Gas Chromatographic Determination of Metalaxyl in Soils and Sunflower

A gas–liquid chromatographic method using a nitrogen-selective detector for the determination of Metalaxyl in soils and sunflower foliage is described. The best and most consistent recovery rates were obtained when acetonitrile was used as the solvent for extracting the fungicide from soils. Sweep codistillation as a purification process for routine determination has yielded good results. Recoveries of Metalaxyl varied for soils from 94% to 106% in the 0.1–5-ppm range and for sunflower foliage from 89% to 105% in the 0.05–5-ppm range. The sensitivity was such that Metalaxyl could be detected down to a level of 0.05 ppm.

Metalaxyl [DL-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl)alanine methyl ester], as Ridomil (Ciba Geigy), is a fungicide registered for use on a variety of vegetables (Urech et al., 1977; Garibaldi and Morando, 1978; De Baets, 1979; Cohen et al., 1979; Bor and Wiertsema, 1979). As a result of its wide usage, a need has arisen to monitor plant materials and soils for residues of the fungicide. At the present time the only available method is that of the manufacturers (Ramsteiner, 1976), in which Metalaxyl, after extraction with methanol, and cleanup by water–methanol/dichloromethane partitioning and alumina column chromatography, is determined by gas–liquid chromatography using alkali flame ionization or Coulson electrolytic conductivity detection. The limit of detection is 0.05 ppm, and recoveries from fortified soil, potatoes, beans and grapes are 98% with an absolute standard deviation of ±12% in the 0.05–0.5-ppm range.

In our preliminary trials this method gave low recoveries when applied to soils. Moreover, the procedure was not rapid enough to allow a large number of samples to be analyzed.

Therefore, the purpose of the present paper is 2-fold. First, it presents results of our efforts toward establishing a satisfactory procedure for extracting the fungicide from soils. Second, we wanted to shorten the time required for the analysis, which involved a too long cleanup procedure.

EXPERIMENTAL SECTION

Apparatus. A Perkin-Elmer Model 900 gas chromatograph equipped with a nitrogen-selective detector and a Hitachi Perkin-Elmer 196 5-mV recorder was employed. A glass column, 2 m × 6 mm, packed with 1.5% cyclohexanedimethanol succinate on 80–100-mesh Gas-Chrom Q was used at 190 °C. General operating conditions were as follows: carrier gas, helium; flow rate, 25 mL/min; hydrogen and air flow rates, 5 and 90 mL/min; injector temperature, 240 °C; chart speed, 5 mm/min.

Reagents. All organic solvents were of "pure grade" and were distilled in glass before use. Celite 545 was obtained from BDH Chemicals, Poole, U.K.; silica gel RS (0.05–0.20-mm i.d.) was from C. Erba, Milan, Italy.

Extraction. (*a*) *From Soil.* Air-dried soil (100 g) was passed through a 1.00-mm sieve, placed into a preextracted thimble, and covered with a plug of preextracted glass wool. Soil and thimble were placed in the Soxhlet extraction tube. The boiling flask was charged with 3 or 4 beads and 150–200 mL of acetonitrile. The Soxhlet apparatus was assembled, placed on a heater, and allowed to cycle for 6 h, after the first condensation occurred, at the rate of three to four exchanges per hour. At the end, when the volume of the solvent in the extraction flask was at a minimum, the extraction flask was removed from the extraction apparatus. After the addition of 1 g of Celite (Thornburg, 1963), the acetonitrile extract was evaporated to dryness in a rotary evaporator at 45 °C. The residue was taken up in three (20-mL) portions of ethyl acetate and filtered. The filtrate was concentrated to 1–2 mL.

(*b*) *From Vegetable Matter.* Chopped vegetable matter (20 g) was blended with 100 mL of methanol in a Sorvall omnimixer at medium speed (setting 5) for 5 min. The supernatant was filtered into a 250-mL flask. The blender jar and the residue were rinsed with methanol (3 × 30 mL) and the washings were combined into the flask. The methanol extracts were evaporated to dryness in a rotary evaporator at 45 °C. The residue was taken up in ethyl acetate (100 mL) and, after adding 1 g of Celite, evaporated to dryness. This last process was then repeated twice to remove the water completely. The extraction residue was taken up in three (20-mL) portion of ethyl acetate and filtered through a G3 fritted glass filter (3.5 cm i.d.) in which 2 g of Celite were stratified. The filtrate was concentrated to 2–3 mL by using a rotary evaporator at 40 °C.

