Quantitative Gas Chromatographic Analysis of Dimethoate and Several Metabolites in Rape, Quail, and Grasshopper Tissues by Derivatization

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A method for the simultaneous quantitative analysis of the organophosphorus insecticide, dimethoate, and its oxon, acid, and *O*-demethyl metabolites has been developed. Recovery data have been collected using spiked samples of rape, quail, and grasshopper tissues. Analysis of the extracts, without prior cleanup, was conducted after derivatization with diazoethane. Total analysis time for all four compounds in a mixture is approximately 12 min.

Dimethoate (*O,O*-dimethyl *S*-(*N*-methylcarbamoyl)-methyl phosphorodithioate) is a systemic organophosphorus insecticide registered in Canada for use against a wide variety of pests on crops, trees, and ornamental plants; for control of houseflies around livestock pens, processing plants, and human dwellings; for control of grasshoppers on livestock forage, and for control of grasshoppers in grain destined for human consumption (Agriculture Canada, 1973).

Metabolic studies carried out by Dauterman et al. (1959 and 1960), Santi and Giacomelli (1962), Hacskaylo and Bull (1963), and Lucier and Menzer (1968) have shown that both the type and the amounts of dimethoate metabolites can vary according to the substrate. For example, the recovery of 17 dimethoate metabolites from bean plants was reported by Lucier and Menzer (1968), whereas ten metabolites were found by Hacskaylo and Bull (1963) in cotton leaves. Six of the first and seven of the second lot of metabolites were positively identified.

Although a number of analytical methods for dimethoate residues have appeared in the literature based on such techniques as colorimetry (Chillwell and Beecham, 1960; Giang and Schechter, 1963; Van Middelem and Waites, 1964; George et al., 1966), paper chromatography (Enos and Frear, 1962), total phosphorus determination (Steller and Curry, 1964), and gas chromatography (Storherr and Watts, 1969; Steller and Pasarela, 1972; Woodham et al., 1974a,b), these methods were designed either for dimethoate alone, or for dimethoate and its oxygen analogue (dimethoxon, omethoate). In some cases, the methods require that dimethoate and dimethoxon be analyzed separately, while several of the methods quantitate without distinguishing between the two.

Shafik and Enos (1969) described a method for the simultaneous gas chromatographic (GC) analysis of six dialkyl metabolites, common to many organophosphorus pesticides, in human blood and urine using the derivatization procedure described by Stanley (1966). Three of these compounds have been reported as dimethoate metabolites, namely *O,O*-dimethyl phosphate (Hacskaylo and Bull, 1963), *O,O*-dimethyl diphosphate, and *O,O*-dimethyl dithiophosphate (Lucier and Menzer, 1968). These and the *O,O*-diethyl homologues are metabolites of many organophosphorus pesticides.

To date, however, no quantitative analytical method has been reported for any metabolite isolated in metabolic studies conducted with dimethoate, other than the dimethyl metabolites of Shafik and Enos (1969), and dimethoxon. The following procedure was developed to facilitate the simultaneous GC analysis of dimethoate (**1**), dimethoxon (**2**), the *O*-demethyl dimethoate (**3**), and the carboxylic acid of dimethoate (**4**) (see Figure 1). Work is now under way to extend this method to include several additional dimethoate metabolites.

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EXPERIMENTAL SECTION

The analysis of dimethoate and metabolites was carried out using a Tracor Micro Tek MT 220 gas chromatograph equipped with a Melpar Flame Photometric Detector (FPD) operated in the phosphorus (526 nm) mode.

Reagents. (a) Standards of dimethoate, dimethoxon (*O,O*-dimethyl *S*-(*N*-methylcarbamoyl)methyl phosphorothioate), the dimethoate carboxylic acid (*O,O*-dimethyl *S*-(carboxymethyl) phosphorodithioate), and *O*-demethyl dimethoate (*O*-methyl *S*-(*N*-methylcarbamoyl)methyl phosphorodithioate) were supplied by American Cyanamid Co., Agricultural Division, Princeton, N.J. (b) *N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine was obtained from Aldrich Chemical Co., Milwaukee, Wis. (see cautionary note 1 below). (c) Chromosorb W HP, 80/100 mesh, coated with 3% OV-17, was obtained from Chromatographic Specialties, Brockville, Ontario.

