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A METHOD FOR THE DETERMINATION OF TOTAL UC 51762 RESIDUES IN COTTON AND SOYBEAN SEED

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DETERMINATION OF TOTAL UC 51762 RESIDUES IN COTTON AND SOYBEAN SEED

UC 51762 = Dimethyl-N,N'-[thiobis[(methylimino)carbonyloxy]]bis[ethanimidothioate]

INTRODUCTION

Total toxic UC 51762 residues consist of methomyl and UC 51762. Methomyl oxime is quantitated by this procedure but is not considered to be a significant residue due to its relatively low toxicity and concentration.

The residues are extracted with acetone and two separate clean-up steps are employed. A partitioning between acetonitrile and hexane removed a portion of the extracted oil and the use of open-column chromatography allows additional removal of endogeneous material.

A basic hydrolysis of the residue converts the methomyl and UC 51762 to methomyl oxime which is quantitated by gas chromatography utilizing a flame photometric detector selective for sulfur-containing compounds.

Typical chromatograms for standard solutions, untreated control samples, and cotton seed containing residue are shown in Figure 1. Similar chromatograms are obtained for soybean seed. Residues are quantitated by reference of the peak height to a previously prepared calibration curve derived from injection of methomyl oxime standard solutions (see example calibration curve in Figure 2). The results are obtained as methomyl oxime equivalents and can be calculated as UC 51762 equivalents. It may be necessary to prepare more than one calibration curve during the day since instrument response may change over a period of time.

The method has been validated by fortification of untreated cotton and soybean seed before extraction with the components, methomyl oxime, methomyl, and UC 51762. The average recovery is about 85 percent (Table I) and method sensitivity is about 0.02 ppm.

The adequacy of the extraction technique is demonstrated by the exhaustive extraction of cottonseed containing residues. The data in Table II show that over 95 percent of the free residues are extracted by the residue method.

Reagents

a. Acetone, Analytical Grade or equivalent
b. Acetonitrile, Analytical Grade or equivalent
c. Iso-hexane, Analytical Grade or equivalent
d. Methylene chloride, Analytical Grade or equivalent
e. Sodium hydroxide, 2.5N in water
f. Sulfuric acid, 4.0N in water
g. Hyflo Super-cel, Mallinckrodt, Analytical Reagent
h. Sodium chloride, Mallinckrodt, Analytical Reagent
i. Silica gel, 70/230 mesh, E. Merck
j. Solvent mixture I, 55/40/5 (v/v/v) ethyl ether, isohexane, methanol

Standard Solutions

a. Weigh 0.1000 g of methomyl oxime into a 100-ml volumetric flask, dilute to the mark with methanol and shake until solution is complete. Concentration of standard is 1000 µg/ml.

b. Withdraw a 10-ml aliquot with a pipet and dilute to 100 ml with methanol in a second volumetric flask. Concentration of standard is 100 µg/ml.

c. Remove 5 ml of stock solution (b) and dilute to 100 ml as above. The standard solution now contains 5 µg/ml of methomyl oxime.

d. By further dilutions, prepare solutions containing 3, 2, and 1 µg/ml.

e. Store solutions at -5°C.

Apparatus

a. Varian Model 3700 Gas Chromatograph (Varian, Inc., Analytical Instruments Division, Palo Alto, California) equipped with a Varian flame photometric detector incorporating a 394 nm filter selective for sulfur-containing compounds, or equivalent.

b. Chromatographic glass column, 3 feet x 1/4 inch o.d., 2 mm i.d. containing 5 percent FFAP (Wilkins Instrument and Research, Inc., Walnut Creek, California) on 100-120 mesh high performance chromosorb W, acid washed and dimethyldichlorosilane treated (Applied Science Laboratories, Inc., State College, PA.).

NOTE: The inlet and exit ends of the column which extends into the injection port and detector port, respectively, are packed with glass wool to minimize exposure of the packing to high temperatures. The column should be conditioned for 48 hours at 225°C with carrier gas flowing. This conditioning can either be done in a separate oven specifically for this purpose or done in the instrument oven with the effluent disconnected from the detector.

Conditions -		
	Oven temperature	170°C
	Injector temperature	170°C
	Detector temperature	200°C
	He flow	22 cc/min
	Air flow	250 cc/min
	H_2 flow	140 cc/min
	Analysis time	about 3 minutes

c. Waring Laboratory Blendor (or equivalent) with an explosion-proof motor and a one quart glass jar equipped with a four-arm stainless steel blade.

d. Open column chromatography - 200 x 13 mm I.D. glass column equipped with a 2 mm Teflon stopcock and a 250 ml solvent reservoir.