Cleanup Procedure. Soil or vegetable matter extract was injected, 0.3 mL each time at 1-min intervals by using nitrogen as the carrier gas at a flow rate of 500 mL/min, onto a column of silanized glass beads (Kontes Co., Vineland, NJ) heated to 200 °C in a Sweep Co-Distiller K 500 750 (Kontes Co., Vineland, NJ) (Figure 1). The volatilized solvent containing the pesticide, which had been separated from nonvolatile constituents, was condensed in a coil immersed in a bath at 0 °C and then passed through a silica gel column (3 cm × 5 mm). Three to four milliliters of ethyl acetate was injected to elute Metalaxyl from the column. The eluate was concentrated in the graduated concentrator tube to 0.1–1 mL by using a gentle stream of nitrogen. Aliquots of this final solution (0.5–1 μL) were injected into the gas chromatograph.

Table I. Properties of the Soils

	soil A	soil B
pH (H_2O)	7.7	7.5
organic matter, %	4.4	11.1
mechanical analysis, g/kg		
sand	486	818
silt	357	64
clay	157	118

RESULTS AND DISCUSSION

Recoveries were conducted by adding known amounts of Metalaxyl in solvent to untreated soils and fresh sunflower foliage prior to the addition of the extraction solvent. The solvent was evaporated, and the samples were then analyzed as described above. Metalaxyl was estimated from a standard linear calibration curve constructed by plotting from 5 to 100 ng of the fungicide against peak area and controlled daily.

The chemical and physical properties of the A (typic *Eutrochrept*) and B (*Mollic psammaquent*) soils used in our experiments are given in Table I. Acetonitrile and methanol were both examined for their capacity to extract Metalaxyl from soils, but because acetonitrile consistently extracted more Metalaxyl from the soils, the use of

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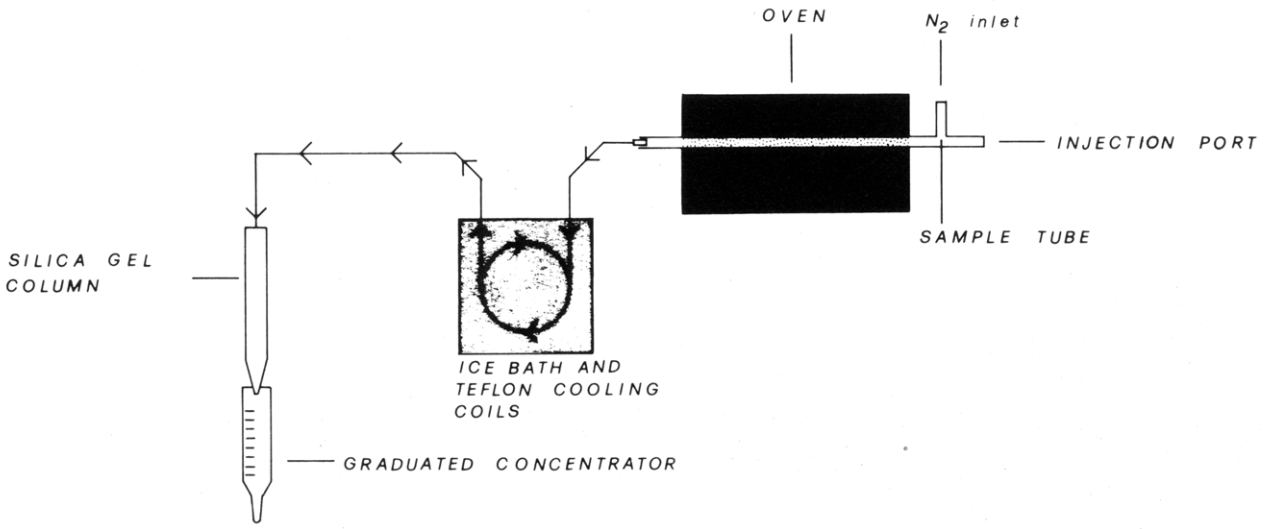

Figure 1. Sweep codistillation apparatus.

