Gas Chromatographic Determination of Metalaxyl in Lettuce

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Two methods are described. In method I lettuce extracts are directly injected into a gas chromatograph containing a SIL 5 and a BP 10 column and two nitrogen-selective detectors. A twin-hole ferrule holds the inlets of both columns. In this construction one injection is split between the two columns, saving time and autoinjector equipment. Method II is based on the same chromatographic system but includes a cleanup step with SEP-PAK silica cartridges. Methods I and II yield recoveries of 88–108% and 74–98%, respectively, in the 0.05–0.5 mg/kg range. The detection limit of metalaxyl with methods I and II is 0.04 and 0.01 mg/kg, respectively. Comparison of both methods applied to field-treated samples yields a correlation coefficient of 0.9996 for 23 observations. Method I takes less time but is slightly less specific than method II.

Metalaxyl [methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-alaninate] is a systemic fungicide developed by Ciba-Geigy. It is recommended for use on lettuce against *Bremia lactucae*. In The Netherlands there is a maximum residue limit of 0.1 mg of metalaxyl/kg of lettuce. In the Federal Republic of Germany, a main importer of Dutch vegetables, there should be no detectable trace of metalaxyl in lettuce. To ensure exportability of lettuce, there is a need for a rapid analytical method suitable for monitoring metalaxyl in large series of samples.

Metalaxyl residues have been determined by several workers by different techniques. Singh and Tripathi (1980) developed a thin-layer chromatographic method to estimate metalaxyl residues. However thin-layer chromatography is a semiquantitative technique. Newsome (1985) described an enzyme-linked immunosorbent assay (ELISA) for residues of metalaxyl in food. This method does not need a sample cleanup and is suitable for analysis of large series. However, Newsome reported that metalaxyl cross-reacted with the herbicides metolachlor and diethatyl ethyl and to a lesser extent with the fungicide furalaxyl.

Several workers have determined crop residues by gas chromatography (Waliszewski and Szymsczynski, 1983; Caverly and Unwin, 1981; Tafuri et al., 1981; Speck and Dirr, 1980; Ernst et al., 1984). However, they used methods containing a rather time-consuming cleanup procedure, and none of them used a second column for identification.

Our purpose was to develop a rapid, specific, and quantitative method for the gas chromatographic determination of metalaxyl in lettuce. Separation and detection were done in two columns with different polarity, which were connected with the same autoinjector and were fitted with a nitrogen-selective detector.

Sample preparation was carried out in two ways. In method I lettuce was directly injected into the gas chromatograph after extraction, and in method II the extracts were first cleaned by solid-phase extraction. The methods have been validated by recovery experiments and compared by analyzing several field-treated samples by both methods.

EXPERIMENTAL SECTION

Reagents. All reagents were of analytical quality. Ethyl acetate was obtained from Merck (Darmstadt) and hexane from Promochem (Wesel). The solid-phase extraction (method II) was done with SEP-PAK silica cartridges

obtained from Waters Associates. Metalaxyl standard solutions of about 0.05 and 0.5 mg/L were prepared by diluting aliquots of a stock standard solution of metalaxyl (about 1 g/L) in ethyl acetate. The standard solutions were stored in the dark at 4 °C. The standard solutions contained diethatyl ethyl, obtained from Hercules (Wilmington), as internal standard at concentrations of 0.5 mg/L for method I and 2.5 mg/L for method II.

Extraction Procedure. Lettuce was chopped with a cutting machine (Stephan). Exactly 50 g of chopped lettuce was weighed into a 250-mL centrifuge tube. For method I, exactly 100 mL of ethyl acetate containing 0.5 mg/L diethatyl ethyl was added. The mixture was macerated with an Ultra Turrax mixer (Janke & Kunkel) for 1 min. A 10-μL aliquot of the lettuce extract was injected into the gas chromatograph. For method II, exactly 100 mL of ethyl acetate was added to the centrifuge tube containing 50 g of chopped lettuce. The mixture was macerated with an Ultra Turrax for 1 min. The lettuce extract was centrifuged at 1000 rpm for 1 min. Exactly 20 mL of the supernatant was transferred to a round-bottom flask and evaporated to dryness on a rotary evaporator (Buchi 011) at 40 °C. The residue was resolved in 1 mL of 30% ethyl acetate in hexane and added to SEP-PAK silica cartridges that had been flushed with 4 mL of the same solvent mixture. The round-bottom flask was washed two times with 1 mL of 30% ethyl acetate in hexane, and the contents of the flask were added to the cartridge. The cartridge was eluted first with 4 mL and then with 10 mL of 30% ethyl acetate in hexane. The latter fraction was evaporated to dryness on a rotary evaporator and the residue resolved in 1 mL of ethyl acetate containing 2.5 mg/L diethatyl ethyl. Of this solution 2 μL was injected in the gas chromatograph.

