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Simplified high-performance liquid chromatography method for the simultaneous analysis of tebuthiuron and hexazinone

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ABSTRACT

A simplified, high-performance liquid chromatography (HPLC) method for the simultaneous measurement of the herbicides tebuthiuron and hexazinone was developed. Separation was achieved on a Nova-Pak® phenyl (4 μm) 10 × 0.8 cm column with methanol–water (50:50 v/v) as eluent and on-line detection at 254 nm for tebuthiuron and 249 nm for hexazinone. At a flow-rate of 2.5 ml min^{-1}, the retention times were approximately 4.5 and 6.3 min for tebuthiuron and hexazinone, respectively. The procedure was used successfully for the analysis of residues of these herbicides in soil and plant tissues. A comparison with published procedures for the individual analysis of tebuthiuron and hexazinone is presented.

INTRODUCTION

The urea herbicide tebuthiuron {N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N′-dimethylurea} and the triazine herbicide hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] are used on non-cropland areas for the control of grasses, broadleaf weeds, and woody plants for brush control [1]. Because they have similar applications, there is the potential for plant and soil samples from sites undergoing brush control to contain residues of both tebuthiuron and hexazinone. High-performance liquid chromatographic (HPLC) methods have been developed for the separate analysis of residues of these herbicides in water, soil, and leaf tissue [2–6]. Two HPLC methods have been developed for the analysis of tebuthiuron, one utilizing a normal-phase column and the other a reversed-phase C_{18} column [2,3]. All the HPLC methods developed for analysis of hexazinone residues utilize reversed-phase columns with C_8 or C_{18} stationary phases, the latter methods requiring a column heater [4–7].

Although applicable for the individual analysis of tebuthiuron or hexazinone, the methods that use reversed-phase columns without column heaters did not adequately separate these analytes when both were present in the sample. This report presents a simple HPLC procedure for the separation and simultaneous analysis of tebuthiuron and hexazinone residues in water, soil and plant samples.

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EXPERIMENTAL[a]

Plant propagation and treatment

Pigweed (*Amaranthus retroflexus* L.) plants were started from seed in 0.32-l pots filled with 240 g of sandy-loam, greenhouse soil (pH 7.0, 3.9% organic matter). Plants were grown under a 16-h photoperiod with a photosynthetic photon flux density of 335 μmol m^{-2} s^{-1} and a day/night temperature of 25/20°C, watered as needed, and fertilized (after seedling establishment) every other watering with a dilute solution of 20-20-20 (N-P-K) fertilizer (0.25 g l^{-1}). Two weeks after emergence, the plants were thinned to 1 plant per pot.

Sixty-day-old plants (three replicates per treatment) were soil-treated with commercial formulations of tebuthiuron (Spike® 20 P, Elanco Products, Indianapolis, IN, U.S.A.) and hexazinone (Pronone® 75 P, Pro-Serve, Memphis, TN, U.S.A.) at 2.75 mg active ingredient (a.i.) per pot, which is equivalent to 3.36 kg ha^{-1} a.i. based on the surface area of the pot. Seven days after treatment, when the leaves began to abscise, the remaining leaves were removed from the stem, dipped into liquid nitrogen, freeze-dried, and stored at -5°C over silica gel. Stem tissue was removed at the soil line and discarded. A subsample of soil was dried at 105°C to determine moisture content, the roots separated from the soil, and the remaning soil stored at -5°C. Roots were washed with deionized water, lightly blotted dry, freeze-dried, and stored over silica at -5°C.

Extraction and partial purification

Soil (20 g equivalent dry weight) was extracted with 75 ml methanol–water (80:20, v/v) at room temperature by shaking for 1 h. The slurry was suction filtered through a Whatman 934-AH glass fiber filter, the soil re-extracted with 25 ml methanol–water (80:20, v/v) for 15 min, suction filtered, and the two extracts combined. The combined extracts were reduced to 2–3 ml at 45°C on a rotary evaporator, brought to a final volume of 4 ml with methanol, and filtered through a 0.45-μm polytetrafluoroethylene (PTFE) membrane syringe filter.

Plant tissue was milled to 0.5 mm in a Brinkmann (Westbury, NY, U.S.A.) ZM-1 centrifugal grinding mill, and extracted twice with shaking for 1 h in methylene chloride at a ratio of 1:100 (tissue–methylene chloride, w/v) at room temperature. The extracts were filtered through Whatman No. 1 filter paper, combined, evaporated to dryness under vacuum at room temperature, resuspended in 10 ml methylene chloride–hexane (50:50, v/v), and filtered through a 5-μm PTFE syringe filter. Semipurification of extracts was obtained using gel permeation chromatography (GPC), [8] with 5 ml of extract loaded on a 2.5 mm (I.D.) GPC column packed with 60 g of Bio-Beads® SX-3 (Bio-Rad, Richmond, CA, U.S.A.) in methylene chloride–hexane (50:50, v/v), providing a final bed length of 39 cm; the eluting solvent was methylene chloride–hexane (50:50, v/v) at a flow-rate of 5 ml min^{-1}. The first 125 ml of eluting solvent was discarded and the following 30 ml collected, evaporated to dryness under

[a] Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

vacuum at room temperature, resuspended in 5 ml methylene chloride, evaporated to dryness under vacuum at room temperature, resuspended in 500 μl of methanol, and filtered through a 0.2-μm PTFE syringe filter.