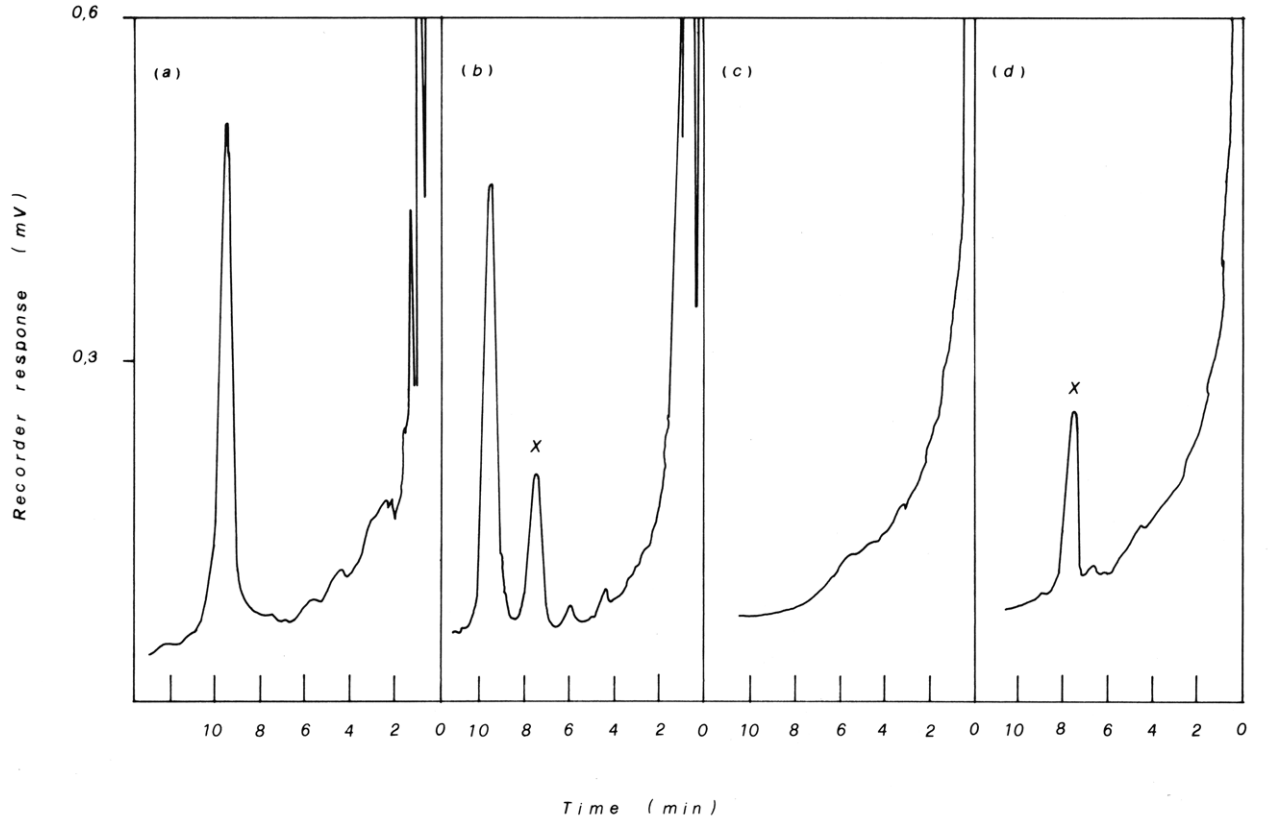

Figure 2. Chromatograms of sunflower and soil extracts concentrated to 0.2 and 1.0 mL. 1-μL samples were injected. (a) Unfortified control sunflower; (b) sunflower fortified at 0.2 ppm; (c) unfortified control soil; (d) soil fortified at 0.2 ppm of Metalaxyl (peak X).

Table II. Recovery Studies of Metalaxyl Added to Soils

	recovery from soils, %[a]			
	soil A		soil B	
metalaxyl added, ppm	hot extraction with methanol	hot extraction with acetonitrile	hot extraction with methanol	hot extraction with acetonitrile
0.1	81 ± 3.0 (4)	92 ± 2.6 (4)	70 ± 2.8 (4)	103 ± 1.9 (5)
0.3	86 ± 3.3 (3)	102 ± 2.1 (5)	80 ± 2.6 (4)	106 ± 3.1 (3)
1.0	80 ± 3.2 (3)	98 ± 2.3 (3)	75 ± 3.2 (3)	95 ± 2.3 (4)
5.0	84 ± 2.9 (4)	94 ± 2.5 (4)	79 ± 3.4 (3)	97 ± 2.9 (3)