Procedure

1. Weigh 50 grams of ginned cottonseed or soybean seed into a blender jar and grind to a meal. The addition of dry ice may be necessary for pulverizing. Add 280 ml of acetone and blend for 15 minutes. Vacuum filter the contents of the jar through Whatman No. 1 paper in a 9 cm Buchner funnel into a 1000 ml vacuum flask.

2. Transfer the filter cake from (1) back to the blender jar and extract again with 280 ml of acetone for 15 minutes. Filter as in (1) combining the filtrates. Wash the filter cake with 25 ml of acetone.

3. Transfer the combined filtrates into two 500 ml Erlenmeyer flasks, immerse into a 35°C water bath and evaporate to a volume of 25 ml using a gentle stream of air. Combine the contents of the two flasks, add 30 ml of acetonitrile, and evaporate to a 5 ml volume.

4. Remove the flask from the water bath and add acetonitrile until the volume is about 50 ml. Transfer to a separatory funnel and partition with 60 ml of iso-hexane. Transfer the lower layer to a second clean separatory funnel. Again partition with 60 ml of clean iso-hexane. Drain the lower layer into a 125 ml Erlenmeyer flask. Carefully evaporate just to dryness in a water bath as before. Discard the upper layers of iso-hexane.

5. To the residue add 25 ml of 2.5N aqueous sodium hydroxide and using a magnetic stirrer hot plate heat to 60°C for 30 minutes with stirring. Remove from bath and cool. Add 16 ml of 4N aqueous sulfuric acid, let stand two minutes with occasional swirling. Saturate mixture with sodium chloride (usually about 8 gm), transfer to a 250-ml separatory funnel and partition with 25 ml of methylene chloride. Drain the lower organic layer through a bed of anhydrous granular sodium sulfate (about 2 inches deep) supported in an 8 cm glass funnel with a plug of glass wool into a 250 ml Erlenmeyer flask. Repeat the extraction three additional times, combining the lower organic layers.

6. Evaporate just to dryness in a water bath at 35°C using a gentle stream of air. Remove from bath and dissolve the residue in 5 ml of Solvent Mixture I.

7. See the Apparatus Section (d) for description of column chromatography equipment. Add 6 inches of silica gel with a glass wool plug on both the top and bottom of gel. Add 25 ml of Solvent Mixture I to the column and discard the eluate.

8. Place a clean 125 ml Erlenmeyer flask under the column and deposit the sample in step 6 on the column and elute. Add an additional 105 ml of solvent mixture I using the standard chromatography practice of first rinsing the flask's contents into the column with small increments of the solvent.

9. As the sample from (8) is being evaporated, insoluble material forms in the flask. To alleviate this problem initially add 10 ml of acetone and continue evaporation. Intermittently, add acetone while evaporating to maintain a clear solution. The final residue should be almost completely soluble in 2 ml of acetone. Transfer this solution to a calibrated centrifuge tube. Rinse flask twice with one ml of acetone, transferring each time to the centrifuge tube.

10. Accurately inject 4 µl of the supernatant liquid into the chromatograph and quantitate the methomyl oxime by referring the peak height to a standard curve derived by injection of methomyl oxime standard. If the peak is off-scale, dilute further with a known amount of acetone until a measurable peak is obtained. If no peak is evident, the solution must be evaporated to 1.0 ml or less and a 4 µl sample of supernatant again injected. The response of sulfur is not linear with concentration, and the peak must be quantitation at the same attenuation used for the standards. The response of the instrument is subject to variation; therefore, the standard curves should be prepared daily or more often if necessary.

11. Calculation:

By comparing the peak heights to the standardization curve, the following calculation can be made.

$$\frac{\mu g \text{ of methomyl oxime X ml}}{0.59 \text{ X } 50} = \text{equivalents of UC 51762, ppm}$$

TABLE I

RECOVERIES OF UC 51762 RESIDUES FROM FORTIFIED COTTON AND SOYBEAN SEED

(50 gram sample)

Amount of Component Added		Methomyl Oxime Recovered		Methomyl Recovered		UC 51762 Recovered	
µg	ppm	µg	%	µg	%	µg	%
A. Cottonseed							
1	0.02					0.86	86
2	0.04	1.6	80				
5	0.1					4.3;4.2	85;84
10	0.2					9.5	95
100	1.0			87	87	83	83
B. Soybean seed							
2	0.04			1.56;1.56	78;78	1.52;1.69	76;85
5	0.10	1.4	73	3.7	74	3.8	76
10	0.20	8.0	80	7.7	77	7.6	76
100	2.0			92	92	85	85

TABLE II

EXTRACTION EFFICIENCY OF THE METHOD
FOR UC 51762 RESIDUES FROM COTTONSEED

(50 gm samples)

Extraction Number	Residues Found, ppm[b]	% of Total
Test No. 1 (field treated)		
1+2[a]	1.73	95.2
3	0.07	3.8
4	0.02	1.0
Test No. 2 (100 µg/ml of UC 51762)		
1+2[a]	83.0	99.0
3	0.76	1.0
4	<.02	0.0
Test No. 3 (100 µg/ml of Methomyl)		
1+2[a]	87.6	98.7
3	0.69	0.8
4	0.46	0.5

a) Represents combining the first two extracts as required by the residue method.

b) Residues are calculated as UC 51762.

