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HPLC Methods for Detection of Uniconazole-P in Soils and Plant Tissues

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Abstract: High-performance liquid chromatographic (HPLC) methods have been developed for the detection of uniconazole-P [(E)-1-(4-chlorophenyl)-4,4,-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol; XE-1019; the active ingredient in Prunit and Sumagic] in soil and plant tissue samples. Methanolic extracts of soil and plant samples were dried to the aqueous phase, the pH adjusted to 11, and partitioned against methylene chloride. The methylene chloride phases were washed with pH 11 water and then passed through C-18 solid phase extraction (SPE) columns. The soil extracts were then dried and the residues taken up in 1 ml acetonitrile of which 20 μl were injected directly onto a C-18 reverse phase analytical column for HPLC analysis. Plant tissue extracts were purified by partitioning and passing through a sequence of Florisil/C-18/Florisil SPE columns before HPLC analysis. Recovery of uniconazole-P was ~70% from soils and ~40% from plant tissues. Quantitative detection of 10 parts per billion (ppb) uniconazole-P in plant tissues and soil samples was feasible following these procedures. The soil cleanup procedures were also used to detect uniconazole-P in leachates collected from container-grown plants.

Uniconazole-P [(E)-1-(4-chlorophenyl)-4,4,-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] is one of the more desirable growth retardants for the control of growth in greenhouse, orchard, and nursery crops, and trees in the landscape (Knox and Norcini 1987; Izumi et al. 1984; Sachs et al. 1989; Steffens 1988; Valent 1988). However, methods of application of this compound need to be improved to increase its usefulness, particularly on ornamentals. The amount

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of compound entering the plant via soil applications or bark painting should be followed to perfect formulation, dosage, timing of application, etc. Also, potential movement of the compound into ground water supplies, following any method of application, is essential information to meet environmental regulations. Radiolabeled uniconazole-P, which is easily detected using liquid chromatographic techniques coupled with image scanning or scintillation spectrometry for quantification (Sterrett 1988), would not be readily adapted to field-based studies. The relatively simple methodology described in this study for measuring uniconazole-P concentrations was achieved by developing cleanup procedures for plant and soil extracts that permitted high-performance liquid chromatographic (HPLC) separation from interfering natural products.

Materials and Methods

All solvents were of HPLC grade, including water, unless otherwise noted. All percentages are expressed on a volume/volume basis with the unspecified solvent being water.

Sample Preparation

Soil

Soil samples were dried before extraction. Ten milliliters of 60% methanol was added to a centrifuge tube containing 1 or 5 g of soil. After agitating on an orbital shaker for 30 min, the samples were centrifuged at 3000 rpm for 5 min, and the supernatant removed. The sample was extracted two more times, the supernatants were then combined and reduced in volume under a stream of air to the aqueous phase. Potassium hydroxide was used to adjust the remaining aqueous phase to pH 11 before partitioning three times with methylene chloride. The volume of methylene chloride should be one half or more of the aqueous sample. The aqueous phase was discarded and the combined methylene chloride phases were washed three times with equal volumes of pH 11 (adjusted with potassium hydroxide) glass-distilled water. The methylene chloride phase was then evaporated to dryness under a stream of air. The residue was redissolved in 0.9 ml of methanol, agitated as necessary, and 2.1 ml water was added to make a 30% methanol solution.

A 500 mg C-18 solid phase extraction (SPE) column (Alltech Associates, Inc., Deerfield, IL, USA; stock no. 205350) was conditioned with 3 ml of 100% methanol followed by 3 ml of 30% methanol. The soil extract was then loaded onto the C-18 SPE column and rinsed with an additional 3 ml of 30% methanol. Uniconazole-P was eluted with 6 ml of 60% methanol that was collected, dried, and the residue redissolved in acetonitrile for HPLC analysis. The flow chart in Fig. 1 illustrates a condensed form of the extraction and cleanup protocol developed for uniconazole-P analysis.