Chromatographic Conditions. A 1.8 m × 4 mm i.d., silanized, Pyrex column was packed with Chromosorb W HP, 80/100 mesh, coated with 3% OV-17: temperatures, inlet 250 °C; detector, 200 °C; column, 180 °C (isothermal); flow rates, nitrogen carrier, 80 ml/min; hydrogen, 180 ml/min; oxygen, 20 ml/min; air, 95 ml/min; chart speed, 0.1 in./min; input attenuation, 10^4; output attenuation, 8; injection volume, 2 μl.

Preparation of Diazoethane. The preparation of the diazoethane (cautionary note 2) is based on the method of McKay et al. (1950), later modified by Stanley (1966). Potassium hydroxide (KOH) (2.3 g; 0.04 mol) was dissolved in 2.3 ml of distilled water in a 125-ml Erlenmeyer flask, and the flask cooled to room temperature. Diethyl ether (25 ml) was added and the flask cooled in an ice bath for approximately 5 min. *N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine (1.6 g; 0.01 mol) was added in portions over a period of several minutes, shaking vigorously after each addition. The ether layer was decanted from the aqueous slurry that forms into a tightly capped bottle and stored in the freezer until required. The yield was 15–20 ml of ether solution (cautionary note 3).

Cautionary Notes. (1) The diazoethane precursor, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine, should not be allowed to contact the skin as it is both a carcinogen and potent mutagen. Extreme care must be exercised during storage and handling of this compound. Cleanup of small spillages and decontamination of implements can be carried out using a strong KOH solution.

(2) Diazoethane preparation, and subsequent usage, should be conducted in a fume cupboard using protective

Table I. Recoveries of Dimethoate and Metabolites after Derivatization with Diazoethane[a]

Compd (peak no.)	Retention time,[b] min	Limit of detectability, ng	Recovery ±2.0, %					
			Plant		Quail		Grasshopper	
			Individ.[c]	Mix[d]	Individ.	Mix	Individ.	Mix
Dimethoate (III)	9.50	0.6	86.1	88.1	98.8	79.2	97.1	74.9
Dimethoxon (II)	5.95	2.5	102.9	95.0	95.3	103.9	100.1	98.3
Dimethoate acid (I)	3.97	0.1	99.1	77.9	93.7	79.5	78.6	77.5
O-Demethyl dimethoate (IV)	10.95	2.0	96.2	53.6	78.1	55.7	55.0	59.7

[a] Retention times and limit of detectability for dimethoate and metabolites are also included. [b] Retention times for unknown peaks were as follows: peak V, 1.07; VI, 2.53; VII, 3.18; VIII, 6.98; IX, 8.00 min. [c] Indicates recovery of compound from substrate in which it alone is present. [d] Indicates recovery of compound in the presence of the other three compounds.

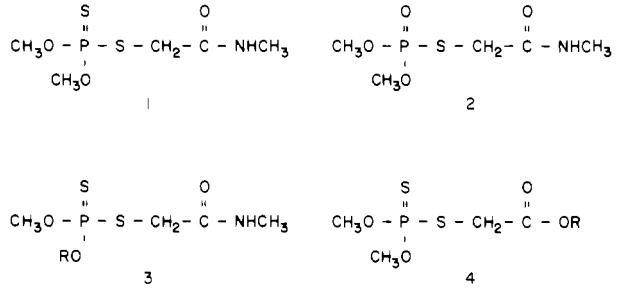

Figure 1. Structural formulas of dimethoate (**1**), dimethoxon (**2**), *O*-demethyl dimethoate (**3**), and dimethoate carboxylic acid (**4**).

hand and face coverings. Diazoethane is toxic and potentially explosive, and should not be exposed to excessive temperatures or to ground glass joints.

(3) If larger quantities of diazoethane are required, the amounts of chemicals used may be increased, but the proportions must not be changed. Too high a concentration of the diazoethane might lead to an explosion. Only diethyl ether should be used as the solvent for diazoethane (Stanley, 1966).

