High-Performance Liquid Chromatography for the Determination of Pencycuron Residues in Several Vegetables

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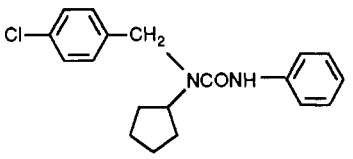
A normal-phase high-performance liquid chromatographic method is described for the determination of pencycuron, a new fungicide, in salads, potatoes, tomatoes, and soils. Residues of pencycuron are extracted from samples with an acetone–water mixture. Purification is performed by liquid partitioning from aqueous solution into dichloromethane followed by a Florisil column chromatography. The extracts are analyzed by HPLC using a cyanopropyl-bonded stationary phase with a UV detection at 239 nm. Recoveries, established at various fortification levels, ranged from 80% to 100% for all sample types. The method ensures a minimum detectable amount above 1.5 ng and detection limits of about 0.06 ppm for salads, 0.03 ppm for tomatoes, and 0.01 ppm for potatoes and soils.

Pencycuron, 1-(4-chlorobenzyl)-1-cyclopentyl-3-phenylurea, is the active ingredient of the fungicide discovered by Nihon Tokushu Noyaku Seizo K.K in Japan and developed by Bayer AG under the trade name MONCEREN.



This nonsystemic fungicide is used to control plant diseases caused by *Rhyzoctonia solani* such as sheath blight of rice, black scurf of potatoes, and damping-off of ornamentals and crops (Koster, 1983; Jager and Velvis, 1986; Yamada, 1986; Sumner, 1987).

Metabolic and environmental transformations have been reported (Ueyama et al., 1982; Yamada, 1986).

Previous studies have reported that, with a relatively long persistency, pencycuron is well retained by plants as a parent form and no major metabolite was found in a great amount (Yamada, 1986). The physical and chemical properties have been reviewed (Yamada, 1986).

This new fungicide is especially used in European countries to protect potato plants, but applications could soon be considered for salads.

Since pencycuron is a new fungicide, few studies are available among research works and only two methods have been reported for the analysis of residues, both using gas chromatography procedures.

Generally, phenylurea compounds are thermally unstable, but previous studies on phenylurea herbicides have carried out that derivatization could allow gas chromatography detection (Kobori et al., 1978).

Alkylation with sodium hydride and methyl iodide in dimethyl sulfoxide was performed, giving specific derivatives of phenylurea pesticides that could be detected by gas chromatography using a flame ionisation detector (Kobori et al., 1978).

Previous work has confirmed thermal degradation of pencycuron during injection and has reported a residue method using the alkylation reaction. After extraction from plant material and partitioning into water and dichloromethane, extacts are cleaned up on Florisil column chromatography. The method then involves methylation with sodium hydride and methyl iodide in dimethyl sulfoxide, and the derivative products, extracted with hexane, are quantified by gas chromatography using a flame ionization detector (Kobori et al., 1978).

Kobori et al. have developed this method for the analysis of pencycuron in hulled rice, rice straw, and soil. Recoveries were in the range 70–100%, and detectable limits reported were 0.01 ppm for rice samples and 0.02 ppm for soil samples.

According to Kobori et al., Bayer AG has described a method for analysis of pencycuron residues in plant material, soil, and water (Vogeler, 1982). In that one, there is no cleanup step on Florisil column but purification is performed after the alkylation reaction by gel permeation chromatography on Bio Beads S-X3 polystyrene gel.

This method ensures a quantitative recovery of the extracted residues and a detection limit of about 0.01 mg/kg for potatoes and lettuces, 0.02 mg/kg for soils, and 0.005 mg/kg for water.

On account of the difficulties met in our laboratory in the use of derivatizing reagents, the gas chromatographic procedures did not prove satisfactory.

In order to eliminate the derivatization step, a high-performance liquid chromatography (HPLC) method was considered. A library search indicates that no liquid chromatography has been reported until today. This paper described a simple, less time consuming method for the determination of pencycuron in various plants. Residues are quantified with a good sensitivity by normal-phase HPLC using a UV detector.

EXPERIMENTAL SECTION

Chemicals and Reagents. *Pencycuron standard*, 99.5% pure, used for fortification and quantitation was supplied by Bayer AG.

Solvents. Acetone, hexane, dichloromethane, and ethyl acetate (SDS, Peypin, France) were glass-redistilled while all solvents used for HPLC were HPLC-grade solvents (SDS; Prolabo), filtered through a 0.45-μm Durapore filter (Millipore Corp.).