FIGURE 1. TYPICAL CHROMATOGRAMS FOR

A - Untreated Control Sample of Cottonseed
B - Cottonseed fortified with 2.04 µg Methomyl Oxime
C - Methomyl Oxime Standard, 2.04 µg/ml

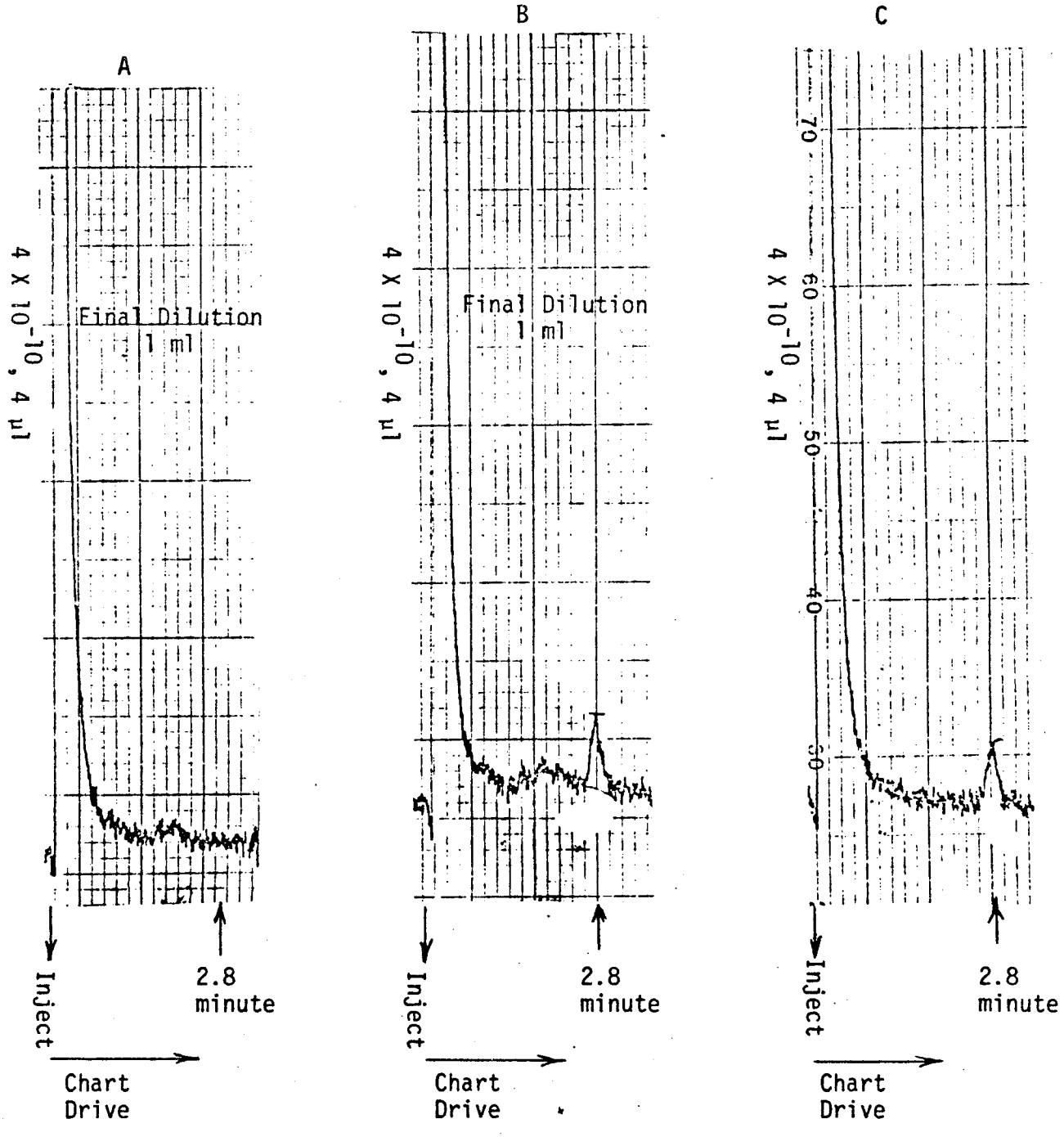


