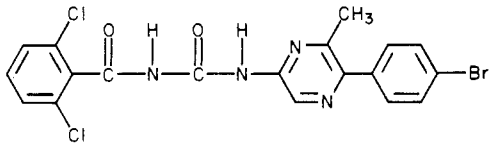
Development of an Analytical Procedure for an Insect Growth Regulator (EL-494) Employing High-Pressure Liquid Chromatography and Its Application on Residues in Alfalfa

Abdalla H. Abdel Monem and Ralph O. Mumma*

A procedure was developed for the analysis of an insect growth regulator (EL-494) employing high-pressure liquid chromatography (LC). The plant tissue was washed and extracted with acetone. The extract was concentrated and cleaned up by using a Florisil column. The concentrated eluate was analyzed by high-pressure LC employing a μBondapak C_{18} column and a methanol-water solvent system. EL-494 was detected by ultraviolet adsorption and the lower limit of detection was 10 ng. For demonstration of the practical application of this procedure, samples of alfalfa, field treated with a 50% wettable powder formulation of EL-494 at three concentrations (283.5, 567.0, and 1134.0 g of AI/ha), were analyzed over a 9-week period. Recoveries of spiked samples varied from 94.9 to 96.2% EL-494 (from 50 to 5000 ng) with a lower limit of detection of 0.025 ppm.

EL-494, *N*-[[[5-(4-bromophenyl)-6-methyl-2-pyrazinyl]amino]carbonyl]-2,6-dichlorobenzamide (I)



I

(Elanco Products Co., Indianapolis, IN) is a molt-inhibiting insect growth regulator. EL-494 has demonstrated insecticidal properties against the southern armyworm, *Spodoptera eridania* (Cramer), tobacco budworm, *Heliothis virescens* (F.), fall armyworm, *S. frugiperda* (J. E. Smith), yellow fever mosquito, *Adedes aegypti* (L.), housefly, *Musca domestica* (L.) (Lilly Research Laboratory, 1977), and gypsy moth *Lymantria dispar* (L.) (Abdel-Monem et al., 1980).

Several residue procedures have been developed for the analysis of a chemically related insecticide. DiPrima et al. (1978) reported the analysis of diflubenzuron, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, in water, soil, sediment, aquatic and forest foliage, fish and shellfish, agricultural crops, milk, eggs, and animal tissue. The samples were extracted with acetonitrile, cleaned up with a Florisil–sodium sulfate solumn, and analyzed by high-pressure liquid chromatography (LC) employing ultraviolet detection. Schaefer and Dupras reported the stability and persistence of diflubenzuron in water (Schaefer and Dupras, 1976) and determined the residues of diflubenzuron in pasture soil, vegetation, and water following aerial application (Schaefer and Dupras, 1977), utilizing high-pressure LC. Oehler and Holman (1975) analyzed the residue of diflubenzuron in bovine manure by high-pressure LC, and the samples were cleaned up by liquid–liquid partition and elution through a Florisil column.

We now report the development of an analytical procedure for EL-494 utilizing high-pressure LC. The practical application of this procedure was demonstrated in the analysis of EL-494 residues on alfalfa.

MATERIALS AND METHODS

Reagent. All solvents were distilled in glass (Burdick and Jackson) and filtered through a 0.45-μm Millipore filter before use. Water was doubly distilled and also filtered prior to use. Florisil (60–100 mesh) was manufactured by Floridin Co., Hancock, WVA.

Instruments. A Model ALC/GPC 244 high-pressure liquid chromatograph equipped with a 6000-A pump, a U6K injector, and a 440 UV detector (Waters Associates, Inc., Milford, MA) was used. Absorbance of eluted compounds was recorded at 254 nm. A 30 cm × 4 mm i.d. μBondapak C_{18} column was used with a methanol–water (80:20 v/v) solvent at a flow rate of 1.0 mL/min. Chromatography was conducted at room temperature using a pressure of 1000 psi.

Spray Application. Samples (leaves and stems) were collected from treated and untreated alfalfa fields (The Pennsylvania State University, Rock Spring, PA). A single application (in triplicate) of three concentrations of 50% wettable powder EL-494 (283.5, 567.0, and 1134.0 g of AI/ha) was applied on June 22, 1979, under good to excellent conditions with a compressed-air hand sprayer (six nozzles). The controls were sprayed with water. A completely random design with three blocks and four treatments was used in the alfalfa field. The experimental unit used was a 20.2-m^2 plot. Replicate samples were randomly collected weekly from each plot for residue analysis for the entire 9-week period. Samples were stored under refrigeration (ca. −20 °C) prior to analysis.

