Determination of Methomyl Residues Using Microcoulometric Gas Chromatography

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A method is described for determining residues of the insecticide, methomyl, *S*-methyl-*N*-[(methylcarbamoyl)oxy]thioacetimidate, in animal and plant tissues and soil. The procedure is based on the gas chromatographic measurement of methyl-*N*-hydroxythioacetimidate after extraction of methomyl from the substrate with an organic solvent and subsequent alkaline hydrolysis to the volatile, more stable fragment. Programmed temperature operation of the gas chromatographic column is used in conjunction with the highly selective microcoulometric sulfur detector to obtain the sensitivity and selectivity required for low-level residue measurements. Intermediate solvent-partitioning cleanup steps are required to minimize possible interfering compounds. Sensitivity of the method is about 0.02 p.p.m. based on a 25-gram sample, with an average recovery of about 93% for all tissues investigated. Somewhat lower recoveries are obtained with soils.

A sensitive analytical method has been developed for determining residues of methomyl, *S*-methyl-*N*-[(methylcarbamoyl)-oxy]thioacetimidate, in plant and animal tissues and in soil. This compound is the active ingredient of Lannate Methomyl Insecticide (formerly Du Pont Insecticide 1179, E. I. du Pont de Nemours & Co.) which controls a broad spectrum of insects when used as a foliar spray. Methomyl is extracted from the sample with organic solvent, and the extract is purified by a liquid-liquid partitioning process. Methomyl (I) is then hydrolyzed to the corresponding oxime, methyl-*N*-hydroxythioacetimidate (II), by the reaction:

$$CH_3\text{-}S\text{-}C(CH_3)=N-OCONHCH_3 \xrightarrow{OH^-} CH_3\text{-}S\text{-}C(CH_3)=N-OH$$

(I) (II)

Compound II is then extracted into an organic solvent and selectively measured by microcoulometric gas chromatography.

The analytical method is sensitive to 0.02 p.p.m. of methomyl based on a 25-gram sample, and satisfactory recoveries have been demonstrated at this level on a variety of subtrates. Average recoveries of about 93% have been obtained on all of the tissue samples investigated. Recoveries from soils are somewhat lower.

APPARATUS AND REAGENTS

The gas chromatograph used was a Model MT-220, Micro-tek Instruments, Inc., Baton Rouge, La., equipped with a Dohrmann microcoulometric titrating system, consisting of a C-200 microcoulometer, T-300P sulfur titration cell, and a S-100 sample inlet combustion unit. The coulometric detector was operated according to instructions furnished by the manufacturer. The chromatographic column was 4-foot glass tubing, 1/4-inch o.d., 3/16 inch i.d., containing 10% FFAP (Wilkens Instrument & Research, Inc., Walnut Creek, Calif.) on 80- to 100-mesh High-Performance Chromosorb W, acid-washed and dimethyldichlorosilane-treated (Applied Science Laboratories, Inc., State College, Pa.). Centrifugation was carried out with an International, size 1, type SB centrifuge equipped with 250-ml. bottles. Reference samples of methomyl, *S* - methyl-*N* - [(methylcarbamoyl) - oxy]thioacetimidate, and methyl-*N*-hydroxythioacetimidate were obtained from the Industrial and Biochemicals Department, Agrichemical Sales Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. The solvents used were Distilled-in-Glass, Burdick and Jackson Laboratories, Inc., Muskegon, Mich. Triethylamine was obtained from Eastman Organic Chemicals, No. 616.

EXPERIMENTAL

Gas Chromatographic Calibration. Equilibrate the chromatograph under the following conditions: vaporizer temperature, 235° C.; transfer temperature, 250° C.; furnace temperature, 850° C.; column temperature, 200° C.; helium carrier-gas flow, 100 cc. per minute; helium purge flow, 50 cc. per minute; oxygen flow, 50 cc. per minute.