FIGURE 2. A TYPICAL CALIBRATION CURVE OBTAINED FROM INJECTION OF METHOMYL OXIME STANDARD SOLUTIONS

4 µl injected, attenuation 4 x 10^{-10}

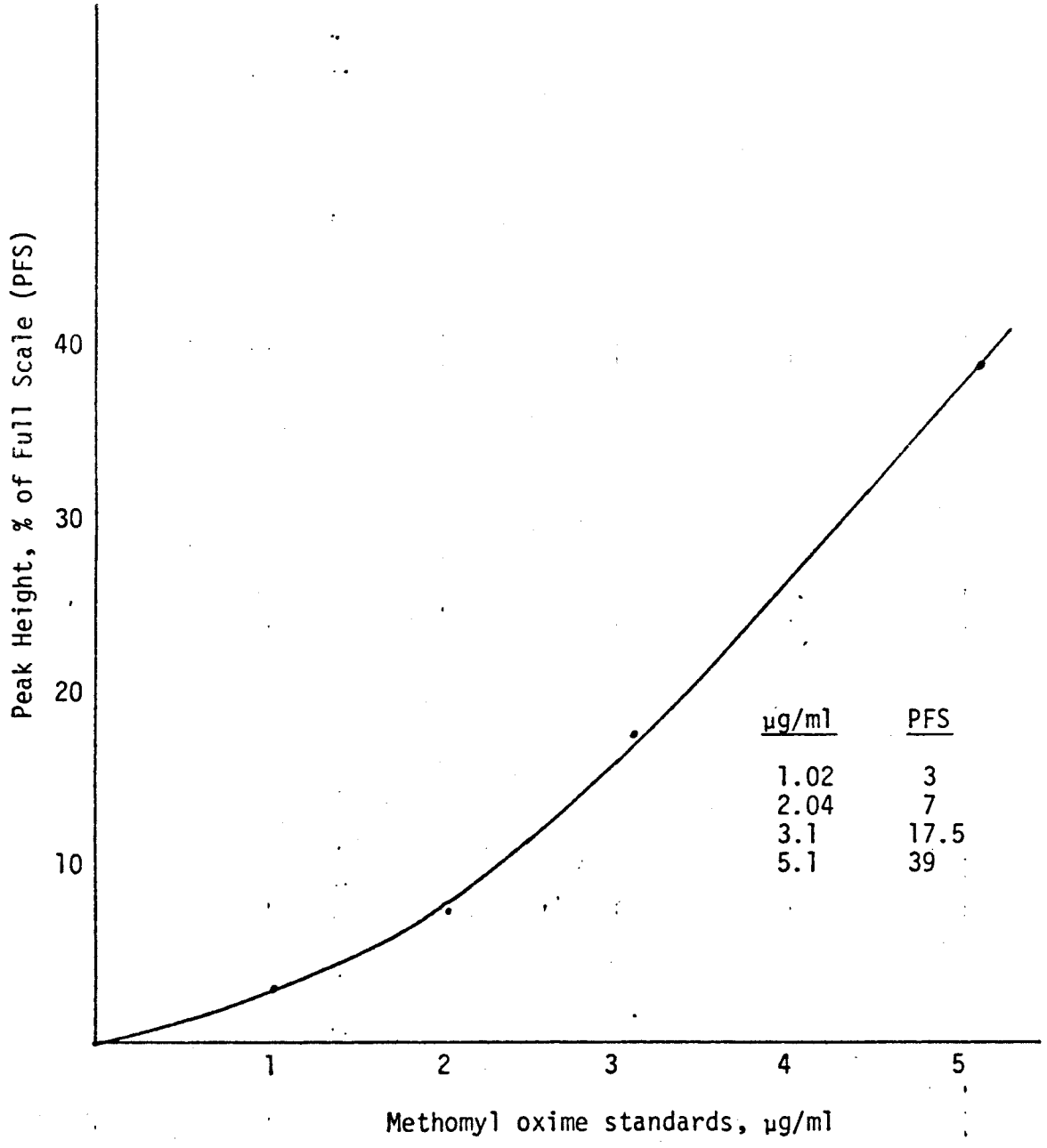
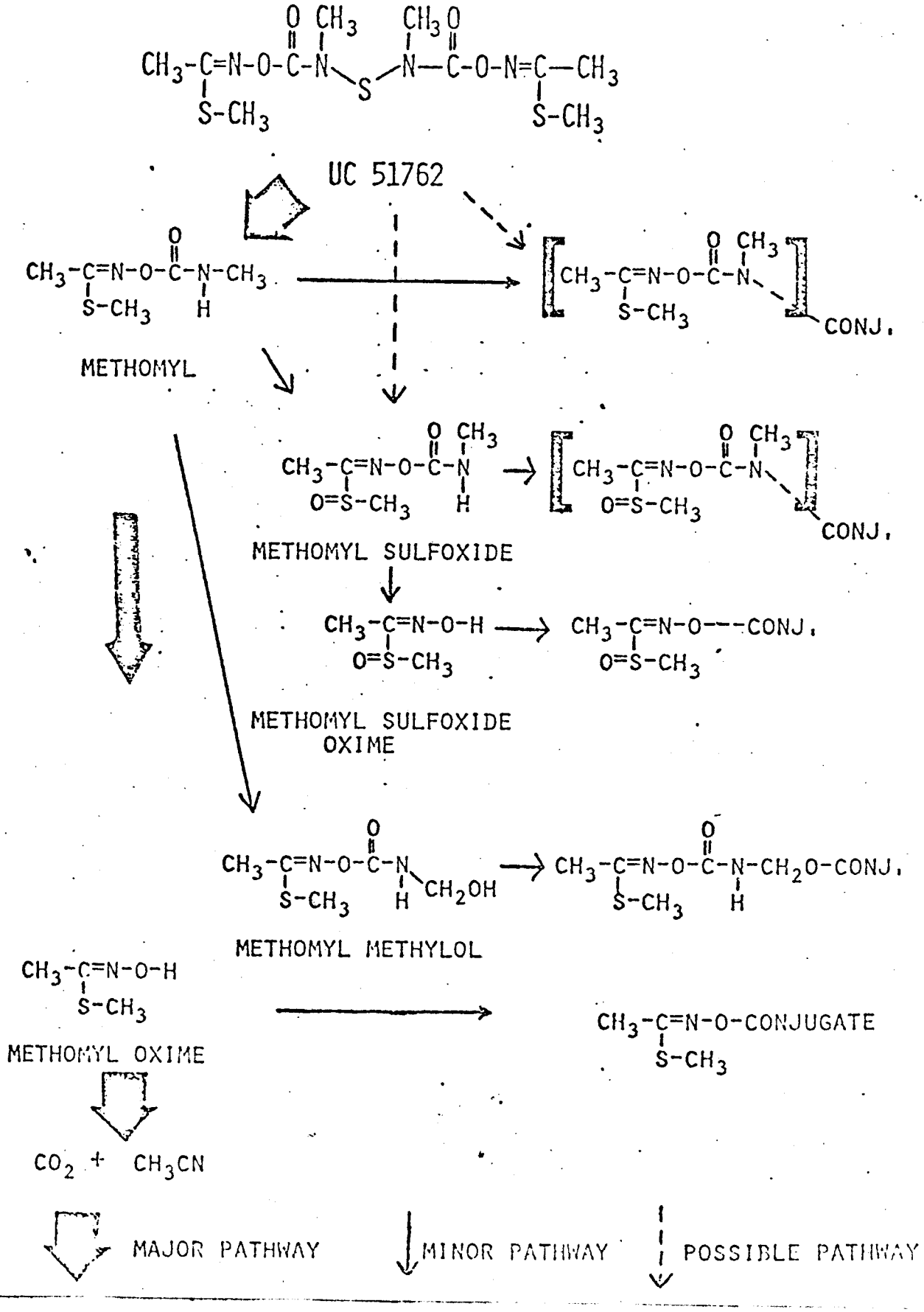