Water was distilled twice and washed with hexane before use.

Anhydrous sodium sulfate was obtained from Prolabo Corp. and was pesticide grade.

Florisil, 60–100 mesh (L. Dreyfus), was activated at 300 °C for 48 h, stored at 130 °C until utilization, and cooled in a desiccator before use.

Liquid Chromatography. The high-performance liquid chromatograph consisted of a Beckman 114M solvent delivery module equipped with a Rheodyne injection valve (sample loop of 50 μL) and a Varian Model 2050 variable-wavelength UV detector operated at 239 nm (0.01 AUFS). Chromatograms were registered with a Linear Model 500 chart recorder (Chart speed of 15 cm/h).

Separations were performed on Chrompack cyanopropyl–silica columns packed with 5-μm particles of Spherisorb CN. The chromatograph was equipped with a modern HPLC system that consisted of interchangeable analytical columns in a stainless steel

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column holder. This holder, containing two cartridges of 10 cm × 3 mm (i.d.), was joined together by a stainless steel column coupling. A guard column packed with polar bonded phase was added in the cartridge holder.

The mobile phase was isooctane–5% 2-propanol at a flow rate of 0.5 mL/min (isocratically). All samples were filtered through 0.45-μm Millex-HV (Millipore Corp.) before injection.

Methods. *A. Salads.* Samples were chopped up into small pieces (Hobbar) and stored at −18 °C. A 25-g subsample was homogenized with 100 mL of a mixture acetone–water (80:20, v/v) in the Ultra-Turrax mixer at maximum speed for a few minutes. The extract was vacuum-filtered through a Buchner funnel containing two Whatman GF/A (9-cm) fiber glass filter papers and a thin layer of Hyflo Supercel. The filter cake was mixed again with 60 mL of the 80% aqueous acetone and finally washed with acetone until near-discoloration.

The combined filtrates were evaporated on vacuum rotary evaporator at about 30 °C, and the remaining aqueous phase was transferred to a 500-mL separatory funnel. The round-bottom flask was washed with 100 mL of 10% sodium chloride solution (added by small portions) that was added to the separatory funnel. Pencycuron was then extracted from the aqueous solution with three 100-mL aliquots of dichloromethane. Each portion was used to wash the round-bottom flask before addition to the separatory funnel. For each partitioning, the two phases were allowed to separate (a small emulsion could appear after the first shaking) and the organic phase (lower layer) was collected.

The combinated extracts were dried over anhydrous sodium sulfate for about 2 h and evaporated to dryness under vacuum on a rotary evaporator (water bath 30 °C). The residue was dissolved in hexane, and the final volume was adjusted to 25 mL.

A glass chromatographic column (40 cm × 2 cm (i.d.)) was packed with 10 g of Florisil (prepared as described above), and the top was covered with a thin layer of anhydrous sodium sulfate. The column was prewashed with hexane. Then, 5 mL of the sample extract was transferred to the column. When sample had drained to the top of the column, two 10-mL aliquots of hexane were added, drained down to the bottom, and discarded. Pencycuron was eluted with 130 mL of hexane–ethyl acetate (80:20, v/v) at the rate of approximately 0.5 mL/min. The eluate fraction was evaporated to dryness, and residues were redissolved in 10 mL of the HPLC mobile phase.

B. Tomatoes. A 100-g sample of finely chopped tomatoes was mixed with 100 mL of acetone–water (80:20, v/v) like salad samples but, in this case, the second blending of the filter cake was performed with 100 mL of the mixture instead of 60 mL because of the volume of the filter cake.

The procedure was then the same than in section A.

The final residue was redissolved in 20 mL of the HPLC mobile phase instead of 10 mL.

C. Soils. Soil samples were ground and a 50-g subsample was shaken on a stirring table for 4 h with 100 mL of acetone–water (80:20, v/v). Filtration and liquid partitioning were then accomplished as described in section A.

After evaporation of the dichloromethane extract, the procedure was a bit different than in section A. Indeed, Florisil column chromatography was performed on the entire extract. So, the residue contained in the round-bottom flask was not adjusted to 25 mL but was directly added to the column with a small volume of hexane. The round-bottom flask was rinced with two 5-mL portions of hexane, which were added to the column. Then, pencycuron was eluted with 130 mL of hexane–ethyl acetate (80:20, v/v). The eluate from the Florisil column was evaporated to dryness, and the volume was adjusted to 20 mL with the HPLC mobile phase.