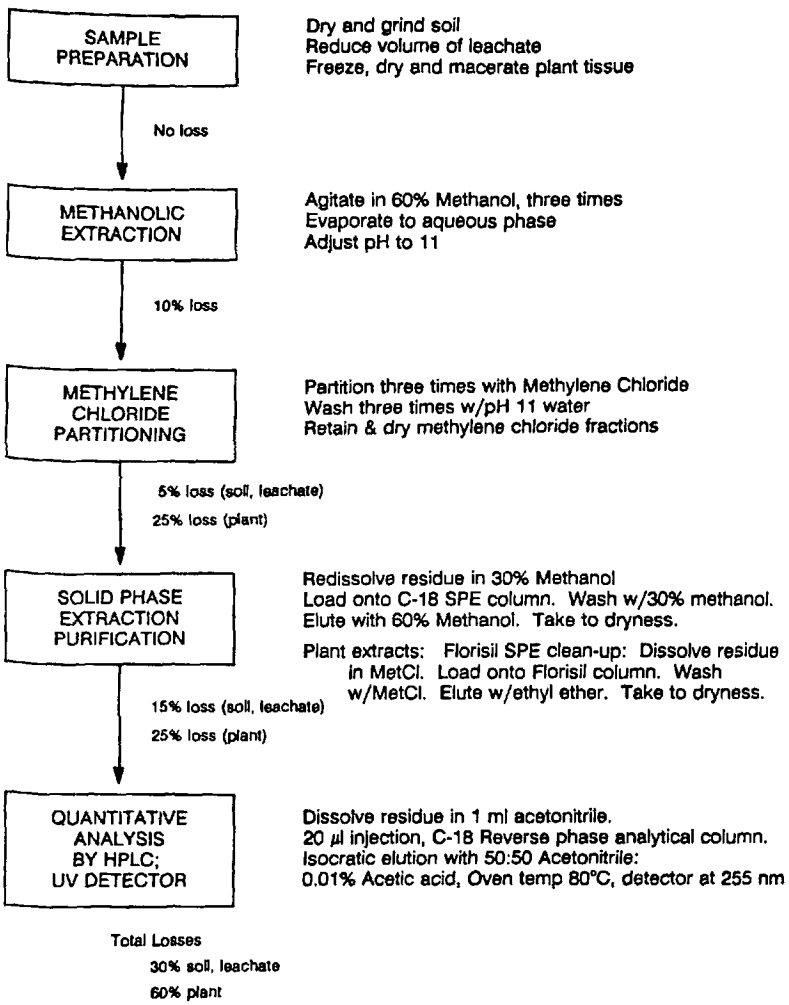

Fig. 1. Flow chart depicting sample processing protocol and losses for analysis of uniconazole-P in soil, soil leachates, and plant tissue.

Soil Leachates

Oleanders about 1-m tall were grown in 15-gallon (56.1 L) containers from July 1987 through March 1988. The containers were fitted with drains (at the base) connected to sampling tubes. Uniconazole-P was applied as a methanolic soil drench (4 g a.i. per plant) in August 1987. Irrigation was supplied by drip emitters, in addition to rainfall, when soil moisture tensiometer readings of -20 centibars were exceeded. Soil leachate samples were analyzed for uniconazole-P content by drying (under air stream and stirring) to a reasonable volume (eg, a 500-ml sample was dried to ~50 ml), adjusting the pH to 11, and partitioning against methylene chloride three times. Leachate extracts were

backwashed and run through C-18 SPE columns before HPLC analysis. HPLC analysis was the same as for soil samples.

Plant Tissues

Samples of leaves, roots, shoot tips, or stems of 5–25 g fresh weight were frozen, then dried overnight in a 55°C oven, before grinding to a fine powder with a mortar and pestle. The tissue was extracted three times with 10–20 ml of 60% methanol. Supernatants were combined and reduced to the aqueous phase. The pH was then adjusted to 11. The aqueous phase was partitioned three times with methylene chloride, and the aqueous phase was discarded. The methylene chloride phase was washed three times with glass-distilled water adjusted to pH 11. The methylene chloride phase was reduced to dryness to remove the remaining water. The residue was redissolved in 3 ml methylene chloride and loaded onto a 500-mg Florisil SPE column (Alltech Associates, Inc.; stock no. 204650). This method was modified from Stahly and Buchanan (1986). The column was first conditioned with 3 ml methylene chloride. The sample was rinsed with 3 ml methylene chloride before eluting the uniconazole-P with 6 ml anhydrous ethyl ether which was collected and dried. This residue was reconstituted in 30% methanol and run through a C-18 SPE column using the soil extract protocol. Plant extracts benefited from an additional pass through Florisil before analysis on HPLC.

HPLC Protocol

Residues were redissolved in 1 ml acetonitrile and 20 μl was injected directly onto a 4.6 mm × 250 mm reverse phase C-18 analytical column held at 80°C for HPLC analysis. An isocratic mixture of acetonitrile:0.01% acetic acid (~50:50) was used to elute the uniconazole-P. The detector was set at 255 nm (the absorption maximum for uniconazole-P). At this setting, 5–10 ng of uniconazole-P were readily detectable—this being equivalent to 250 ppb in the injection solution. Some plant samples remained complex enough to require the use of two analytical C-18 columns in tandem for adequate resolution. Figure 2 depicts typical HPLC chromatograms for analyses of uniconazole-P.

With larger injection volumes or more concentrated final extraction volumes, lower concentrations would be detectable. By extracting 25-g samples, *concentrations as low as 10 ppb are detectable*. For tracking movement of the compound into the environment, larger samples of water or soil must be extracted, and this may require using more elaborate cleanup procedures to remove interfering compounds.