Figure 1.

Metabolic pathways of UC 51762 in plants



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A METHOD FOR THE DETERMINATION OF THIODICARB RESIDUES IN SOYBEAN SEED BY GAS CHROMATOGRAPHY

Method Designation: THIODICARB-FPD-SOYBEANS

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DETERMINATION OF THIODICARB RESIDUES IN SOYBEAN SEED

Thiodicarb = Dimethyl-N,N'-[thiobis[(methylimino)carbonyloxy]]bis[ethanimidothioate]

INTRODUCTION

Thiodicarb residues consist of thiodicarb and its degradation product, methomyl. Methomyl oxime is also quantitated by this procedure, but it is not a significant residue because it is relatively non-toxic and very low in concentration.

Thiodicarb and methomyl are hydrolyzed to methomyl oxime by heating in aqueous base. The total residue in the form of methomyl oxime is then extracted from the acidified aqueous sample with dichloromethane. After concentration and dissolution in acetone, the sample is analyzed by gas chromatography utilizing a flame photometric detector equipped with a filter specific for sulfur and quantitated by comparison with a standard curve.

The validity of the method was tested by fortifying samples of untreated seed with thiodicarb and methomyl and analyzing by the described procedure. Recoveries average 83 percent and sensitivity is 0.02 ppm. Details of recovery experiments for the fortified seed are shown in Table II.

Typical chromatograms for standard solutions, an untreated control, and seed containing residue are shown in Figure 1. Figure 2 is a typical calibration curve.

Laboratory experience has shown that the use of certain brands, grades, or lots of dichloromethane results in a loss of methomyl oxime. At the present time, the reason for the adverse reaction is not known. The available dichloromethane should be tested by the following procedure for any adverse reaction toward the oxime.

