# Extraction and Cleanup Methods to Determine Malathion and Its Hydrolytic Products in Stored Grains by Gas–Liquid Chromatography

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The acidified acetone extraction, cleanup, and the gas chromatographic procedures described in this paper are satisfactory for routinely analyzing samples containing malathion, malathion half ester, and malathion dicarboxylic acid. The acids are quantitatively converted to their methyl esters by reaction with $BF_3$-methanol. *O*-demethyl derivative of malathion apparently cannot be esterified by $BF_3$-methanol, and incomplete esterification was obtained using diazomethane. This method is suitable for determination of malathion, half-ester, and dicarboxylic acid derivatives of malathion in the presence of waxes and pigments of the grain sorghum, corn, and wheat.

Malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) has been used extensively to protect stored grains from insects. Although its metabolism has been studied on different substrates (Corley and Beroza, 1968; Rowlands, 1964; St. John and Lisk, 1968), no work has been done on identifying malathion half-ester (*O,O*-dimethyl dithiophosphate of monoethyl mercaptosuccinate), malathion dicarboxylic acid (*O,O*-dimethyl dithiophosphate of mercaptosuccinic acid), and *O*-demethyl malathion by gas-liquid chromatography. Corley and Beroza (1968) described a procedure for gas chromatographic analysis of malaoxon and malathion. St. John and Lisk (1968) used diazomethane for methylation of metabolites of organophosphorus insecticides but presented no data concerning the aforementioned hydrolytic products of malathion. Rowlands (1964) used paper chromatography to identify these hydrolytic products of malathion because their high polarity and nonvolatility make it difficult to use gas chromatography for analysis. However, gas-liquid chromatography of the methyl esters of the hydrolytic products of malathion provides a rapid and convenient method for their quantitative analysis.

Because the properties of malathion and its hydrolytic products differ, separate methods for their extraction and cleanup were described previously (Koivistoinen *et al.*, 1965; Malathion Panel, 1959; Norris and Kuchar, 1959; Rowlands, 1964).

Reported here is an integrated extraction procedure applicable to malathion residues and its hydrolytic products. For gas chromatographic analysis, boron trifluoride in methanol ($BF_3$-MeOH) was used for methylation of malathion half-ester and malathion dicarboxylic acid. *O*-demethyl malathion derivative was partially esterified using diazomethane.

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## MATERIALS AND METHODS

Analytical grade standards of malathion, malathion half-ester, malathion dicarboxylic acid, and potassium salt of *O*-demethyl malathion, from American Cyanamid Co., Princeton, N. J., were prepared in redistilled acetone.

Boron trifluoride 14% in methanol ($BF_3$-MeOH), boron trichloride 10% in methanol ($BCl_3$-MeOH), and boron trichloride 4% in butanol reagents (Applied Science Laboratories, State College, Pa.) were used as received. All solvents were glass redistilled.

Gas chromatographs included Aerograph with the phosphorus detector kit and Barber-Colman Model 5000 Series equipped with an electron capture detector.

**Extraction and Cleanup.** A 20-gram sample each of wheat, grain sorghum, and corn was fortified with malathion, malathion half-ester, and malathion dicarboxylic acid, using 20 μg. of each in 1 ml. of redistilled acetone. The fortified sample was blended with 100 ml. of acidified acetone (0.5 to 1.0 ml. of 2*N* HCl) using a Sorvall Omnimixer driven at top speed for 5 minutes. The acetone extract was filtered and transferred quantitatively to a 500-ml. round-bottomed flask and concentrated to 2 to 3 ml. under vacuum at 35–40° C. The residue was transferred to a separatory funnel with the aid of 10 ml. of hexane and 10 ml. of acetonitrile. Then 100 ml. of water was added and the funnel shaken vigorously for 1 to 2 minutes. After two layers separated, the lower (aqueous acetonitrile) layer was drawn off into a 200-ml. round-bottomed flask to analyze the hydrolytic products of malathion. The upper (hexane) layer was partitioned with 80% acetonitrile in water to analyze malathion as previously described (Kadoum, 1968).