GC Analysis. GC analyses were carried out on a Carlo Erba HRGC 5300 fitted with two N–P detectors and a splitless capillary injector with autosampler. A twin-hole ferrule connected the injector with two columns: CP-SIL-5 CB, fused silica (12.5 m × 0.22 mm, 0.41-μm film thickness). Helium was supplied as carrier gas to each column at a flow of 3 mL/min. Flows of helium makeup gas, air, and hydrogen to each detector were 35, 350, and 35 mL/min respectively. The oven temperature course was programmed from 100 °C (with 1-min hold) to 180 °C at 20 °C/min and from 180 to 260 °C with 10 °C/min (with 2-min hold at 260 °C). The injector and the detector were maintained at 210 and 280 °C, respectively. Chromatograms were displayed on a Yokogawa recorder. Quantitation was performed by comparing sample peak heights with those obtained for standard solutions. The N–P

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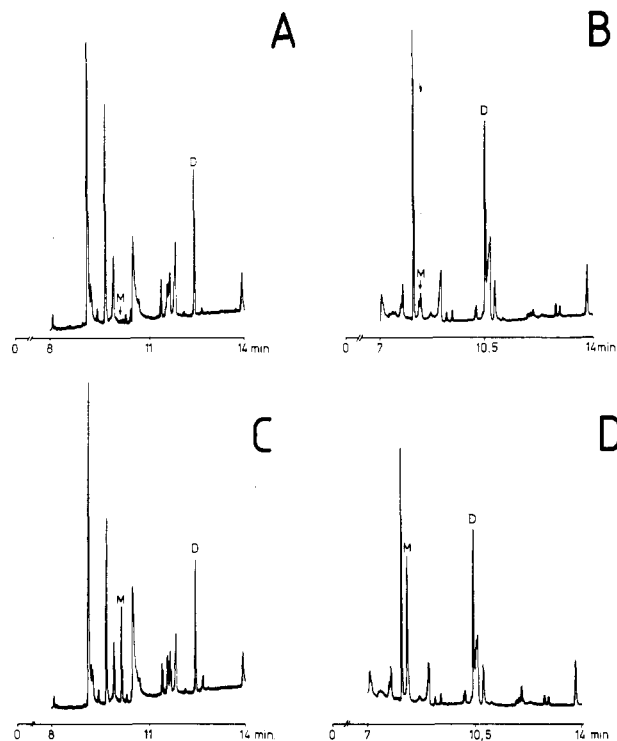
GC Determination of Metalaxyl In Lettuce

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Figure 1. Chromatograms of uncleaned lettuce extracts (obtained with method I) on two columns. Conditions: 10-μL samples were injected; D = diethatyl ethyl (0.5 mg/L); M = metalaxyl. Key: A, unfortified control lettuce on a BP 10 column; B, same on a SIL 5 column; C, lettuce fortified with 0.5 mg of metalaxyl/kg on a BP 10 column [metalaxyl peak represents an injected amount of 1.25 ng of metalaxyl]; D, same on a SIL 5 column.

Table I. Recoveries of Metalaxyl Added to Chopped Lettuce (mg/kg) Estimated with Method I

metalaxyl added	BP 10 column			SIL 5 column		
	control	spiked	% rec[a]	control	spiked	% rec
0.050	–[b]	0.054	108	–	0.044	88
0.050	–	0.049	98	–	0.049	98
0.050	–	0.054	108	–	0.046	92
0.050	–	0.052	104	–	0.049	98
av ± SD			105 ± 4.7			94 ± 4.9
0.50	–	0.52	104	0.070	0.55	96
0.50	–	0.50	100	0.063	0.56	99
0.50	–	0.48	96	0.11	0.59	96
0.50	–	0.49	98	0.075	0.60	105
av ± SD			99.5 ± 3.4			99 ± 4.2