Test for acceptability of dichloromethane: Add 100 µg of methomyl oxime and one drop of ethylene glycol to 100 mL of the dichloromethane to be tested. Carefully evaporate the solution just to dryness with dry air and add 25 mL of acetone. Mix well and quantitate the oxime by GC as explained in the following method. A recovery of greater than 90 percent is acceptable and permits the use of the dichloromethane being tested.

Reagents

(a) Solvent Mixture I, 90/10 - acetone/methanol (v/v)

(b) Acetonitrile, analytical grade

(c) Hexane, analytical grade

(d) Ethylene glycol, analytical grade (used as "keeper")

(e) Sodium hydroxide, 2.5 N aqueous (100 g of sodium hydroxide per liter of solution)

(f) Hydrochloric acid, concentrated

(g) Potassium phosphate, monobasic, analytical reagent

(h) Sodium chloride, analytical grade

(i) Dichloromethane (tested for acceptability)

(j) Sodium sulfate, anhydrous, granular

Standard Solutions

(a) Weigh 0.1000 g of methomyl oxime into a 100 mL volumetric flask, dilute to the mark with acetone and shake until solution is complete. Concentration of standard is 1000 µg/mL.

(b) Withdraw a 10 mL aliquot with a pipet and dilute to 100 mL with acetone in a second volumetric flask. Concentration of standard is 100 µg/mL.

(c) Remove 5 mL of stock solution (b) and dilute to 100 mL as above. The standard solution now contains 5 µg/mL of methomyl oxime.

(d) By further dilutions, prepare solutions containing 3, 2, and 1 µg/mL.

(e) Store standards refrigerated when not in use.

NOTE: This mode of preparation of standards is described only as a guide; any other valid procedure is satisfactory.

Apparatus

(a) Hewlett Packard 5840A Gas Chromatograph (Hewlett Packard, Avondale, PA) or the equivalent, equipped with a flame photometric detector incorporating a 394 nm filter selective for sulfur containing compounds. See Table I for chromatographic conditions.

(b) Super Dispax, SD-45N homogenizer with a G302 generator and Tekmar Power Controller, Model TR-10.

(c) Model 186 Precision Water Bath (GCA Corporation, Chicago, IL) or the equivalent.

(d) Magne-4 four-unit magnetic stirrer with hot plate (Cole Parmer Instrument Co., Chicago, IL) or the equivalent.

(e) Centrifuge, Beckman, J-21C (or equivalent).

Procedure

1. Pregrind the seed to a fine meal in a Waring blender. Weigh 50 g into an 800 mL plastic beaker, add 225 mL of solvent Mixture I and extract at medium speed for 60 seconds with the Tekmar homogenizer. (Adjust the generator for maximum grinding by immersing the head to where all material is agitated from the bottom of the container.)

2. Vacuum filter the contents of the beaker through Whatman No. 1 paper (or equivalent) in a 9 cm Buchner funnel into a 500 mL flask. Return filter cake to the beaker and repeat the extraction and filtration. Rinse the beaker with 50 mL of solvent Mixture I and use as a wash for the filter cake.

3. Transfer the combined filtrate into two 500 mL Erlenmeyer flasks (for quicker evaporation), add two drops of ethylene glycol as keeper to each. Set the flasks in a warm water bath (35-40°C) and evaporate to about 25 mL using a gentle stream of air. Combine the filtrates, add 25 mL of acetonitrile and again evaporate to about 5 mL (to ensure all acetone is gone).

4. Add 45 mL of acetonitrile. Swirl to mix, and transfer to a 250 mL separatory funnel. Add 75 mL of hexane and shake for 30 seconds. Allow layers to separate and discard the top hexane layer. Repeat this clean-up step with an additional 75 mL of hexane, again discarding the hexane. Drain the lower layer into a 250 mL Erlenmeyer flask and add two drops of ethylene glycol. Set the flask in a warm water bath and evaporate just to dryness with a gentle stream of air.

5. To the residue remaining in the flask, add 25 mL of 2.5N aqueous sodium hydroxide and a 2 inch magnetic stirring bar. Set the flask in a water bath positioned on a Magne-4 stirrer-hot plate. Stir and heat for 45 minutes at 60°C.

6. Remove the flask and cool to about 20°C in an ice bath, positioned on a magnetic stirrer. While the solution is being stirred, add 5.0 mL of concentrated hydrochloric acid dropwise, then add 4 g of potassium phosphate monobasic (the pH of the solution should now be buffered between 5 and 6). Saturate the contents of the flask with 10 g of sodium chloride and mix for an additional minute.