HPLC analysis

The HPLC system was composed of Waters Assoc. HPLC components: a system controller (Model 600E), an autosampler (Model 712), and a scanning, photodiode array UV detector (Model 990). The columns, solvents, and flow-rates used in this study are listed in Table I. Sample injection volumes were 50 μl for soils, roots, and leaves (untreated controls only), and 15 μl for leaf extracts from herbicide-treated plants. Detection was monitored from 220 to 300 nm, while the concentrations of tebuthiuron and hexazinone were determined based on their individual absorption maxima, *i.e.*, 254 and 249 nm for tebuthiuron and hexazinone, respectively. Plots of peak response *versus* amount of herbicide in the range of 1.25 to 2000 ng consistently gave linear responses with correlation coefficients of 0.98 or greater for both herbicides. (For presentation purposes, chromatograms were reproduced at an intermediate wavelength, *i.e.*, 252 nm).

RESULTS AND DISCUSSION

The published, reversed-phase methods for the individual analysis of tebuthiuron and hexazinone failed to provide adequate separation of these two compounds (Fig. 1). The two methods utilizing a C_8 column (Fig. 1 A and B) gave partial, but not baseline, separation of tebuthiuron and hexazinone. The C_{18} method resulted in tebuthiuron and hexazinone co-eluting, as determined by the increased peak height and absorption spectra. Of the methods tested, only the phenyl column gave baseline separation of tebuthiuron and hexazinone, with retention times of 4.5 and 6.3 min, respectively. The phenyl column method was used successfully in analyzing residues

TABLE I

HPLC COLUMNS AND SOLVENT SYSTEMS USED IN TESTING FOUR METHODS FOR THE SEPARATION OF TEBUTHIURON AND HEXAZINONE

Columns and prefilters are products of Waters Assoc.

Method	Column dimensions and packing	Eluent (v/v)	Prefilter	Flow-rate (ml min^{-1})	Ref.
A	10 cm × 8 mm (I.D.) Radial-Pak® C_8, 10-μm spherically shaped silica	acetonitrile–water (50:50)	C_{18} Guard-Pak®	1.2	5
B	10 cm × 8 mm (I.D.) Radial-Pak C_8, 10-μm spherically shaped silica	acetonitrile–water (45:55)	none	1.0	4
C	30 cm × 3.9 mm (I.D.) C_{18}, μBondapak®, 10-μm irregularly shaped silica	methanol–water (50:50)	none	1.0	3
D	10 cm × 8 mm (I.D.) Nova-Pak phenyl, 4-μm spherically shaped silica	methanol–water (50:50)	Phenyl Guard-Pak	2.5	This report

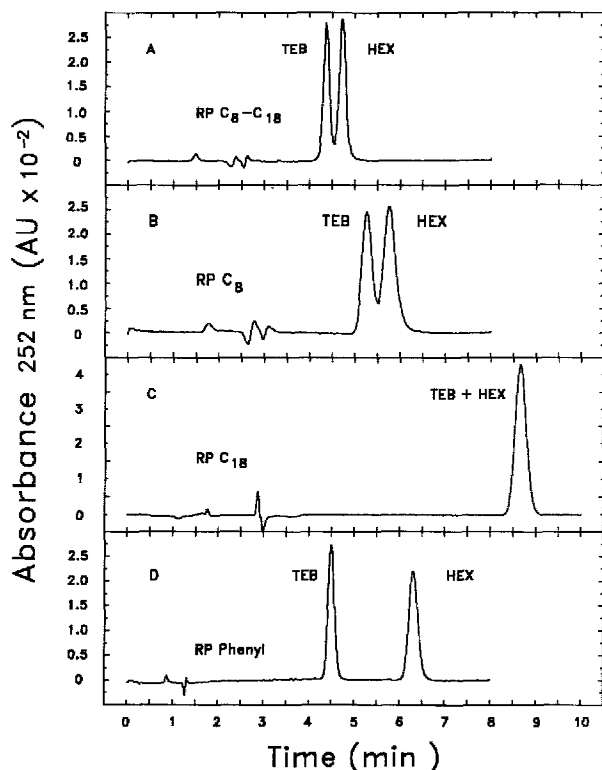

Fig. 1. Chromatographic separation of tebuthiuron (TEB) and hexazinone (HEX) using the HPLC methods described in Table 1. Each chromatogram respresents an injection of 0.12 μg of each herbicide.

of these herbicides in soil and plant tissue (Fig. 2). Given a detection limit (based upon peak response for spiked, control extracts) of 1.25 ng, the amount of material extracted, and the final volume of the extracts, the minimum detection limit for tebuthiuron and hexazinone on the phenyl column system was 0.1, 0.02, and 0.005 μg g^{-1} for roots, leaves, and soil, respectively. The recovery rates of tebuthiuron and hexazinone, from control soil and leaf samples (two replicates each) spiked with 1 μg each of tebuthiuron and hexazinone and extracted and partially purified as described above, were 99 and 100 ± 2% and 98 and 102 ± 2%, respectively. These recovery rates are higher than those reported for other HPLC methods for tebuthiuron [3] and hexazinone [5], but similar to that reported for a gas chromatographic method for hexazinone [7].