Sample Extraction. Leaves and stems (20 g) of alfalfa were washed 3 times (30, 30, and 40 mL) with acetone, and the acetone was combined and worked up separately. The washed plant tissue was placed in a 1.06-L blender jar, 100 mL of acetone was added, and the mixture was blended for 5 min. The homogenate was filtered under reduced pressure (aspirator) through glass wool. The filtrate was transferred to a 250-mL round-bottom flask and concentrated to dryness in a rotary evaporator (aspirator) at 40 °C.

Florisil Column Chromatography. Sodium sulfate (2 g) was added to a glass wool plugged column (1 × 50 cm), followed by 7 g of Florisil and an additional 2 g of anhydrous sodium sulfate. The column was prewashed with 20 mL of methylene chloride and the washings were discarded. The dried extract, contained in the 250-mL flask, was transferred with methylene chloride (15 and 20 mL) to the column. Suction (aspirator) was used to increase the flow rate. The methylene chloride eluate was discarded. The column was then washed with 50 mL of chloroform, and this eluate was collected in a 100-mL round-bottom flask and evaporated to dryness on a rotary evaporator (aspirator) at 40 °C. The residues were dis-

Pesticide Research Laboratory and Graduate Study Center, Department of Entomology, The Pennsylvania State University, University Park, Pennsylvania 16802.

0021-8561/81/1429-0075$01.00/0

Table I. Reproducibility of the Analytical Method for EL-494 at Three Spiked Concentrations

no. of samples	sample wt, g	fortified level, μg	final sample vol, mL	injected vol, μL	$\bar{x}$ (EL-494 recovered), ng	SD	CV, %[a]	% recovery
9	20	2.5	1	20	47.5	2.6	5.5	94.9
9	20	50.0	1	10	474.4	27.7	5.8	94.9
9	20	2500.0	1	2	4810.5	117.0	2.4	96.2

[a] % CV = (SD/$\bar{x}$) × 100.

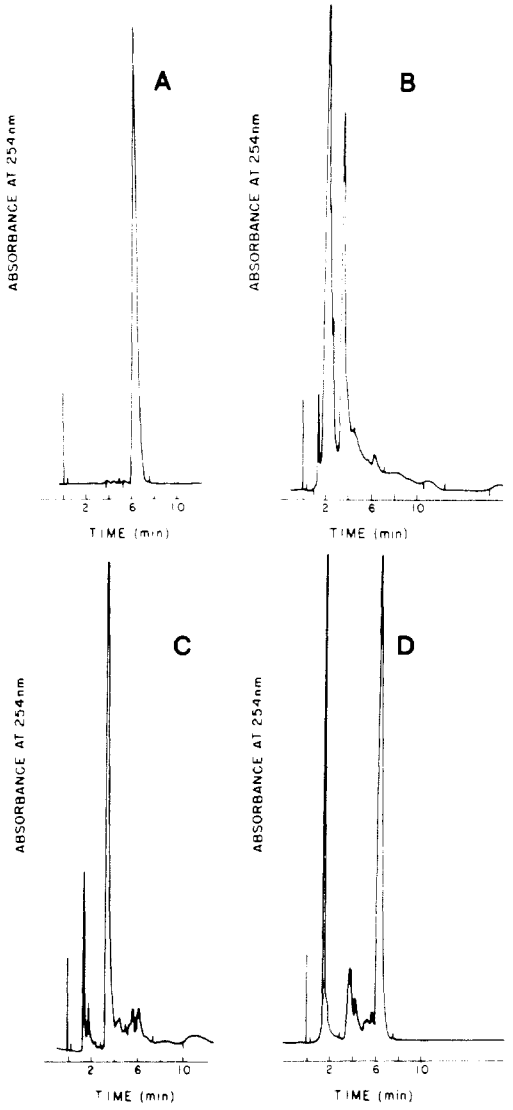


Figure 1. High-pressure liquid chromatograms: (A) EL-494 (standard), (B) an extract of alfalfa plants fortified with EL-494 without cleanup, (C) the methylene chloride eluate from the Florisil column, and (D) the chloroform eluate from the Florisil column. A μBondapak C_{18} column was used with a methanol–water (80:20 v/v) solvent system at a flow rate of 1.0 mL/min.

solved in 1 mL of chloroform, placed in 2-mL vials, and stored at −20 °C prior to high-pressure LC analysis. For recovery tests the untreated control samples were fortified with a known amount of EL-494 before the addition of the extraction solvent.