7. Transfer the solution to a 250 mL separatory funnel and partition with 50 mL of methylene chloride by shaking gently for 30 seconds, venting to release pressure as required. Drain the bottom organic layer through a bed of anhydrous granular sodium sulfate (supported in a 9 cm funnel by a plug of glass wool). Repeat with an additional 50 mL methylene chloride and then twice with 25 mL quantities. Wash the sodium sulfate with 25 mL of methylene chloride and add two drops of ethylene glycol.
   NOTE: To avoid any degradation of the pesticide, the complete hydrolysis procedure should be completed on the same day.

8. Set the flask in a warm water bath (35-40°C) and evaporate just to dryness with a gentle stream of air.

9. Dissolve the residue in the flask in a measured volume of acetone. Inject exactly 4 µL into the chromatograph. Quantitate the methomyl oxime by reference to a previously prepared calibration curve.
   NOTE: Store all sample solutions in a refrigerator or freezer when work stops for the day. This storage should be limited to three days.

10. Determine the thiodicarb equivalents in the original sample using the following values and equation:

$$\text{ppm thiodicarb} = (a \times b)/(c \times d)$$

where:
- a = concentration of methomyl oxime in final solution, µg/ml (from calibration curve)
- b = final dilution in mL
- c = sample size in g
- d = 0.59, a molecular weight conversion factor from methomyl oxime to thiodicarb (210 µg MO/354 µg thiodicarb)

FIGURE 1

TYPICAL CHROMATOGRAMS

A Methomyl Oxime Standard, 0.25 µg/mL

B Methomyl Oxime Standard, 0.75 µg/mL

C Untreated Soybean Seed

D Treated Soybean Seed Containing 0.02 ppm Thiodicarb Residues

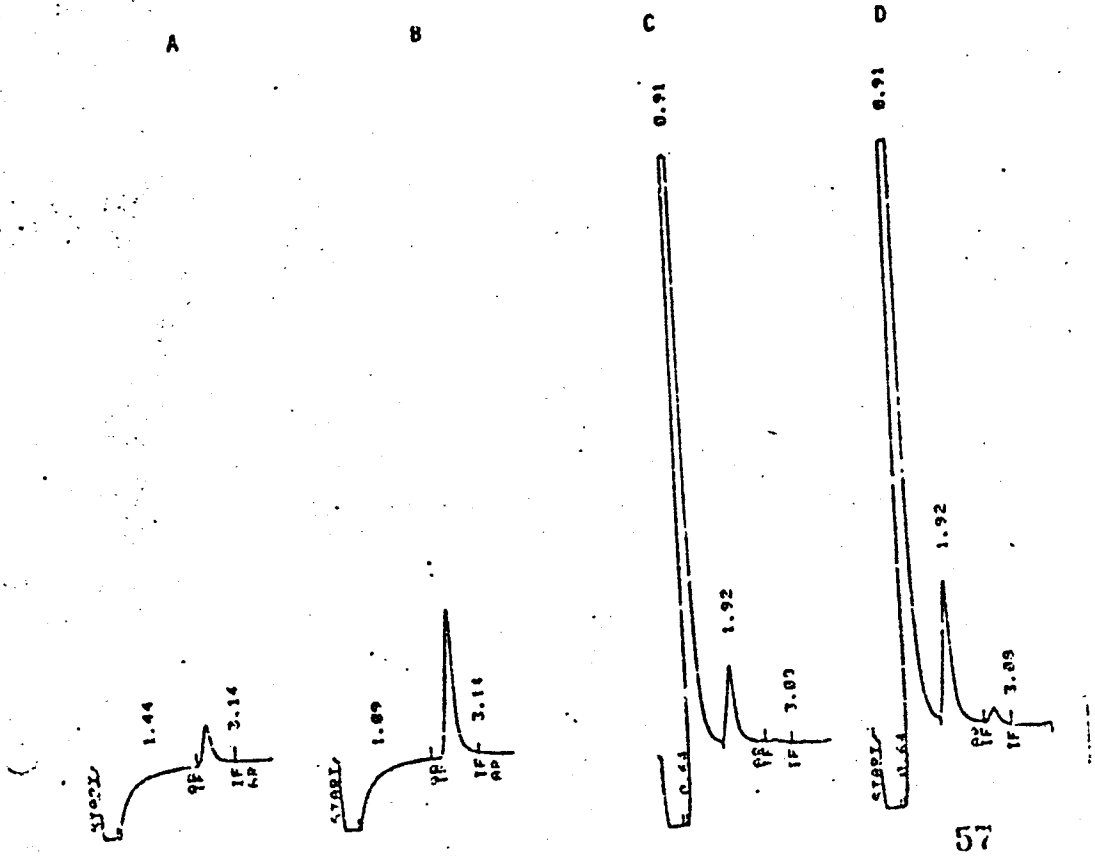
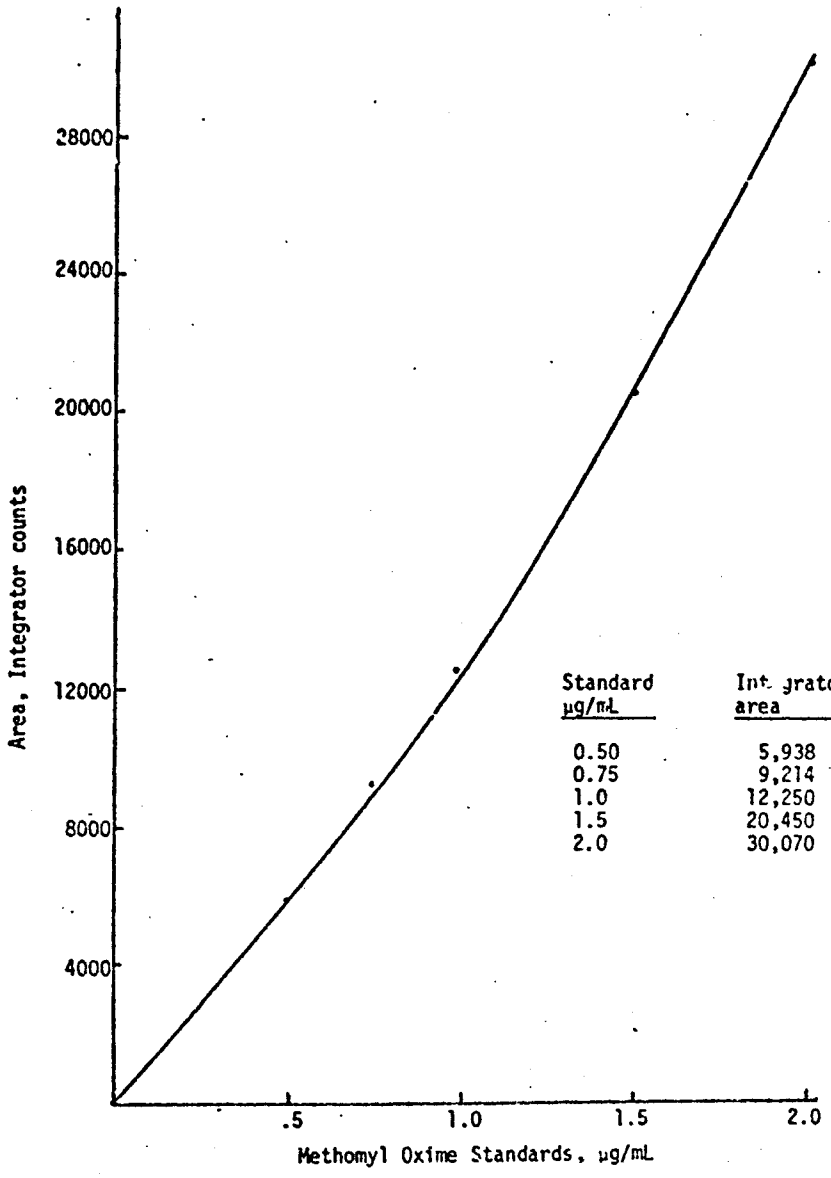