Condition the chromatographic column by maintaining the temperature at 200° C. with carrier gas flowing for at least 48 hours. Set the initial column temperature at 100° C. and the coulometer sensitivity at 100 ohms using the Lo-Gain mode. Inject aliquots of 0.5, 1.0, 5, and 25 μg. per ml. calibration solutions of compound II so that the peak does not exceed full-scale deflection. (Full-scale deflection for II is approximately 0.12 μg.) Wait 2 minutes and increase the column temperature at 7.5° per minute to a maximum of 200° C. Hold the column at this temperature for about 10 minutes. (The retention time for II from the beginning of the programming is about 12 minutes.) Construct a calibration curve

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of micrograms of compound II *vs.* peak height. Chromatograph one or more calibration solutions daily, to ensure that the calibration curve remains accurate.

Isolation. Weigh 25 grams of a representative sample into a Waring Blendor jar, add 100 to 150 ml. of ethyl acetate, cover, and blend at high speed for 5 to 10 minutes. Quantitatively transfer the blended sample to a 250-ml. centrifuge bottle, using several small volumes of ethyl acetate as wash liquid. Centrifuge at 2000 r.p.m. for 10 to 15 minutes and carefully decant the ethyl acetate extract through a cotton plug into a 400-ml. beaker. Add 100 ml. of ethyl acetate to the substrate remaining in the centrifuge bottle, stopper, and shake vigorously for 2 or 3 minutes. Centrifuge this as before and combine the ethyl acetate extracts. Repeat the extraction once more, using 100 ml. of ethyl acetate. Add 50 ml. of distilled water to the combined organic extracts and evaporate the ethyl acetate at room temperature in a hood. (When analyzing a series of samples, this evaporation can be conveniently carried out overnight.) Transfer the water from the 400-ml. beaker to a 250-ml. separatory funnel, using water washes. Dilute to approximately 100 ml. with water and acidify with 5 ml. of 1*N* sulfuric acid. Add 50 ml. of *n*-hexane to the separatory funnel, shake for 2 minutes, and allow the phases to separate. Centrifuge to obtain a clean separation, if necessary. Discard the hexane layer. Repeat the hexane extraction procedure twice more, using additional 50-ml. portions of solvent. Discard the hexane after each wash. Add 50 ml. of chloroform to the aqueous layer remaining in the separatory funnel, and shake for 2 minutes. After a clean separation of the layers (centrifuge if necessary), drain the chloroform extract through cotton into a 150-ml. beaker. Repeat the extraction twice more, using 50-ml. portions of chloroform. Combine the chloroform extracts and carefully concentrate in a hood at room temperature to about 20 ml.

Add 50 ml. of 0.1*N* sodium hydroxide to the concentrated extract and heat on a steam bath with occasional stirring to remove the residual chloroform. Cover and continue to heat on the steam bath for an additional 15 minutes. Cool, acidify with 1*N* sulfuric acid, and quantitatively transfer the solution to a 125-ml. separatory funnel, using water washes. Extract the aqueous hydrolyzate with three 30-ml. portions of ethyl acetate, using about 2-minute shaking periods for each extraction. Allow the phases to separate and filter the ethyl acetate phases through anhydrous sodium sulfate into a 100-ml. beaker. Add 0.1 ml. of triethylamine to the combined extract and carefully concentrate the solvent to about 20 ml. by evaporation at room temperature in a well ventilated hood. Quantitatively transfer the concentrated extract to a 30-ml. beaker, add 0.1 ml. more of triethylamine, and concentrate the solution to about 0.5 ml. by evaporation as above. [Caution. Because of the volatility of the hydrolyzate compound II, it is necessary to use extreme care in the solvent concentration steps. Do not allow the solvent to go to dryness.] Transfer the concentrated extract to a 1-ml. volumetric flask, using a finely drawn out dropper with several small washes of ethyl acetate. Dilute the volumetric flask to volume with ethyl acetate and mix thoroughly.

Gas Chromatographic Analysis. Equilibrate the instrument and chromatograph an aliquot of the above prepared extract as described under Calibration. Measure the peak height of compound II and determine the micrograms of this material in the aliquot, using the calibration curve previously prepared. Calculate the amount of methomyl, *S*-methyl-*N*-[(methylcarbamoyl)oxy]-thioacetimidate, in parts per million by dividing the micrograms of compound II found, corrected for the molecular weight conversion (1.54), aliquot, and recovery factors, by the sample weight in grams.