D. Potatoes. A 50-g subsample of chopped-up potatoes was used, and analysis was performed according to the method described in section A with only one difference: After the liquid partitioning step, the dichloromethane extracts were evaporated to dryness with a vacuum rotary evaporator and residue was dissolved in 20 mL of hexane (instead of 25 mL) while the aliquot transferred to the Florisil column was 10 mL (instead of 5 mL).

Then, the samples were purified by the Florisil column procedure as described in section A, and the eluate fraction was dissolved, after evaporation to dryness, in 10 mL of the HPLC mobile phase.

Table I. Percent Recoveries of Pencycuron from Fortified Samples of Salad, Tomato, Potato, and Soil

sample type	added, ppm	N	% recovery range	% recovery av	% recovery SD
salad	0.06	4	90–96	92.7	±2.7
	0.2	6	86–94	88.8	±3.4
	0.4	6	78–95	90.2	±6.6
	1	2	95–97	96.0	±1.4
tomato	0.03	2	96–110	103.0	±9.9
	0.05	5	89–93	89.6	±2.1
	0.1	5	86–94	91.0	±4.1
	0.25	2	90–91	90.5	±0.7
soil	0.012	2	96–98	97.0	±1.4
	0.04	5	92–103	97.4	±4.3
	0.08	3	96–98	97.0	±1.0
	0.2	3	85–89	87.0	±2.0
potato	0.012	3	70–88	81.3	±9.1
	0.04	4	95–104	99.7	±3.8
	0.08	4	85–98	89.2	±6.1
	0.2	4	82–86	84.0	±2.0

RESULTS AND DISCUSSION

Validation of the Method. In order to evaluate the described method, recovery tests were carried out for whole sample types. Untreated samples were fortified with various amounts of pencycuron from the limit detection level to greater levels. For these last ones, solutions were diluted before injection to prevent off-scale response of the detector. The recoveries thus obtained are shown in Table I, and overall recoveries averaged 90.2 ± 4.8 (N = 16) for salads, 92.1 ± 5.9 (N = 14) for tomatoes, 94.8 ± 5.2 (N = 13) for soils, and 88.6 ± 8.7 (N = 15) for potatoes.

The limit of detection depended on the sample type because interfering peaks due to sample coextractives were different according to the vegetable material. The weight of the subsample submitted to the analysis, the volume of the aliquot transferred to the Florisil column, and the final volume of HPLC mobile phase in which the residue was dissolved were adjusted according to the sample type. The detection limit for the HPLC analysis was about 1.5 ng of pencycuron. It was defined as 3 times the base line and gave a peak height above 10 mm, easily measurable. This detection limit corresponds to practical limits of detection of 0.06 μg/g for salads, 0.03 μg/g for tomatoes, and 0.01 μg/g for potatoes and soils.

For salads, this limit could be improved down to 0.06 μg/g because some salads were found to be cleaner than others, giving less interference, but it was generally necessary to take very small subsamples (25 g) to prevent interfering peaks from coextractives, especially chlorophylls. For other sample types, the described procedures allowed good sensitivity, and chromatograms were almost free from interfering compounds. Two varieties of potatoes were used for the extraction of residues, but significant differences were not noticed on the chromatograms.

Chromatograms for standard, untreated samples and samples fortified at two different levels of pencycuron (one fortification was performed at the limit detection level) are reported in Figures 1–4.

The straight-line calibration curve was realized, the detection system appeared to be linear up to at least 20 ng injected (pencycuron solution of 0.4 μg/mL), and the correlation coefficient of the linear regression analysis was 99.8%.

Analysis Conditions. A number of solvents have been used to elute pencycuron from Florisil. Acetone was used but was unacceptable because all pigmented compounds were eluted with the fungicide and the detection was impossible. Then, a mixture hexane–diethyl ether was found to be selective enough to elute pencycuron without ex-

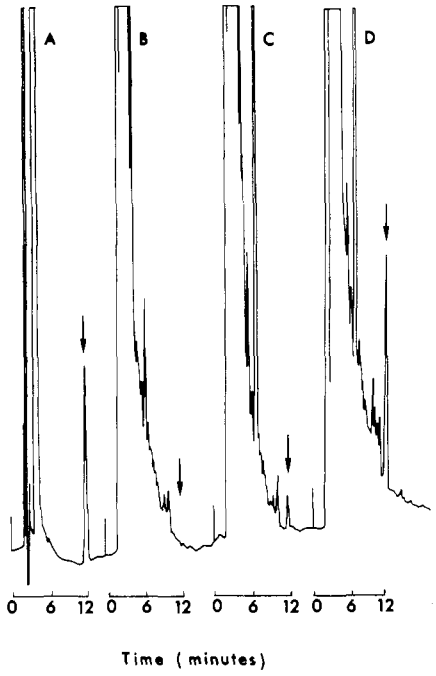

Figure 1. HPLC chromatograms of salads (the arrow indicates pencycuron): (A) pencycuron standard, 10 ng (equivalent to 0.4 ppm); (B) untreated control salad; (C) control salad fortified with 0.06 ppm of pencycuron (94% recovery); (D) control salad fortified with 0.4 ppm of pencycuron (92% recovery).