High-Pressure LC Analysis. The mobile phase used for high-pressure LC was methanol–water (80:20 v/v) with a flow rate of 1.0 mL/min. A solution containing 10 ng/μL EL-494 was used as a standard to determine the retention time and establish operating conditions. The injection volume was usually 2–20 μL. A series of standards ranging from 10 to 20 000 ng was used to determine the response vs. nanograms of EL-494. The peak areas were determined

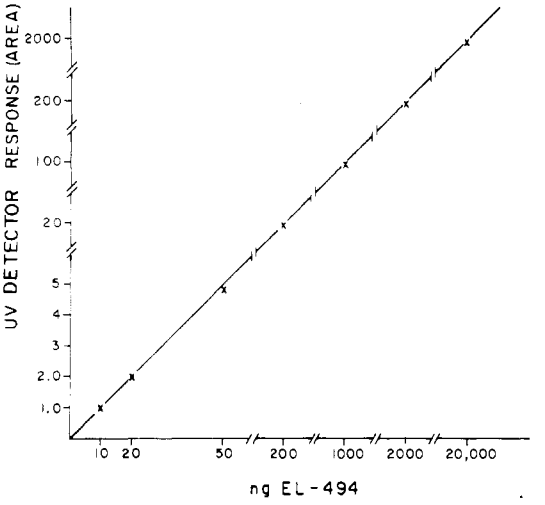


Figure 2. Response of the UV detector to EL-494.

electronically via a Spectra Physic Autolab Minigrator and the nanograms of EL-494 were calculated from the calibration curve.

RESULTS AND DISCUSSION

EL-494 can be readily analyzed by high-pressure LC employing a reverse-phase column (μBondapak C_{18}) with a methanol–water solvent system (80:20 v/v). These conditions produce a sharp EL-494 peak in ~6 min (Figure 1A). The lower limit of detection is 10 ng of EL-494 and corresponded to a lower limit of 0.025 ppm of EL-494 in the 20-g samples of alfalfa that were used for analysis. A linear response was obtained over the entire concentration range (10–20 000 ng) (Figure 2).

Plant samples (20 g) were washed with acetone and subsequently blended in acetone to determine if a washing technique would be sufficient to remove all of the EL-494 residue. Both extracts were analyzed separately. A scheme of the extraction procedure and Florisil column cleanup is illustrated in Figure 3. The acetone wash did not require cleanup prior to analysis by high-pressure LC. Figure 1B shows a chromatogram of an extract of a fortified sample before cleanup on a Florisil column. The sample contained many large peaks representing the impurities (mostly early peaks), and EL-494 was detectable but as a minor peak. The methylene chloride eluate of the Florisil column removed most of the impurities (Figure 1C) but no EL-494. The chloroform eluate, following the methylene chloride eluate, contains primarily EL-494 (Figure 1D). Nine samples each were spiked with three concentrations of EL-494 (2.5, 50, and 2500 μg) to determine the recovery and the reproducibility of this technique (Table I). The recovery ranged between 94.9 and 96.2%, and the coefficient of variation (CV) varied from 2.4% at 2500 μg to 5.5% at 2.5 μg (Table I).

For demonstration of the applicability of the method, plots in an alfalfa field were sprayed with three concentrations of EL-494, and samples were collected weekly over a 9-week period. Table II shows the percentage of EL-494 found in the acetone wash and the acetone extract. At