RESULTS AND DISCUSSION

Methomyl cannot be directly gas-chromatographed at the levels required for a sensitive residue method. Consequently, the analytical procedure is based on the gas chromatographic measurement of a characteristic fragment, compound II. The derivative compound is easily formed by a simple quantitative hydrolysis in dilute aqueous solution as described by the equation shown above. Compound II gas-chromatographs unchanged, about 75% of the theoretical sulfur being titrated by the coulometric detector. The fact that the calibration curve continues to be strictly linear, even at the smallest levels of compound II chromatographed, indicates that this material passes through the chromatographic system without significant degradation.

The applicability of the gas chromatographic method for determining residues of methomyl has been demonstrated on a variety of substrates. Recovery of this compound added to untreated control tissue samples averaged about 93% over the range of 0.02 to 4.0 p.p.m. (Table I). Recoveries from soil were lower, reflecting the usual difficulty of extracting polar compounds from soil with organic solvents. Recoveries were conducted by adding known amounts of methomyl to the untreated controls contained in a Waring Blendor jar. Analyses were carried out as described in Experimental.

Table I. Methomyl Recovery Data

Crop	Residue Level, P.P.M.	No. of Detns.	Recovery, % Av.	Recovery, % Range
Cabbage	0.02 to 4.0	9	98	86–110
Sweet corn kernels	0.02 to 0.26	11	96	83–115
Sweet corn stalk	0.04 to 0.20	3	92	83–100
Soybeans, leaves	0.02 to 0.22	7	89	80–98
Soybeans, forage	0.02 to 0.20	4	90	84–95
Cottonseed	0.02 to 0.20	4	81	75–86
Tobacco	0.02 to 0.40	5	92	86–95
Snap beans, pods	0.02 to 0.40	8	97	87–104
Snap beans, forage	0.02 to 0.40	6	90	83–104
Tomatoes	0.02 to 1.0	6	86	80–96
Celery	0.02 to 2.0	7	102	96–110
Lima beans	0.02 to 0.26	6	93	79–115
Potatoes	0.02 to 0.26	6	91	83–108
Peaches	0.02 to 0.42	5	105	87–115
Liver	0.08 to 1.0	2	94	88–100
Kidney	0.08 to 0.20	2	98	90–105
Muscle	0.02 to 0.08	3	92	85–96
Fat	0.04 to 0.12	2	96	93–98
Urine	0.20 to 1.4	4	90	80–97
Soil	0.04 to 4.0	12	63	58–67

The use of the sulfur coulometric detector and the FFAP chromatographic column provides for a highly selective measurement of the desired compound in a wide variety of substrates. Naturally occurring organic sulfur compounds will vary in identity and quantity in different crops; therefore, three substrates were selected for illustration to show typical variations which can occur in backgrounds when this method is employed. Figure 1 shows chromatograms obtained on extracts of soybeans, the upper curve obtained on a sample fortified with 0.02 p.p.m. of methomyl, the lower curve representing a control soybean extract. Extracts of control soybeans are virtually free from materials which are detected in the chromatographic analysis. Other substrate extracts, such as those of control and fortified sweet corn kernels shown in Figure 2, show significant amounts of unknown components as chromatographic peaks; however, these peaks do not interfere with the analytical method. Figure 2 shows that 0.02 p.p.m. of methomyl fortified in sweet corn kernels can readily be detected and measured when compared with the control extract shown in the lower curve.

Of all of the substrates tested, cabbage presented the most difficulty in the application of the proposed procedure. Because of the relatively large amount of organic sulfur compounds extracted from cabbage, it is necessary, when working at very low residue levels, to modify the chromatographic procedure to measure residues of methomyl in this crop accurately. To eliminate the buildup of high-boiling, sulfur-containing compounds which accumulate in the column, the chromatographic column must be conditioned after every five or six analyses, by maintaining the column temperature at 200° C. for at least 1 hour. If accumulated interferences are not removed by this treatment, they interfere with successive chromatographic measurements. Cabbage presents an extreme case, and this extra column-conditioning step is not required for the rest of the substrates investigated. Figure 3 shows the chromatograms of fortified and control cabbage extracts obtained with the recommended procedure and indicates the feasibility of measuring methomyl at the 0.02-p.p.m. level in this substrate.