The aqueous acetonitrile layer was evaporated under vacuum at 35–40° C. and the residual water was transferred quantitatively to a 250-ml. separatory funnel using small portions of ethylacetate totaling 30 ml. An additional 25 ml. of water and 1 ml. of 2*N* HCl were added to the separatory funnel which was shaken thoroughly for 1 to 2 minutes.

After the two layers separated, the aqueous layer was discarded and the ethyl acetate layer was collected in a 100-ml. round-bottomed flask for concentration under vacuum at 35–40° C. to 2 to 3 ml. of ethyl acetate. The concentrate was transferred quantitatively to a 15-ml. centrifuge tube using small portions of ethyl acetate totaling 5 ml. The solvent was evaporated under a stream of nitrogen and the residue was methylated.

**Methylation and Cleanup of Diacid, Half-Ester, and *O*-Demethyl Derivatives of Malathion.** Standard curves were developed for diacid and half ester derivatives of malathion as follows: different aliquots of standard solutions of the compounds mentioned were transferred to a series of 12-ml. centrifuge tubes and evaporated to dryness with a gentle stream of nitrogen. One-half milliliter of $BF_3$-MeOH reagent was added to each tube and the reaction mixture was allowed to boil for 3 minutes. The boiled mixture was transferred to a 60-ml. separatory funnel using small portions of benzene totaling 7 ml., and about 25 ml. of water was added. The contents were mixed thoroughly by vigorous shaking of the separatory funnel. After the two layers separated, the aqueous layer was drained off and discarded. The benzene layer was washed with an additional 25 ml. of water and dried by passing through a layer of anhydrous sodium sulfate placed on a 2-inch funnel containing a glass wool plug. The separatory funnel and sodium sulfate layer were washed three times with small portions of benzene totaling 3 ml. The total benzene extract was evaporated to dryness and the residue was dissolved in hexane for microcolumn chromatographic cleanup using high purity grade Silica gel 950 as previously described (Kadoum, 1967) except that 5% ethyl acetate in benzene was the eluting solvent which resulted in total recovery of compounds studied. "*O*-demethyl malathion" derivative, which did not react with $BF_3$-methanol reagent, was partially methylated with diazomethane following the procedure of Schlenk and Gellerman (1960). Diazomethane was prepared from Diazald (Aldrich Chemical Co., Inc., Milwaukee, Wis.) according to Kirkland (1961).

**Gas Chromatographic Analysis.** Operating conditions were as follows: gas chromatograph equipped with an electron-capture detector: column, 6-foot glass column of 3% DC-11 on 60 to 80-mesh silinized Gas Chrom P; carrier gas, nitrogen, 36 ml. per minute; temperature, column 200° C., detector cell 220° C., injector 240° C., volume injected, 4 $\mu$l. of the extract in hexane.

Gas chromatograph with phosphorus detector kit: column, $^1/_8$ inch × 5 feet borosilicate glass packed with 5% QF-1 on 100- to 120-mesh aeropack 30; temperature, column 175° C., detector cell 195° C., injector 180° C., carrier gas, nitrogen 20 ml. per minute, and other gases (air, 175 ml. per minute; hydrogen, 16 ml. per minute); volume injected; 2 $\mu$l. of the extract in hexane.

## RESULTS AND DISCUSSION

Results obtained from acetone extraction of five replicates of 20-gram samples of grain sorghum, wheat, and corn, fortified with a mixture of malathion and its hydrolytic products yielded 97 ± 2, 95 ± 3, and 96 ± 2.5% recovery for malathion, malathion half-ester, and malathion dicarboxylic acid, respectively. This rapid and convenient extraction method gave the highest results when compared to hexane extraction of malathion (58 ± 2.5%) and Soxhlet extraction, which gives a high recovery of malathion but takes more time (Norris and Kuchar, 1959). The addition of 0.5 to 1.0 ml. of 2*N* HCl to acidify the acetone used for extraction only increased recovery percentage of half-ester and dicarboxylic acid derivatives of malathion. Therefore, it is not necessary to acidify the acetone which will be used for extracting malathion only.