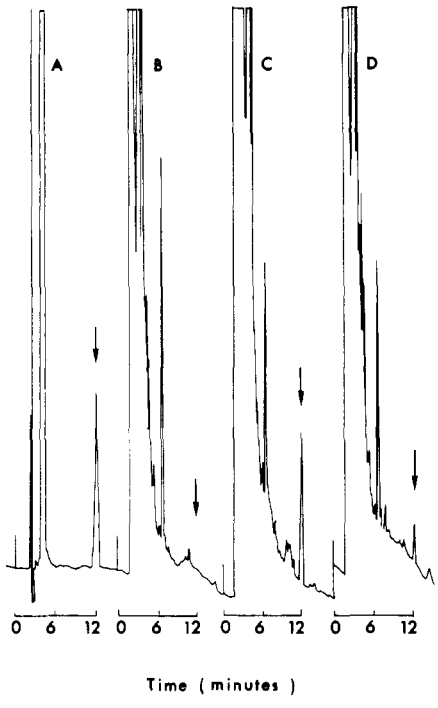

Figure 2. HPLC chromatograms of tomatoes: (A) pencycuron standard, 10 ng (equivalent to 0.1 ppm); (B) untreated control tomato; (C) control tomato fortified with 0.1 ppm of pencycuron (88% recovery); (D) control tomato fortified with 0.03 ppm of pencycuron (97% recovery).

cessive sample coextractives but reproducibility of recoveries on Florisil column chromatography did not prove satisfactory after several tests. Finally the mixture hexane–ethyl acetate (80:20, v/v) proved to be the most convenient for quantitative recovery and to be selective enough.

Trials have shown that Florisil was more efficient than other adsorbents such as alumina, and the adsorption cleanup was more suitable when the Florisil was used dry,

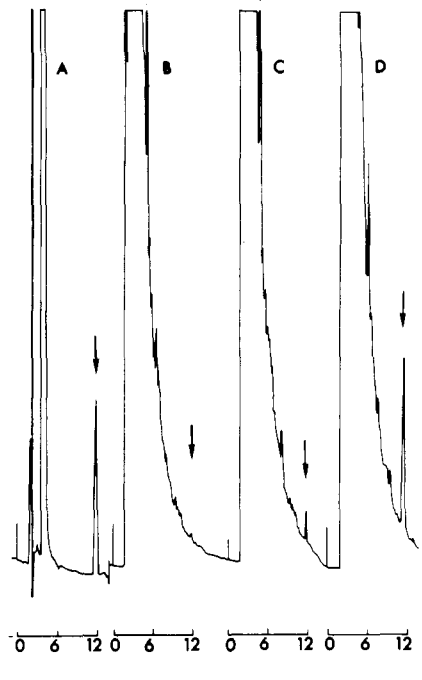

Figure 3. HPLC chromatograms of soils: (A) pencycuron standard, 10 ng (equivalent to 0.08 ppm); (B) untreated control soil; (C) control soil fortified with 0.01 ppm of pencycuron (98% recovery); (D) control soil fortified with 0.08 ppm of pencycuron (97% recovery).