Sample Spiking. Duplicate samples of rape (*Brassica napus*), quail (*Couturnix couturnix japonica*), and grasshopper (*Melanoplus bivittatus*) tissue were spiked with individual standards of dimethoate, or one of the three metabolites, in acetone, to give samples containing the equivalent of 28.5 μg of dimethoate, 61.3 μg of dimethoxon, 6.2 μg of dimethoate carboxylic acid, or 39.6 μg of *O*-demethyl dimethoate. In addition, duplicates of each tissue type were spiked with a mixture of dimethoate and the three metabolites, in acetone, to give the equivalent of 28.5 μg of dimethoate, 61.3 μg of dimethoxon, 6.2 μg of dimethoate carboxylic acid, and 28.4 μg of *O*-demethyl dimethoate. Assuming 100% extraction efficiency by the method described below, extracts will contain 14.3 ng/μl dimethoate, 30.7 ng/μl dimethoxon, 3.1 ng/μl dimethoate carboxylic acid, and 14.2 ng/μl *O*-demethyl dimethoate. These concentrations were arbitrarily chosen to yield similar peak heights for each principal compound in the chromatograms of a derivatized standard mixture.

Sample Extraction. Glacial acetic acid (0.5 ml) was added to all samples, other than the rape tissue samples, just prior to extraction.

Rape Tissue. Ten grams of tissue, 30 g of Na_2SO_4, and 25 ml of 1% glacial acetic acid were homogenized together in a Sorval blender for 5 min at a setting of 4. The liquor was decanted through glass wool into a separatory funnel and extracted with three 15-ml portions of acetonitrile. The acetic acid was repeated three times. The rape tissue homogenate was then extracted twice with 25 ml of acetonitrile. The acetonitrile extracts were combined and evaporated almost to dryness on a rotary evaporator at 40 °C. The residue was taken up in acetone, and transferred to a septum vial for storage to await derivatization.

Grasshopper and Quail Tissues. Two grams of whole grasshopper, 10 g of Na_2SO_4, and 25 ml of acetonitrile (or 10 g of quail tissue, 40 g of Na_2SO_4, and 50 ml of acetonitrile) are homogenized in a Sorval blender for 5 min at a setting of 4. The extract was filtered through glass wool into a round-bottomed flask. This procedure was repeated three times. The extracts were combined and the procedure then followed that for the rape tissue.

Derivatization Procedure. The sample was quantitatively transferred to a 10-ml graduated test tube and enough diazoethane (1–2 ml) was added to cause a distinct yellow color (greenish-yellow in the case of the rape extract) in the extract and the sample was allowed to stand for several minutes. The sample volume was reduced to approximately 1 ml under nitrogen. More acetone is added and the volume reduced once more to approximately 1 ml to ensure that all traces of ether and diazoethane have been removed. The sample volume was then adjusted to 2.00 ml and a 2.0-μl aliquot was injected into the gas chromatograph for analysis.

RESULTS AND DISCUSSION

Figure 2 is representative of the chromatograms obtained from the substances both before and after derivatization with diazoethane. No evidence of interference by co-extractives from any of the three tissue types was observed. Figure 2b shows a number of lesser peaks present, which to date, remain unidentified. These are thought to be further breakdown products of the metabolites. Peaks VI and VII are associated with dimethoxon and do not appear to be affected by derivatization. Peak VII, however, is also common to derivatized extracts containing the *O*-methyl-*O*-ethyl dimethoate. As with peaks V, VIII, and IX, however, it appears only in the derivatized extract, but not in a derivatized standard. Further work is under way to relate these unknown peaks to other known dimethoate metabolites. Retention data for these unknowns are listed in Table I.

Preparation of derivatives of the dimethoate carboxylic acid and the *O*-demethyl dimethoate was performed with diazomethane before investigating the use of diazoethane. Both compounds reacted successfully to the treatment, yielding the dimethoate acid methyl ester, and dimethoate. Dimethoxon was unaffected by diazomethane. However, simultaneous analysis by gas chromatography of the four compounds was not possible due to the aforementioned conversion of the *O*-demethyl dimethoate to the parent compound. Consequently quantitative analysis of the *O*-demethyl dimethoate would have necessitated separate analysis of either the parent compound or the derivatized metabolite. Use of diazoethane eliminated the need for such a step.