**Table I. Retention Times and Limits of Detection of Compounds Studied**

| Compound | Retention Time (Min.) Determined by Gas Chromatograph with | | Minimum Detectable Amounts, Ng.[a] | |
|---|---|---|---|---|
| | Phosphorus thermionic detector | Electron capture detector | Phosphorus thermionic detector | Electron capture detector |
| Malathion | 9.75 | 2.25 | 0.05 | 0.1 |
| Malathion half-ester (methylated) | 8.00 | 1.88 | 0.2 | 0.4 |
| Malathion dicarboxylic acid (methylated) | 6.70 | 1.50 | 0.2 | 0.4 |

[a] Sensitivity of detection of standard methylated derivatives is the same as that of malathion, but the limits of detection of the derivatives recorded result from contaminants of the extracts that increase background noise.

In preliminary experiments $BF_3$-methanol, $BCl_3$-methanol, and $BF_3$-butanol were used to esterify 2 $\mu$g. of malathion diacid. Following cleanup and injection into the gas chromatograph, these peak heights were obtained: 21.6, 6.7, 1.8 cm. for samples esterified with $BF_3$-MeOH, $BCl_3$-MeOH, or $BF_3$-butanol, respectively. Hence, $BF_3$-MeOH was used instead of the other two reagents.

To determine optimum conditions to esterify malathion half-ester and dicarboxylic acid derivatives, temperature, time of esterification, and amount of $BF_3$-MeOH required were studied. Esterification of separate 8 $\mu$g. of dicarboxylic acid samples resulted in peak heights of 30–35, 80–82, 75–77, 65–72, and 0.00 cm., respectively, with the mixture boiled for 1, 3, 5, 10, or 20 minutes. With excessive heat, no peaks were obtained after injection of the solution. The results indicated that boiling the mixture 3 minutes in a water bath was optimum to esterify microgram quantities of dicarboxylic acid. One-half milliliter of $BF_3$-MeOH was satisfactory to esterify up to 500 $\mu$g. of malathion half-esters and dicarboxylic acid derivatives.

The esterification mixture after cleanup and adjustment to the desired concentration was injected into the gas chromatograph. A linear relationship was obtained between peak height and corresponding nanogram amounts of each methylated compound injected. Reproducible and consistent results were obtained from four replicates of each amount of compound analyzed. Table I lists the compounds studied, retention time of their methyl esters, and their limits of detection. The sensitivity of the procedure, which depends on the processes involved in the analysis of each compound, was estimated as the amount of nanograms injected to produce a peak at least 10 times the noise level.

Early repeated trials for methylation of *O*-demethyl malathion derivative using $BF_3$-methanol failed to produce any esterification. Diazomethane then was used despite its explosive and toxic nature, but only incomplete recovery of *O*-demethyl malathion derivative was achieved. Methylation of 50 $\mu$g. resulted in two compounds with peak heights of 7 and 3 cm. with retention times of 2.20 and 3.20 minutes,

respectively, using an electron-capture detector with the aforementioned operating conditions.

The method reported herein is a gas chromatographic technique that is more accurate, faster, and more convenient than Rowland's (1964) other chromatographic procedure. It is well suited to resolving mixtures of malathion and its hydrolytic products after they are converted to stable volatile derivatives in wheat, grain sorghum, and corn, and it should apply equally well for other stored grains and their products. St. John and Lisk (1968) describe a method for analysis of other metabolites of malathion that cannot be methylated by $BF_3$-methanol such as dimethyl phosphate, dimethyl thiophosphate, and dimethyl dithiophosphate.

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