With certain crops containing troublesome interferences, it is sometimes necessary to make additional hexane washes of the initial acidified aqueous solution to prepare a cleaner extract. These additional washes are indicated if appreciable color is still being extracted with the third hexane wash. With some crops, such as cottonseed and tobacco, it is advantageous to increase the volume of hexane washes to 100 ml. to avoid emulsion difficulties that sometimes occur. Such adaptations in the procedure are mostly matters of judgment, and their use should be determined for the particular substrate to be analyzed.

The analytical method is based on measurements of peak height, rather than peak area, since this measurement provides higher selectivity from possible interferences such as those shown in the chromatograms of sweet corn kernels and cabbage. Measurements based on peak height also provide maximum freedom from errors owing to minor base line shifts inherent at high sensitivity with the detector system used. Peak heights have been reproducible over extended periods of time with the method employed, and linear calibration curves extrapolating to

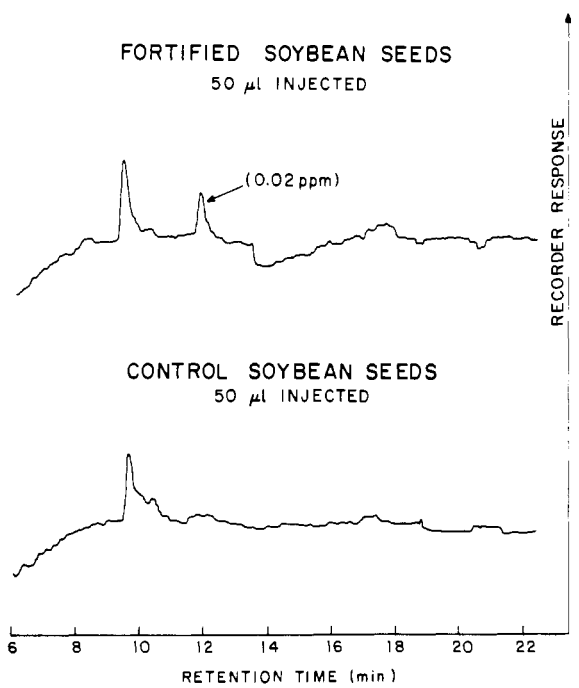

Figure 1. Chromatograms of soybean extracts

Upper. Soybeans fortified with 0.02 p.p.m. of methomyl
Lower. Soybean control

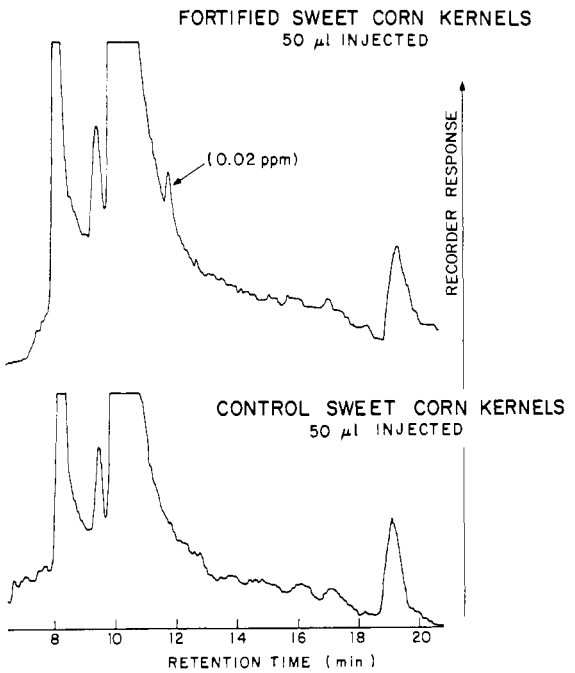

Figure 2. Chromatograms of sweet kernel extracts

Upper. Sweet corn kernels fortified with 0.02 p.p.m. of methomyl
Lower. Sweet corn kernels control