FIGURE 2. TYPICAL CALIBRATION CURVE OBTAINED FROM INJECTION OF METHOMYL OXIME STANDARD SOLUTIONS



Standard µg/mL	Integrator area
0.50	5,938
0.75	9,214
1.0	12,250
1.5	20,450
2.0	30,070

TABLE 1

EXPERIMENTAL CHROMATOGRAPHIC CONDITIONS FOR THE HEWLETT PACKARD 5840A GAS CHROMATOGRAPH AND THE COLUMN USED

column temperature, °C	175
injector temperature, °C	185
detector temperature, °C	225
gas flow rates, mL/min	
helium	20
hydrogen	48
oxygen	8
air	50
injection volume, µL	4
attenuation	2^4
glass column	
length, cm	100
inner diameter, mm	2
packing	
stationary phase	5% SP-1000
support	Supelcoport 100/120
aged	
time, h	48
temperature, °C	250

TABLE II

RECOVERIES OF THIODICARB AND METHOMYL FROM FORTIFIED SOYBEAN SEED

(50 gram samples)

AMOUNT OF COMPONENT ADDED		METHOMYL RECOVERED		THIODICARB RECOVERED	
µg	ppm	µg	%	µg	%
1.0	0.02	-	-	0.92	92
		-	-	0.93	93
		-	-	0.84	84
		-	-	0.93	93
2.0	0.04	1.6	80	1.5	75
		1.6	80	1.7	85
		-	-	1.5	75
5.0	0.10	3.7	74	3.8	76
10	0.20	7.7	77	7.6	76
		9.2	92	8.2	82
		8.5	85	8.5	85
		-	-	7.3	73
		-	-	8.3	83
100	2.0	92	92	85	85
		Average	83%		83%