oily co-extractives but still resulted in an HPLC chromatogram showing unacceptable interferences in quantitation. However, further cleanup using silica gel chromatography effectively removed most of the interfering co-extractives in the region of the retention times of the four pesticides under study (curve 2b and 2c). Figure 3 shows the HPLC chromatograms of the spiked sesame seed extracts after liquid−liquid partitioning and silica gel column cleanup. No pesticides were detected in the hexane eluates from the silica gel chromatography (curve 3a). The unknown peaks in the acetonitrile eluates did not interfere with the pesticide peaks used in quantitation (curve 3b). Thus, the cleanup procedure developed in this study sufficiently removed oily co-extractives for HPLC analysis at sub-part-per-million concentrations.

Recovery studies were performed using the control sesame seed samples fortified with a mixture of four pesticides in triplicate at each concentration (Table 1)

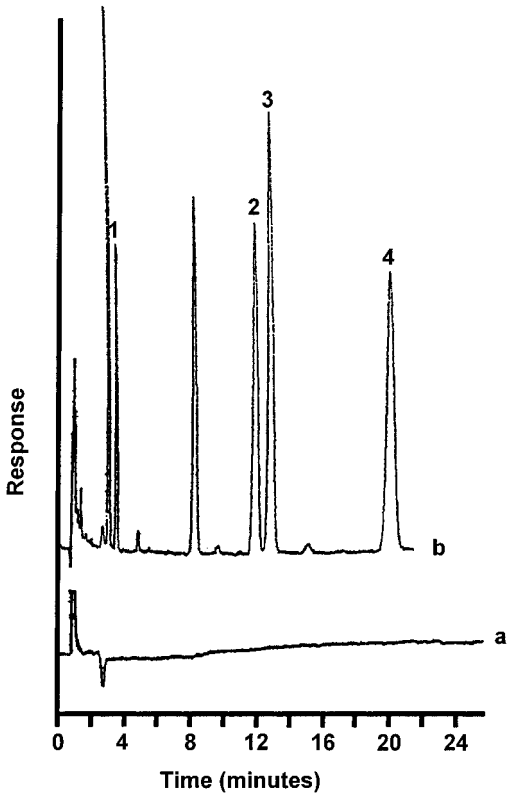

Figure 3. HPLC chromatograms of the Soxhlet extracts of fortified sesame seeds following liquid−liquid partitioning and silica gel cleanup in the (a) hexane and (b) acetonitrile fractions. (Refer to Figure 1 for peak labels.)

Table 3. Mean Percent Recoveries (±Sd) of Pesticides from Fortified Sesame Seeds at All Spike Levels

	pesticide			
sample no.[a]	carbaryl	malathion	fenitrothion	diazinon
1	85.9 ± 3.8	83.7 ± 4.7	80.6 ± 7.8	88.5 ± 2.8
2	82.2 ± 8.0	89.1 ± 7.9	86.2 ± 6.7	90.2 ± 6.9
3	81.0 ± 6.6	84.2 ± 7.5	86.8 ± 6.1	86.1 ± 5.8
4	83.7 ± 4.5	85.0 ± 6.5	88.3 ± 8.0	88.6 ± 3.8
overall mean	83.2 ± 5.7	85.5 ± 6.6	85.5 ± 7.2	88.4 ± 4.8

[a] For spiking levels of each sample see Table 1.

and the results are shown in Table 3. The mean recoveries ranged from 80 to 90%, and standard deviations among the replicate determinations ranged between 3 and 8%.

The method described above was applied to the simultaneous determination of carbaryl, malathion, fenitrothion, and diazinon residues in a sesame sample collected from the treated field in Ethiopia. In compliance with the protocol followed for the monitoring program in Ethiopia the sample was obtained from the treated field three weeks after the last application of the pesticide. No residues were detected in the field-treated sample under the experimental conditions described in this method. Figure 4 shows the HPLC chromatogram of the extract of the sesame sample from the treated crop in the field. It appears that any residues present after the last application had declined to levels below the detection limit at the harvest time. Although no information is available on the maximum residue limit (mrl) of the pesticides under study in sesame crop, it is worth noting that the Joint FAO/WHO Meeting of Experts on Pesticide Residue (JMPR) recommended the mrl guideline levels of 1 to 20 μg/g of carbaryl, malathion,

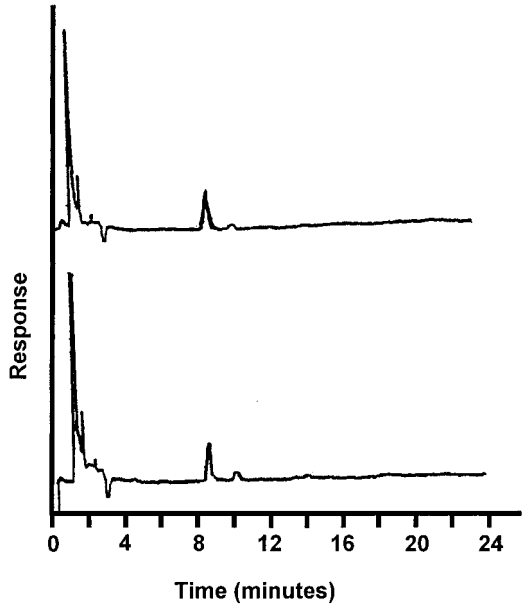

Figure 4. HPLC chromatograms of the field-treated sesame seeds after cleanup in the (a) hexane and (b) acetonitrile silica gel fractions.

fenitrothion, and diazinon in grain and milled products from grain (Snelson, 1987).

CONCLUSION

The method described presents a valuable tool for monitoring pesticide residues in the sesame crop in Ethiopia. Sesame seeds represent a particularly difficult matrix for pesticide analysis because of their high lipid contents. The method is efficient and is readily applicable to a program involving routine monitoring of carbaryl, malathion, fenitrothion, and diazinon residues in oily crops. The experimental conditions for the HPLC system and the reported cleanup procedure used in this study, coupled with the recovery data and limit of detection described, should prove a valuable addition to the methodology available for monitoring pesticide residues in sesame crop.

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