[a] Difference between the amounts found in spiked and control samples, expressed as a percentage of the amount of metalaxyl added. [b] Below detection limit (0.04 mg/kg).
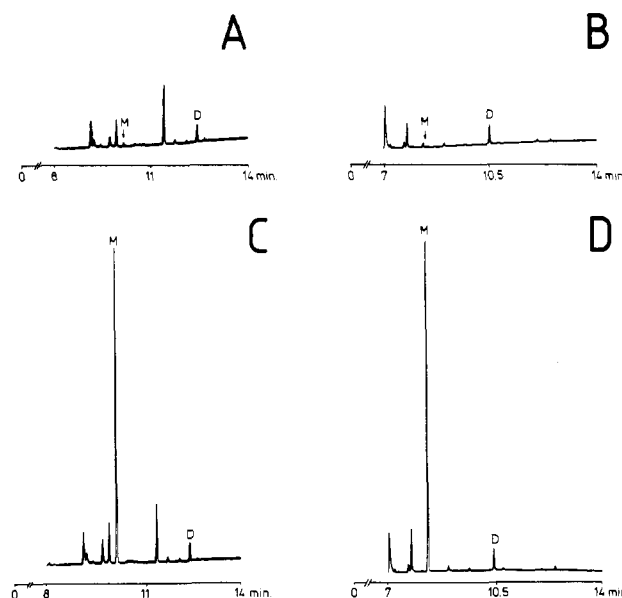

Figure 2. Chromatograms of cleaned lettuce extracts (obtained with method II) on two columns. Conditions: 2-μL samples were injected; D = diethatyl ethyl (0.5 mg/L); M = metalaxyl. Key: A, unfortified control lettuce on a BP 10 column; B, same on a SIL 5 column; C, lettuce fortified with 0.5 mg of metalaxyl/kg on a BP 10 column [metalaxyl peak represents an injected amount of 5 ng of metalaxyl]; D, same on a SIL 5 column.

detector was linear over the range of 0.05–10 ng/injection. Internal standard was added to correct for injected amounts and/or retention shifts.

Recovery Experiments. Thirty-two portions of 50 g of untreated chopped lettuce each were weighed into centrifuge tubes. Sixteen portions were kept untreated. To eight portions were added 220-μL aliquots of metalaxyl standard solutions (11.3 mg/L) with a microsyringe (Hamilton), corresponding to 0.05 mg/kg. To the remaining eight portions were added 220-μL aliquots of metalaxyl standard solutions (113 mg/L) corresponding to 0.5 mg/kg. Next, for both methods 100 mL of ethyl acetate was added after which the samples were analyzed as described above. Recoveries (%) were calculated as the difference between the amounts of metalaxyl found in the spiked and in the nonspiked samples expressed as a percentage of the amount of metalaxyl added.

RESULTS AND DISCUSSION

Figure 1 shows chromatograms of uncleaned lettuce extracts with and without metalaxyl. Peaks could be identified by comparing the relative retention times of peaks in the sample chromatograms with those in the standard chromatograms. Thanks to a twin-hole ferrule that divides the amounts injected equally over two columns, the metalaxyl peak can be confirmed in one run, so that analysis time can be reduced substantially. The detection limit, defined as 3 times the base-line noise was estimated to be 0.10 ng/injection, which corresponds to a metalaxyl content of 0.04 mg/kg. Separation on the SIL 5 column gave matrix peaks interfering with those of diethatyl ethyl and metalaxyl (Figure 1B,D). After subtraction of the nonspiked level from the spiked level, the results are still quantitative (Table I). For field-treated samples analyzed with method I, the SIL 5 column was used for identification only. Quantification was done with

the BP 10 column, where no matrix peaks interfering with those of diethatyl ethyl and metalaxyl were observed.

Although much plant residue is injected with method I it appears from experiments that hundreds of injections can be done before the columns deteriorate. Then the column performance can be restored by cutting approximately 15 cm of the front end of the column.

Figure 2 shows chromatograms with less peaks from the matrix and a higher metalaxyl peak, as a result of cleanup and concentration (method II). Solid-phase extraction with SEP-PAK silica cartridges yields satisfactory results. Only one elution mixture (30% ethyl acetate in hexane) is needed. Amounts of chemicals and labor needed are less than with self-made columns. We did not observe any overlapping peaks. Quantification was done by calculating the average of the results obtained with both columns. The detection limit was estimated to be 0.025 ng/injection or 0.01 mg of metalaxyl/kg.

The experimental results of a recovery study for both methods are given in Tables I and II. Recovery of method I is close to 100% and for method II somewhat lower because of some loss in the cleanup, but still satisfactory. The lettuce sample spiked with 0.50 mg of metalaxyl/kg

Table II. Recoveries of Metalaxyl Added to Chopped Lettuce (mg/kg) Estimated with Method II

metalaxyl added	BP 10 column			SIL 5 column		
	control	spiked	% rec[a]	control	spiked	% rec
0.050	–[b]	0.044	88	–	0.043	86
0.050	–	0.043	86	–	0.048	96
0.050	–	0.038	76	–	0.044	88
0.050	–	0.037	74	–	0.043	86
av ± SD			81 ± 7.0			89 ± 4.8
0.50	–	0.44	88	–	0.41	82
0.50	–	0.49	98	–	0.45	90
0.50	–	0.44	88	–	0.47	94
0.50	–	0.42	84	–	0.43	86
av ± SD			89.5 ± 6.0			88 ± 5.2

[a] Difference between the amounts found in spiked and control samples, expressed as a percentage of the amount of metalaxyl added. [b] Below detection limit (0.01 mg/kg).

gives overlapping matrix peaks on the SIL 5 column (Figure 1B,D).