[a] Mean ± standard error (number of determinations in parentheses).

methanol was discontinued (Table II). The poor performance of methanol is probably due to the fact that the organic matter in our soils may have been higher than in that reported by Ramsteiner (1976). Indeed, when methanol was used, recoveries decreased from soil A to soil B, as the organic matter content increased from 4.4 to 11.1%. Exhaustive Soxhlet extraction by acetonitrile improved recoveries by 14–24% on samples of soils with a

Table III. Mean Recoveries of Metalaxyl Added to Sunflower Foliage before Extraction

metalaxyl added, ppm	recovery from sunflower foliage, %[a]
0.05	89 ± 2.2 (5)
0.1	98 ± 2.5 (5)
0.2	103 ± 3.1 (4)
1.0	105 ± 1.9 (5)
5.0	91 ± 2.9 (4)

[a] Mean ± standard error (number of determinations in parentheses).

fair content of organic matter.

Sweep codistillation as a purification process for routine determination has yielded good results. With regard to the lifetime of silanized glass bead column, we find that it can be used for three sunflower foliage analyses or for ten soil analyses. The silica gel microcolumn has been used successfully to clean up Metalaxyl extracts from soils and sunflower foliage. The efficiency of the analytical methods for sunflower foliage is indicated in Table III by the recovery of the fungicide added to untreated samples.

When the chromatograms of the numerous blanks tested are taken into account (five from soils; seven from sunflower foliage), the limit of sensitivity was 0.05 ppm. Typical chromatograms obtained by injecting extracts of fortified samples are shown in Figure 2.

As the operator need not be present during the 6 h of Soxhlet extraction of Metalaxyl from the soil, this method is less time consuming than the Ramsteiner (1976) procedure.

The most significant advantage of the method presented here is that it is simple, straightforward, and reproducible.

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Hydrolysis of High Levels of Naringin in Grapefruit Juice Using a Hollow Fiber Naringinase Reactor

Grapefruit juice that has excessive naringin bitterness is generally unmarketable. This paper describes a procedure for reducing the naringin content of grapefruit juice containing very high levels of naringin using a commercially available naringinase restrained in a hollow fiber reactor. Juice containing 885 μg/mL naringin was debittered to a level acceptable to a sensory evaluation panel sensitive to bitterness. Single-strength unclarified juice from a frozen concentrate was recycled through the hollow fiber reactor at temperatures up to 45 °C to achieve different levels of naringin hydrolysis. Parameters affecting naringin hydrolysis to prunin and naringenin such as flow rates, hollow fiber membrane surface area, temperature, and enzyme loadings were investigated in order to improve debittering rates. The 4-ppm limonin content of the juice did not contribute to the excessive bitterness and was found to be unchanged by the debittering process. Juice containing 885 μg/mL naringin could be debittered to 285 μg/mL at a rate of 14 mL/min at 45 °C, with no adverse effects from the particulate matter in the juice.

Grapefruit juices with high levels of naringin are extremely bitter, and a method of lowering the naringin concentration to a more acceptable level would permit the blending of these juices into a product without excessive bitterness. This is dependent upon a suitably low level of limonin (Guadagni et al., 1973, 1974), which at normal levels contributes a small amount to total grapefruit bitterness.

The use of hollow fiber immobilized naringinase (from *Aspergillus niger*) has been shown to be a feasible method for reducing the naringin content of grapefruit juice from 290 μg/mL to any desired lower concentration (Olson et al., 1979). In this work, juice with an extremely high, almost 900 μg/mL, naringin level was used to explore debittering parameters. In addition, the objective was to obtain the highest debittering rate possible and to determine the acceptability of the treated juice.

EXPERIMENTAL SECTION

Hollow Fiber. The Romicon HF 1.1-43-PM10 hollow fiber (HF) cartridge, manifolds, and pumping system for flow rates up to 1.0 L/min used in this work are the same as those reported previously (Olson et al., 1979). Plumbing was entirely Eastman semirigid EVA-66 food-grade tubing. The large (up to 10.0 L/min) pumping capacity unit used was the Romicon HFXS MKII Ultrafiltration System incorporating the PM10 HF cartridge. This system employed two centrifugal pumps: a $^3/_4$-hp stainless steel