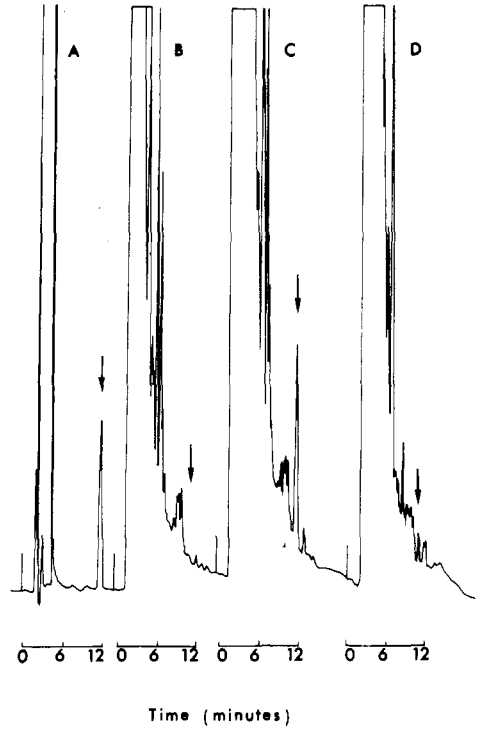

Figure 4. HPLC chromatograms of potatoes: (A) pencycuron standard, 10 ng (equivalent to 0.08 ppm); (B) untreated control soil; (C) control potato fortified with 0.08 ppm of pencycuron (100% recovery); (D) control potato fortified with 0.01 ppm of pencycuron (85% recovery).

without deactivation by added water.

A great part of this study and the research of optimal conditions for extraction and purification steps were performed under HPLC conditions entirely different from those described above. It was an adsorption high-performance liquid chromatography using a silica stationary

phase while the mobile phase was hexane–2% 2-propanol. But, since silanols of the silica phase are deactivated by water, this stationary phase was very sensitive to water traces eventually present in the injected samples and this induced important variations of the retention time of the pencycuron. For this reason a cyanopropyl bonded phase was preferred. Less sensitive to water, this stationary phase, used as a normal phase, allowed quite similar separations. Furthermore, this bonded phase requires less time of equilibration.

Optimization of the mobile phase has been considered to improve HPLC performance, and many modifications of the mobile phase have been studied. Under the conditions used, isooctane–5% 2-propanol has been found to give the most convenient separation between pencycuron and coextractives eluting at the beginning of the chromatograms. Further investigations, using a gradient elution system could be considered later to determine whether it is possible to improve this.

However, we have observed that the retention time of the pencycuron tends to increase, thus inducing a small variation in the peak height of standard. This is why frequent injections of the standard were required before quantification analysis. Moreover, pencycuron solutions appeared to be unstable at low concentrations, and standard solutions must be prepared very often.

Performance of the Method. This method has been successfully applied to the analysis of pencycuron in several salad samples, cabbage lettuce, and curly lettuce, supplied by various producers from different regions. We must notice that reproducibility has not always been satisfactory, but this seemed to be due to the vegetable itself. Indeed, salad samples supplied by different firms were certainly badly homogenized. Consequently, some 25-subsamples contained a great proportion of white leaves (or internal leaves) while others were principally composed of green parts (external leaves). This could explain the lack of reproducibility because internal leaves, protected by external ones during the pesticide treatment, contained less residues.

If we compare previous gas chromatographic methods and this liquid chromatographic one, reproducibilities and recoveries are quite similar. Of course, the detection limits are a little higher when using high-performance liquid chromatography, especially for salads: 0.06 $\mu g/g$ with liquid chromatography instead of 0.01 $\mu g/g$ with gas chromatography (Vogeler, 1982). But for other samples, detection limits are in the same range, using liquid chromatography or gas chromatography. For potatoes, the method described in this paper ensures the same detection limit (0.01 $\mu g/g$) as those obtained by Vogeler (1982), and in all cases, HPLC analysis avoids the derivatization step which is time consuming.

As was mentioned previously, efficiency and sensitivity of this method might be increased with use of a gradient elution HPLC system, especially by eliminating the interfering peaks from the first part of the chromatograms.

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Diagnostic Ion Series for the Identification of Amadori Rearrangement Products by MS Techniques Based on Electron-Impact Ionization

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The importance of Amadori rearrangement products in food and biological systems led us to investigate their high-resolution electron-impact fragmentations. On the basis of the fragmentations of a large number of Amadori products, we propose two fragmentation pathways in which the ions retain fragments from the two moieties sugar and amino acid and as such can be used as diagnostic ion series for the identification of Amadori rearrangement products by mass spectrometric techniques with electron-impact ionization.

The established importance of the decomposition products of 1-(amino acid)-1-deoxy-D-fructoses or Amadori rearrangement products (ARPs, 1; Scheme I) in food (Nursten, 1980) and biological systems (Monnier et al., 1984) led us to investigate their fragmentations under high-resolution electron-impact conditions (Yaylayan and Sporns, 1988). Since EIMS fragmentations are based on ground-state solution chemistry, as supported by a wide body of experimental evidence (Budzikiewicz et al., 1967), the fragmentation schemes obtained could be extrapolated to actual decompositions of Amadori products taking place

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