Gas Chromatographic Determination of Malathion and Its Oxygen Analog, Mala-oxon

A rapid gas chromatographic method for determining malathion and its oxygen analog mala-oxon with a single injection requires minimum cleanup and gives recoveries ranging from 90 to 100% from spinach, range grass, tomatoes, milk, and fat at the 0.05- to 2-p.p.m. level. The compounds are detected by flame photometry after chromatography on a column lightly loaded with diethylene glycol succinate. Diazinon and its oxygen analog can be determined by using the same procedure.

Mala-oxon (diethyl mercaptosuccinate *S*-ester with *O,O*-dimethyl phosphorothioate) is known to be a metabolite of malathion. Interest in determining the residues of this oxygen analog stems from the fact that it is a more active cholinesterase inhibitor than the parent compound. Although many methods are available for determining residues of malathion, few are also capable of determining residues of mala-oxon. Coffin (1966) separated malathion and mala-oxon by thin-layer chromatography of a lettuce extract and estimated recoveries of the pesticides at 91 to 98%. McCaulley (1965) indicated that mala-oxon could be determined by infrared spectroscopy, but presented no data on recovery. Suffet *et al.* (1967) partially separated microgram amounts of malathion from mala-oxon by gas chromatography on Reoplex-400 in their analysis of water. Their method is not applicable to residue analyses of the substances analyzed in the present work without a thorough cleanup, and it was not stated whether the procedure is quantitative.

A highly sensitive, rapid gas chromatographic method for determining malathion and mala-oxon simultaneously has now been devised. The compounds are separated on a 61-cm. column containing 2% diethylene glycol succinate on Gas Chrom Q and detected with the Melpar flame photometric detector (Brody and Chaney, 1966) set up to sense phosphorus. Recoveries of malathion and mala-oxon were 90 to 100% from spinach, tomatoes, range grass, animal tissues and fat, and milk. A minimum of cleanup was required with samples fortified at the 0.05- to 2-p.p.m. level.

Diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] and its oxygen analog diazoxon also can be determined by the present procedure.

EXPERIMENTAL

A Barber-Colman Co. (Rockford, Ill.) Model 5220 gas chromatograph equipped with the flame photometric phosphorus detector of Brody and Chaney (1966) (526-mμ interference filter) was used with a 1-mv. strip chart recorder. The detector is now available from MicroTek Instrument Corp., Baton Rouge, La.

Ethyl acetate, acetonitrile, and methylene chloride were distilled-in-glass solvents from Burdick and Jackson Laboratories, Inc., Muskegon, Mich.

The pesticides were analytically pure standards supplied by the manufacturers.

Sample Treatment. All samples were kept frozen until ready for analysis. They were then treated as follows.

Whole samples of spinach and range grass were chopped in a Hobart food cutter and were mixed well. Fat was rendered on a steam bath and mixed well. Cores or slices of tissue from various locations of bulk samples were cut into small pieces and mixed well. Several tomatoes were quartered to make up a 100-gram sample.

Extraction and Cleanup. SPINACH, RANGE GRASS, AND TOMATOES. Blend in a quart blender jar (Lourdes Instrument Co., Brooklyn, N. Y.) at high speed for 3 minutes 50 grams of plant material (100 grams of tomatoes) with 200 ml. of acetonitrile. Filter by gravity through Whatman No. 12 fluted filter paper, transfer 100 ml. of the extract to a 1000-ml. round-bottomed flask, and concentrate to several milliliters in a rotating evaporator. Transfer the residue with 50 ml. of acetonitrile-water (4 to 1, v./v.) to a 250-ml. separatory funnel, and extract the solution three times with 50-ml. portions of isooctane which are discarded. Add 50 ml. of methylene chloride to the

acetonitrile-water mixture, mix gently, and allow the layers to separate. Filter the lower layer (acetonitrile–methylene chloride solution) through 50 grams of anhydrous sodium sulfate, rinse with two 25-ml. portions of methylene chloride, and concentrate to several milliliters in a rotating evaporator. Take up the residue with 15 ml. of ethyl acetate, and concentrate to 5 ml. in a 50° C. water bath with a gentle stream of dry air. (Concentrate ethyl acetate solutions from the other products in this manner; to concentrate the solutions of all other solvents, use a rotating evaporator.)

ANIMAL TISSUE. Blend 10 grams of chopped tissue with 50 ml. of acetonitrile in a 100-ml. stainless steel blender cup at high speed for three minutes. Filter the mass on a Buchner funnel, and rinse the blender cup with two 25-ml. portions of acetonitrile which are poured successively through the filter cake. Concentrate the combined filtrates to several milliliters and transfer the residue to a 125-ml. separatory funnel with 25 ml. of 4 to 1 acetonitrile-water. Extract with three 25-ml. portions of isooctane which are discarded. Add 25 ml. of methylene chloride to the acetonitrile-water mixture, mix gently, and allow the layers to separate. Filter the acetonitrile-methylene chloride mixture through a plug of ca. 30 grams of anhydrous sodium sulfate, rinse the plug with methylene chloride, and evaporate the filtrate to several milliliters. Take up the residue with 15 ml. of ethyl acetate, and concentrate it to 5 ml. for gas chromatographic analysis.