Method II has been used in our laboratory in the period 1985–1987 for determining residues of metalaxyl in about 10.000 lettuce samples. In 1987 method I was developed. To verify the reliability of method I, 200 field-treated samples were analyzed with both methods. When positive residues of metalaxyl were found, they were identified by both methods I and II. Metalaxyl concentrations of 23 samples were above 0.04 mg/kg. For these 23 observations the correlation coefficient between both methods is 0.9996.

CONCLUSION

Metalaxyl peaks can be identified on two columns in one run. Cleanup by solid-phase extraction saves work and chemicals. Comparing both methods, we may conclude that method II is more specific and more sensitive than method I. However, method I takes less time. Depending on the number of samples to be analyzed and the sensitivity required, a suitable method can be chosen.

LITERATURE CITED

Caverly, D. J.; Unwin, J. Determination of Residues of Furalaxyl and Metalaxyl in Nutrient Solution, Peat Compost and Soil Samples by Gas Chromatography. *Analyst* **1981,** *106,* 389–393.

Ernst, G. F.; Pieterse, C.; Haarsveld, J. E. Onderzoek naar het voorkomen van metalaxyl in kassla. Internal Report IR/71/84/R 119; Keuringsdienst van Waren voor het gebied Utrecht: Utrecht, 1984.

Newsome, W. H. An Enzyme-Linked Immunosorbent Assay for Metalaxyl in Foods. *J. Agric. Food Chem.* **1985,** *33,* 528–530.

Singh, U. S.; Tripathi, R. K. Estimation of the systemic fungicide Ridomil by thin-layer chromatography. *J. Chromatogr.* **1980,** *200,* 317–323.

Speck, M.; Dirr, E. Gas chromatographic determination of Metalaxyl (Ridomil) residues in tobacco. *J. Chromatogr.* **1980,** *200,* 313–316.

Tafuri, F.; Marucchini, C.; Patumi, M.; Businelli, M. Gas Chromatographic Determination of Metalaxyl in Soils and Sunflower. *J. Agric. Food Chem.* **1981,** *29,* 1298–1301.

Waliszewski, S. M.; Szymczynski, G. A. Economical Method for the Determination of Metalaxyl in Tomatoes. *Z. Anal. Chem.* **1983,** *316,* 322–324.

Detection of Adulteration of California Orange Juice Concentrates with Externally Added Carotenoids by Liquid Chromatography

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A liquid chromatographic procedure to separate and quantitate externally added common color adulterants to orange juice concentrates is described. The procedure involved treatment of extracted carotenoids with methanolic HCl to convert carotenoids with 5,6-epoxide end groups to 5,8-epoxides and measurement of HPLC peaks at 465 nm. This procedure allowed the quantitation of fatty acid esters of cryptoxanthin, citraurin, and lutein and free cryptoxanthin and β-carotene, without interference from the 5,6- and 5,8-epoxides, which constituted two-thirds of the total carotenoids in commercially processed orange juice concentrate. Although the total carotenoids in California navel and Valencia orange juice concentrates varied with variety, season, and location, the percentage composition of individual carotenoids remained within a narrow range. The mean concentrations of cryptoxanthin esters in California Valencia and navel orange concentrates were 15.5 and 23.5%, respectively, of the total carotenoids measured at 465 nm. The cryptoxanthin ester concentration in tangerine juice concentrates exceeded 40% of the total. Cryptoxanthin palmitate predominated in navel orange concentrates whereas, in tangerine concentrates, myristate and laurate esters predominated. The HPLC procedure permitted detection of commonly used adulterants in orange juice concentrates.

Color is an important quality aspect in commercially processed orange juice concentrates. The color of citrus

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fruits is due to carotenoids and detection of added colorants to orange juice is of concern to consumers, regulatory agencies, and citrus processing industries. The most common adulterants are synthetic β-carotene and β-apo-8′-carotenal, marigold flower (*Taqetes erecta*) and citrus peel extracts, and tangerine and mandarin juices. Valencia orange is the major source of concentrates produced in the United States. Navel oranges and other citrus fruits are also processed for juice.