Table I lists retention times, limits of detectability, and percent recovery for dimethoate and each metabolite. As

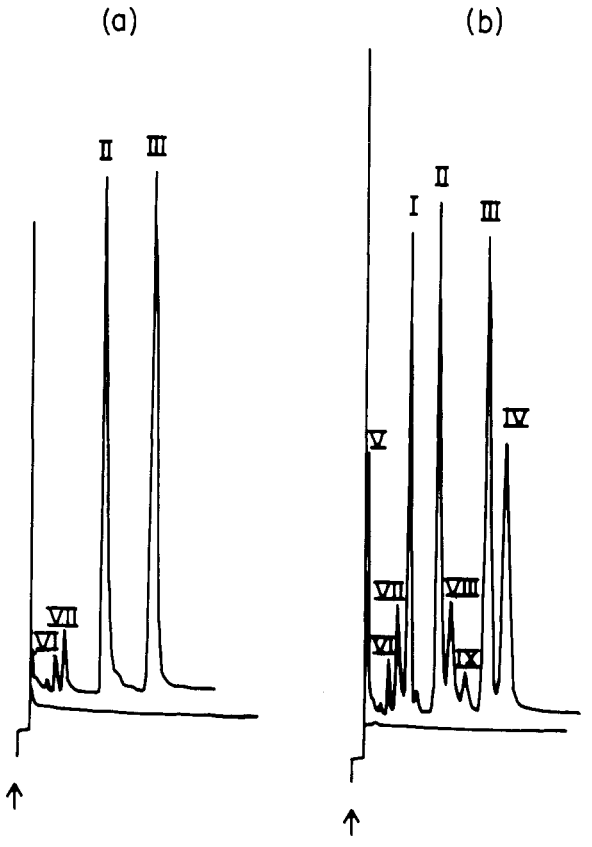

Figure 2. Typical chromatograms of actual extracts, both unfortified and fortified, obtained for dimethoate and its metabolites (a) prior to derivatization and (b) after derivatization with diazoethane. Peak I is the ethyl ester of dimethoate carboxylic acid; peak III is dimethoate; peak IV is *O*-methyl-*O*-ethyl dimethoate; injection volume, 2 μl.

can be seen, the method is extremely sensitive toward both dimethoate and dimethoate acid ethyl ester. Lesser sensitivity is experienced with dimethoxon and *O*-methyl-*O*-ethyl dimethoate. Recovery rates for single compounds were somewhat higher in most cases than recovery rates for the compound in the presence of the other three compounds. Inclusion of the acid treatment step in the extraction procedure was found to improve metabolite recoveries considerably over extractions carried out without acid treatment, probably due to hydrolysis of conjugates formed by the metabolites.

It should be noted that extracts should not be allowed to go to dryness at any time. It has been our experience that loss of the *O*-demethyl metabolite will occur in such cases. The low recoveries experienced for the *O*-demethyl dimethoate cannot, at present, be explained. However, the recoveries obtained were reproducible. Dimethoate and the other metabolites, however, do not seem to be as prone to this phenomenon.

Column age will affect the response of the dimethoxon, causing either complete retention by the column packing, or erratic, but steadily increasing responses which make quantitation impossible. Use of silane reagents for on-column deactivation is not recommended to correct this problem. Such action destroys the column's usefulness by causing the compounds to "skate" off the column within 2 min after injection. We have found that injecting a large quantity of tissue extract containing dimethoxon (usually 50 μl will suffice) will restore column performance and lengthen column life considerably.

In conclusion, the method described is a rapid, sensitive, and reliable method for dimethoate and metabolite analysis, requiring no prior cleanup of samples, and thereby reducing the possibility of losses which are often associated with cleanup procedures.

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Received for review January 15, 1976. Accepted April 19, 1976. Contribution No. 7 from the Pesticide Research Laboratory. Financial support for this project was provided by the Manitoba Department of Agriculture through Dean L. H. Shebeski, Faculty of Agriculture, University of Manitoba. This project was also supported by Agriculture Canada and the National Research Council of Canada.