MILK. Blend 100 grams of milk with 200 ml. of acetonitrile in a 1-quart blender jar for three minutes. Filter the extract on a Buchner funnel, and concentrate the filtrate in a rotating evaporator. Extract the water residue with 200 ml. of methylene chloride, filter the organic layer through ca. 100 grams of anhydrous sodium sulfate, and rinse the sodium sulfate with methylene chloride. (One extraction is adequate for quantitative recovery of malathion and mala-oxon.) After evaporating the methylene chloride, take up the residue with 15 ml. of ethyl acetate, and concentrate it to 5 ml.

FAT. Transfer 5 grams of the liquid fat to a 250-ml. separatory funnel with 25 ml. of acetonitrile-water (4 to 1), and continue as described for the animal tissue starting with the isooctane extraction.

Gas Chromatographic Analysis. Operate the gas chromatograph with the following parameters.

COLUMN. Aluminum, 61 cm. × 6 mm. O.D. (4 mm. I.D.).

PACKING. Diethylene glycol succinate, 2% (w./w.) on 100- to 120-mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.).

CARRIER GAS. Nitrogen at 100 ml. per minute.

OTHER GASES. Oxygen and air, each at 40 ml. per minute; hydrogen at 200 ml. per minute.

TEMPERATURES. Column 160° C., injection port 190° C., detector 150° C. (external).

Condition the column for two days at 200° C., and condition it further (Bowman and Beroza, 1965) by injecting 500-ng. amounts of malathion and mala-oxon into the gas chromatograph until tests with 5-ng. amounts of each give a constant response. Inject 5 μl. of the ethyl acetate solution into the gas chromatograph. Determine the insecticide content by comparing peak heights of unknowns with those of the standards. Typical retention times of malathion and mala-oxon were 6.9 and 9.1 minutes, respectively. Because mala-oxon was seldom found, analyses were speeded by raising the column temperature to 175° C.; retention times then became 4 and 5 minutes for malathion and mala-oxon, respectively. If mala-oxon did appear, the analysis was repeated with the column temperature at 160° C. to assure proper quantitation.

RESULTS AND DISCUSSION

Typical chromatograms are shown in Figure 1, and Table I summarizes the results obtained with malathion and mala-oxon. Individual analyses are given to illustrate reproducibility. Recoveries from fortified samples were

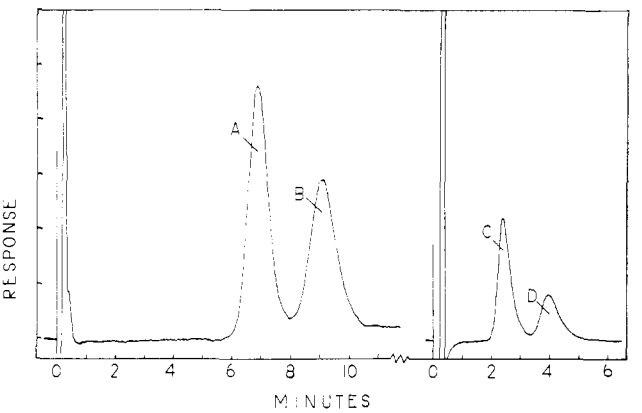

Figure 1. Chromatograms of malathion, diazinon, and their oxygen analogs

Left. 0.2 p.p.m. of malathion, *A*, and 0.5 p.p.m. of mala-oxon, *B*, in extract equivalent to 100 mg. of milk
Right. 0.04 p.p.m. of diazinon, *C*, and 0.08 p.p.m. of diazoxon, *D*, in extract equivalent to 25 mg. of corn

Table I. Typical Recoveries from Samples Fortified with Malathion and Mala-oxon

Product	Level of Fortification, P.P.M.	Per Cent Recovery	
		Malathion	Mala-oxon
Spinach	0.1	90	90
	0.1	90	90
	0.2	95	95
	0.2	95	95
Range grass	0.1	95	92
	0.1	95	90
	0.2	95	92
	0.2	95	90
Tomatoes	0.05	95	90
	0.05	95	90
	0.1	94	90
	0.1	93	92
Milk	0.05	95	90
	0.05	95	90
	0.1	95	90
	0.1	95	90
Fat	0.5	92	90
	0.5	90	92
	1.0	92	100
	1.0	92	100

90 to 100% and concentration was proportional to peak height up to the 100-ng. level. Sensitivity of the procedure depended on the material analyzed and the size of the sample. For samples equivalent to 100 grams of plant, sensitivity (twice noise level) was at least 0.01 p.p.m. for malathion and about 0.03 p.p.m. for mala-oxon. The sample size of fat and animal tissue being much smaller, sensitivity was reduced to 0.5 to 0.1 p.p.m.

Nonpolar gas chromatographic substrates such as DC 200 did not separate the two compounds sufficiently to permit analysis of both on the same sample.

Typical chromatograms of diazinon and diazoxon are also shown in Figure 1. Conditions were the same except that oven temperatures were 165° C. for diazinon and diazoxon. Retention times were 2.4 and 3.9 minutes, respectively.

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Correction

COLORIMETRIC DETERMINATION OF ALAR RESIDUES IN APPLES

In this article by L. G. Edgerton *et al.* [J. AGR. FOOD CHEM. **15,** 812 (1967)], in Table II, line 16 under Application Date(s) should read June 23, 1964 rather than June 23, 1966. All data below line 16 are for 1966, all data above are for 1964.