Results and Discussion

Recovery rates were calculated by adding known amounts of uniconazole-P to plant tissues of several species and to soils of various compositions before

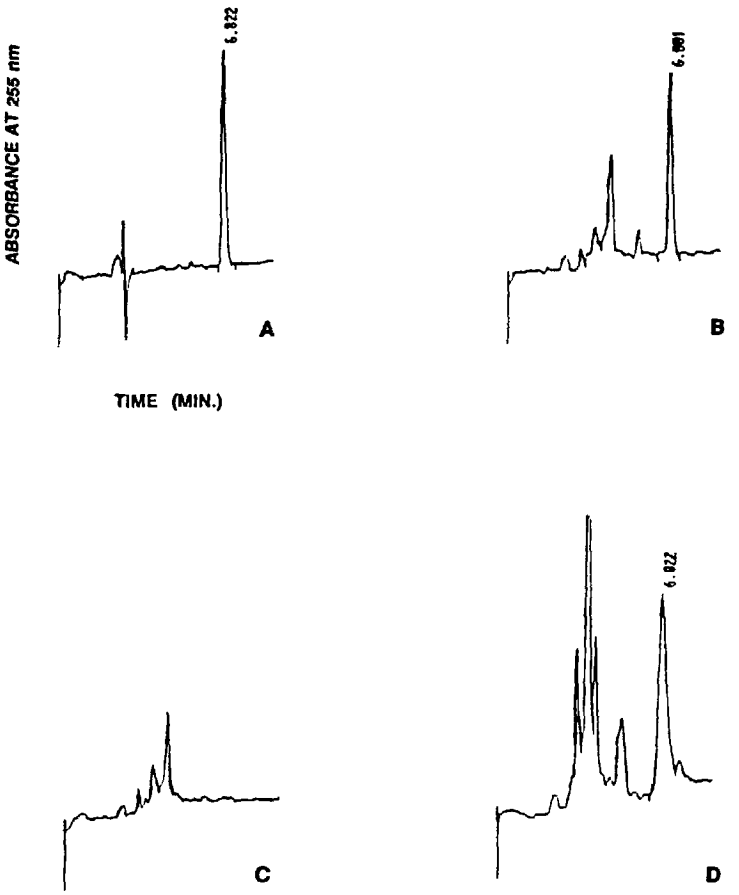

Fig. 2. Typical HPLC chromatograms of uniconazole-P extracted from 5 g of elm leaves. HPLC conditions: 4.6 mm × 250 mm C-18 column; oven temperature 80°C; 1 ml/min 50:50 acetonitrile:0.01% acetic acid. Retention time for uniconazole-P is 6.8 min. (**A**) 50 ng standard; (**B**) 43 ng from spiked sample [5 μg added to 5 g leaves. Chromatogram represents 1/50 of sample (ie, 100 ng if 100% recovery).]; (**C**) untreated sample; and (**D**) 84 ng from a bark-painted tree (25 g leaves).

extracting as described above. Technical grade uniconazole-P was obtained from Chevron Chemical Company for testing purposes. Standards of known amounts of uniconazole-P were analyzed and used as external standards to compare to amounts extracted from samples of soil or plant origin.

Five micrograms of uniconazole-P was added as a methanolic solution to soil or plant tissue and allowed to dry. If a final volume of 1 ml acetonitrile was used and 20 μl was injected (ie, 1/50 of sample), we would expect 100 ng of compound per injection if 100% were recovered. As the extraction protocol was developed and cleanup methods improved, percent recovery was calculated for each experiment. Percent recovery was increased and the presence of interfering compounds reduced by (a) using 60% methanol (versus 80%) for extraction, (b) adjusting aqueous phases to pH 11, and (c) increasing the

Table 1. Percent recovery of uniconazole-P from soil samples.

Experiment no.	Soil type	Reps	pH wash[a]	Initial extraction[b]	Note[c]	% recovery
87-13	UC mix	3	NO	1 × 80%	1PT	58.3
	UC mix	3	NO	2 × 80%	1PT	70.1
87-14	UC mix	3	NO	1 × 80%	1PT	68.2
	UC mix	3	NO	2 × 80%	1PT	78.6
	Yolo	3	NO	1 × 80%	1PT	73.5
	Yolo	3	NO	2 × 80%	1PT	69.6
87-18	UC mix	3	NO	2 × 80%	1PT	60.5
	UC mix	3	NO	3 × 80%	1PT	71.8
	Yolo	3	NO	2 × 80%	1PT	54.3
	Yolo	3	NO	3 × 80%	1PT	65.8
87-24	UC mix	3	NO	3 × 80%	L, 1PT	72.6
	Yolo	3	NO	3 × 80%	L, 1PT	92.9
87-28	Yolo	3	NO	3 × 80%	L, P, 1PT	44.4
87-32	Yolo	3	NO	3 × 80%	L, 1PT	64.0
	Yolo	3	NO	3 × 80%	L, P, 1PT	55.8
87-35	Yolo	3	NO	3 × 80%	1PT	74.6
	Yolo	3	ACID	3 × 80%	1PT	56.7
	Yolo	3	NO	3 × 80%	3PT	87.2
	Yolo	3	ACID	3 × 80%	3PT	63.6
87-42	Yolo	3	ALK	3 × 80%	3PT	73.6
	Yolo	3	NO	3 × 80%	3PT	67.2
87-45	Yolo	3	ALK	3 × 60%	3PT	81.3
	Yolo	3	ALK	3 × 80%	3PT	74.3
88-19	Yolo	12	ALK	3 × 60%	3PT	63.2
88-28	Sand	12	ALK	3 × 60%	3PT	71.2
Avg ± SD						68.5 ± 10.6