Seven days after treatment with 3.36 kg ha^{-1} of each herbicide, tebuthiuron and hexazinone were more concentrated in the leaves than in the roots or soil (Table II). The distribution of tebuthiuron and hexazinone in pigweed was similar to that reported for tebuthiuron in common ragweed (*Ambrosia artemisiifolia* L.) and rye (*Secale cereale* L. 'Elbon') 1 day after treatment [9], but the reverse of that reported for tebuthiuron and hexazinone in bur oak (*Quercus macrocarpa* Michx.) and eastern redcedar (*Juniperus virginiana* L.) 3 days after treatment [10].

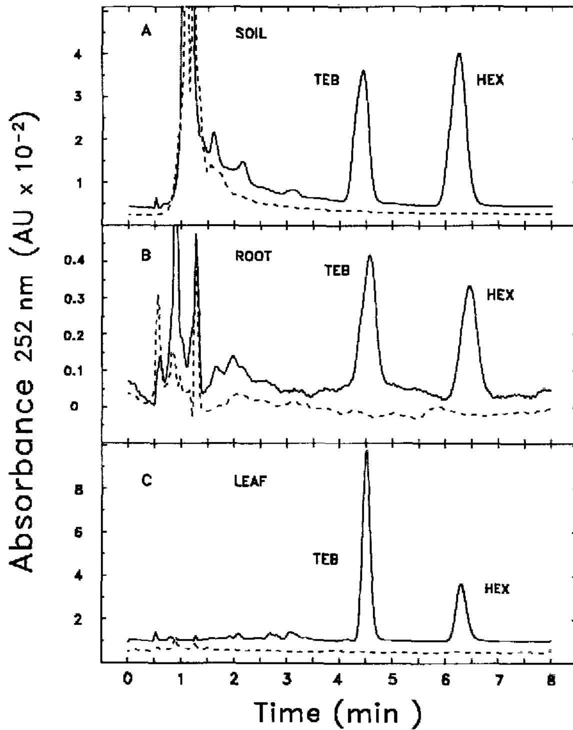

Fig. 2. Chromatograms of extracts from soil (A) and pigweed (*Amaranthus retroflexus* L.) root (B) and leaf (C) tissue 7 days after treatment with 3.36 kg ha^{-1} (a.i.) of soil-applied tebuthiuron (TEB) and hexazinone (HEX). Dotted lines represent untreated control samples and solid lines represent herbicide-treated samples.

TABLE II

RESIDUES OF TEBUTHIURON AND HEXAZINONE IN SOIL AND PIGWEED (*AMARANTHUS RETROFLEXUS* L.) TISSUES SEVEN DAYS AFTER TREATMENT WITH 3.36 kg ha^{-1} (A.I., SOIL-APPLIED) OF EACH HERBICIDE

Values represent the average of three replicates ± 1 standard error of the mean.

Sample		Tebuthiuron ($\mu g\ g^{-1}$)	Hexazinone ($\mu g\ g^{-1}$)
Soil			
	Control	0	0
	Treated	1.83 ± 0.30	2.06 ± 0.30
Roots			
	Control	0	0
	Treated	3.68 ± 0.68	4.72 ± 1.32
Leaves			
	Control	0	0
	Treated	21.4 ± 8.2	8.9 ±2.9

The method described here, which utilizes a reversed-phase phenyl column and an isocratic mobile phase of methanol–water (50:50, v/v), gave excellent separation of tebuthiuron and hexazinone, and is applicable to the simultaneous analysis of these herbicides in soil and plant tissue.

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REFERENCES

1 N. E. Humburg (Editor), *Herbicide Handbook of the Weed Science Society of America*, Weed Science Society of America, Champaign, IL, 6th ed., 1989.
2 A. Loh, R. Frank and O. D. Decker, *Anal. Methods Pestic. Plant Growth Regul.*, 11 (1980) 351.
3 A. E. Smith, Jr., L. M. Shuman and N. Lokey, *J. Agric. Food Chem.*, 32 (1984) 416.
4 C. E. Parker, C. A. Haney, D. J. Harvan and J. R. Hass, *J. Chromatogr.*, 242 (1982) 77.
5 D. C. Bouchard and T. L. Lavy, *J. Chromatogr.*, 270 (1983) 396.
6 T. H. Byast, *J. Chromatogr.*, 134 (1977) 216.
7 C. L. McIntosh, D. D. Schlueter and R. F. Holt, *Anal. Methods Pestic. Plant Growth Regul.*, 13 (1984) 267.
8 M. L. Hopper, *J. Agric. Food Chem.*, 30 (1982) 1038.
9 W. G. Steinert and J. F. Strizke, *Weed Sci.*, 25 (1977) 390.
10 W. K. McNeal, J. F. Strizke and E. Basler, *Weed Sci.*, 32 (1984) 739.