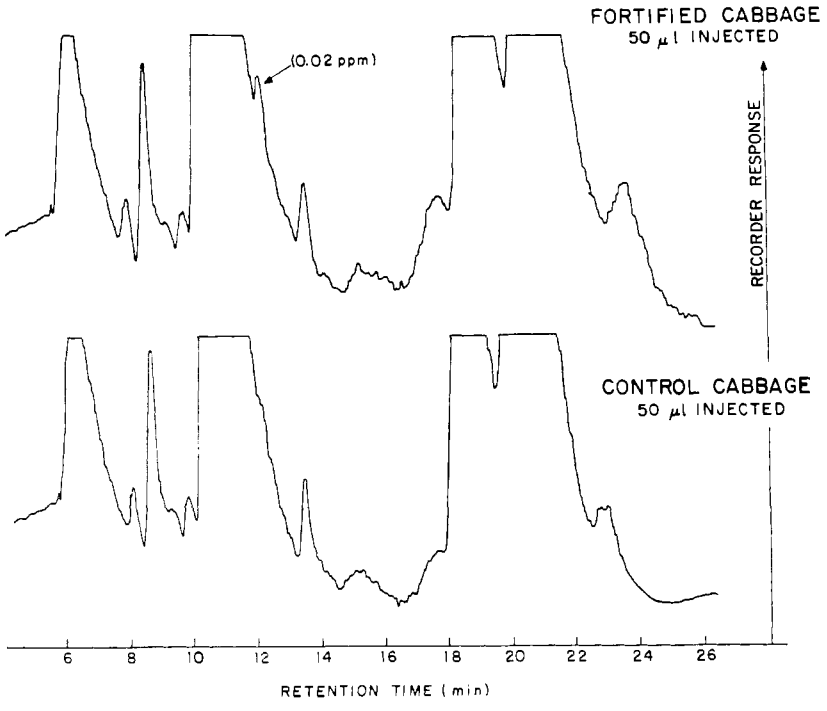

Figure 3. Chromatograms of cabbage extracts

Upper. Cabbage fortified with 0.02 p.p.m. of methomyl *Lower.* Cabbage control

zero concentration are obtained for compound II in amounts up to 0.12 μg.

Errors due to volatilization of the residue are insignificant, providing the procedures described in the Experimental section are carefully followed. Because of the relatively high volatility of the hydrolyzate (compound II), care must be exercised in handling this material to eliminate losses occurring during various solvent concentration and transfer steps. Since compound II is a weak acid, organic solutions of this material may be treated with triethylamine to form the less volatile amine salt.

A series of sulfur-containing pesticides (Table II) was carried through the complete isolation and chromatographic procedure at the 1-p.p.m. level to determine if any of the materials interfere with the determination of methomyl residues.

None of these compounds showed any interference with methomyl determination. Only UC-21149 produced a chromatographic peak by the procedure described and this peak eluted approximately 2 minutes prior to compound II.

The highly selective microcoulometric sulfur detector is recommended for the gas chromatographic analysis of methomyl residues. However, the electron-capture detector might be useful in certain situations. Using an electron-capture detector containing a Ni^{63} radioactive source and the chromatographic column described above, 5 μl. of a 2-μg. per ml. solution of compound II produced a peak about 50 mm. high at approximately 5 minutes' retention with the column operating isothermally at 150° C. Unfortunately, the extracts of many substrates demonstrate components with electron-capturing properties, and these seriously interfere with the quantitative measurement of the desired compound. For instance, very heavy interference was found in sweet corn kernel extracts when the electron-capture detector was used.

Table II. Sulfur-Containing Pesticides

Chemical Name	Generic Name or Code
1. 4-Benzothienyl-*N*-methylcarbamate	MCA-600
2. *N*-Trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide	Captan
3. *O,O*-Diethyl-*O*(and *S*)-2-(ethylthio)-ethyl phosphorothioate	Demeton
4. 6,7,8,9,10,10-Hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide	Endosulfan
5. Ferric dimethyldithiocarbamate	Ferbam
6. *O,O*-Dimethyl-*S*-[2-(ethylsulfinyl)-ethyl]-phosphorothioate	Malathion
7. Manganese ethylenebisdithiocarbamate	Maneb
8. *O,O*-Dimethyl-*O*-*p*-nitrophenyl phosphorothioate	Methyl parathion
9. *O,O*-Diethyl-*O*-*p*-nitrophenyl phosphorothioate	Parathion
10. *O*-Ethyl-*O*-*p*-nitrophenyl phenyl phosphorothioate	EPN
11. *O,O*-Diethyl-*S*-ethylthiomethyl phosphorodithioate	Phorate
12. Carbamic acid, methyl ester with propionaldehyde, 2-methyl-2-methylthiol oxime	UC 21149

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