[a] NO, wash not pH adjusted; ACID, pH adjusted to 3; and ALK, pH adjusted to 11.
[b] 1 × 80%, one extraction with 80% methanol, etc.
[c] L, low levels of uniconazole-P (10 ng/HPLC injection versus 100 ng/injection); P, plant tissue in soil (ie, roots included in sample); and 1PT or 3PT, number of methylene chloride partitions.

number of methylene chloride partitions from one to three. The results for soil samples are listed in Table 1. UC Mix is composed of 33% peat, 33% #20 sand, and 33% redwood sawdust. Yolo loam has the following approximate composition: 26% sand, 26% clay, 47% silt, and 1% organic matter (Huntington et al. 1981).

The average recovery of uniconazole-P from all soil extracts was 68%. Recovery from soil leachates was similar with an average of 65%. Uniconazole-P recovery from plant extracts was only about 40%. More uniconazole-P was lost from plant extracts during partitioning and washing than from soil extracts, as well as during the additional use of Florisil SPE columns. Figure 1 includes calculated losses at each step of the extraction protocol.

Samples of soil, soil leachates from containers, and plant tissues have been collected and analyzed from experiments in which uniconazole-P was applied as bark paints or as soil drenches. Surface (top 2 cm) soil beneath trees, bark painted 1 year previously, contained 0–2 ppm (μg/g dry weight soil) uniconazole-P. Only 0.05 ppm was found in the 12.5–25 cm region, with no detectable

Table 2. Uniconazole-P (4 g applied in August 1987) recovered from soil cores of soil drenched oleanders in 15-gallon containers of field soil.[a]

	Uniconazole-P (ppm)					
	Application site (cm core depth)			Perimeter (cm core depth)		
	0–13	13–25	25–38	0–13	13–25	25–38
November 87	16.3	0.2	4.6	0.2	0.2	NA
March 88	24.2	2.9	0.5	5.7	1.8	2.4

NA, not available due to soil compaction when coring.
[a] Uniconazole-P is shown in ppm and has been corrected for losses (raw data divided by 0.7, see Table 1).

Table 3. Uniconazole-P (4 g applied in August 1987) recovered from plant tissue from soil drenched oleanders. Tissue was collected March 1988.[a]

Tissue	ppm
Tips	0.24
Young leaves	0.26
Mature leaves	0.62
Stems	0.20
Roots	4.93

[a] Amounts shown in ppm (μg/g fresh weight) and have been corrected for losses (raw data divided by 0.04).

uniconazole-P below 25 cm. One inch soil cores (18″ deep, total volume of 170 ml) were collected from 15-gallon (56.1 L) containers of field soil (Yolo loam) that had 4 g uniconazole-P applied as a soil drench. The amounts recovered from soil cores are shown in Table 2. Seven months after soil application, little downward movement of uniconazole-P in this soil type was detected.

One year after bark painting, 11-year-old, 12″ DBH (diameter at breast height) Chinese elm (*Ulmus parvifolia*) trees and 2.5-year-old, 5″ DBH eucalyptus (*Eucalyptus camaldulensis* and *E. grandis*) trees with 3.5% uniconazole-P, 50 ppb (ng/g fresh weight) uniconazole-P was recovered from mature leaf tissue of the three species. Growth inhibition was ~80% for the three species (Sachs et al. 1989). Dosage for elm was 4 g/tree, whereas for both eucalytpus species was 15 g/tree. Dosage varied in proportion to tree diameters and band widths of the application. A 4 g/tree dosage to 5″ DBH eucalyptus trees proved ineffective in reducing stem elongation.

Much higher concentrations, from 240 to nearly 5000 ppb, were found in the shoot and root tissues of oleanders (*Nerium oleander*) 7 months after treatment with a soil drench of 4 g per 15-gallon container (Table 3). The physiological significance of uniconazole-P concentrations in plant tissues, with respect to growth inhibition in the different species and tissues, awaits further experimentation.

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