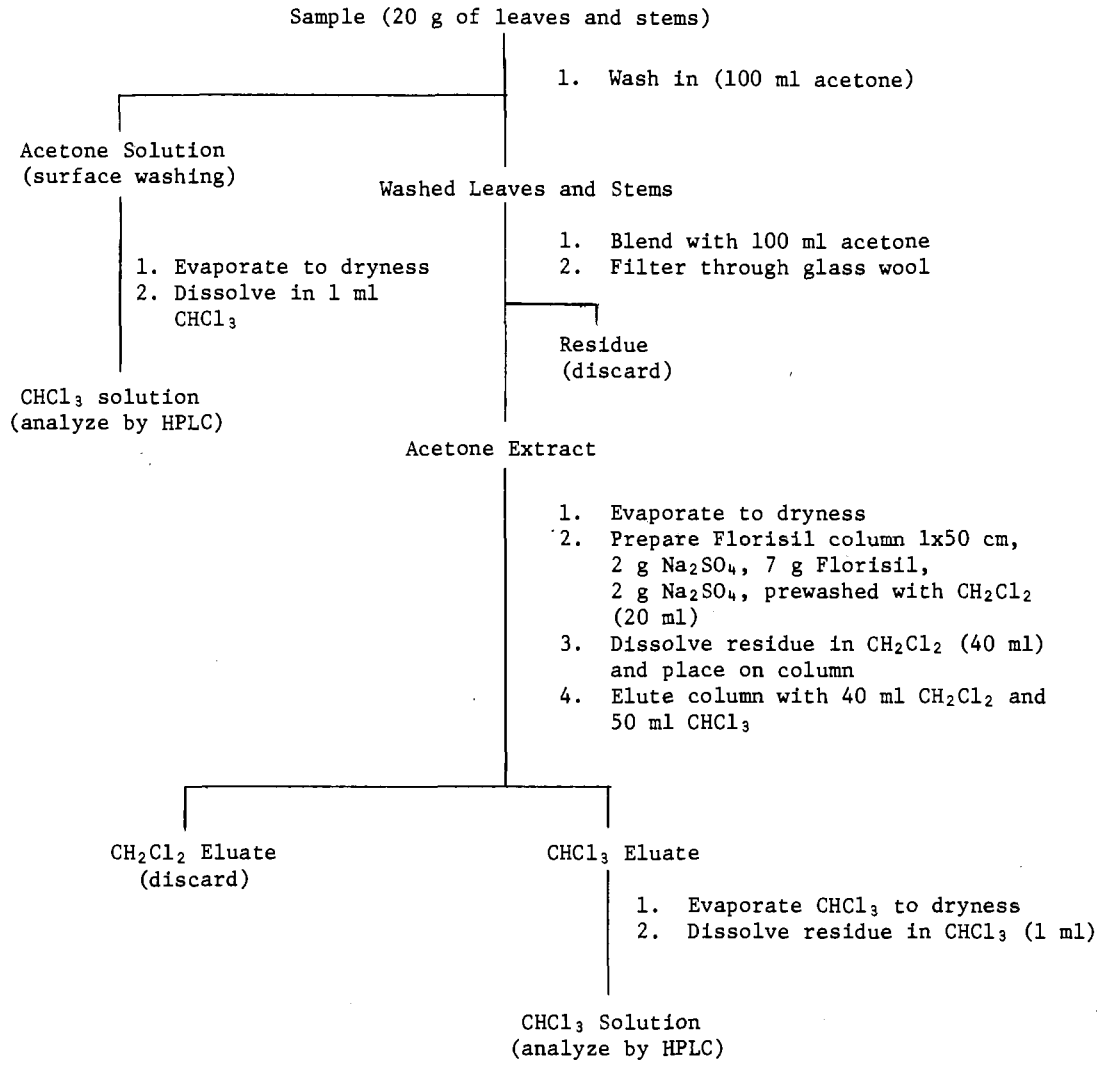


Figure 3. Scheme of the developed extraction and cleanup procedure for EL-494.

Table II. Percentage Composition of EL-494 Residues in the Acetone Wash and the Acetone Extract of Alfalfa

	% residue composition[a]					
	233.5 g of EL-494/ha		567.0 g of EL-494/ha		1134.0 g of EL-494/ha	
time, weeks	acetone wash	acetone extract	acetone wash	acetone extract	acetone wash	acetone extract
0	100.0	0.0	100.0	0.0	100.0	0.0
1	91.5	8.5	93.0	7.0	93.9	6.1
2	30.2	69.8	29.3	70.7	30.0	70.0
3	37.7	62.3	37.6	62.4	40.6	59.4
4	40.4	59.6	36.6	63.4	41.1	58.9
6	40.7	59.3	32.1	67.9	38.5	61.5
9	41.2	58.8	36.8	63.2	38.9	61.1

[a] Each value represents the average of triplicates.

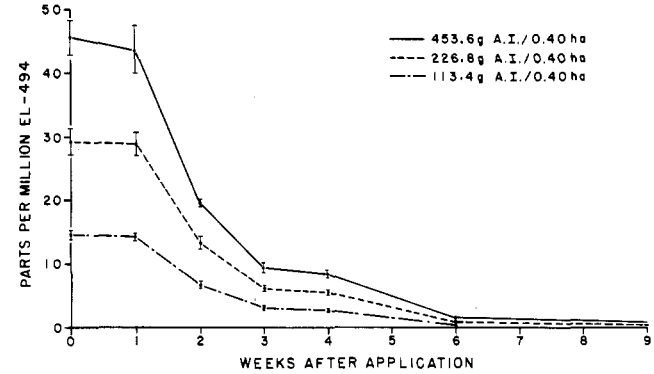


Figure 4. Disappearance curves for three applied concentrations of EL-494 on alfalfa.

application time the acetone wash removed 100% of the EL-494 residues, and after 1 week the acetone wash still removed ~91% of the residue. However, by the second week ~30% of the residue was found in the leaf extract and this increased with time. Figure 4 represents the disappearance curves for the three concentrations of EL-494 in an alfalfa field. After 9 weeks a 20-g sample contained less than 1 ppm at all applied concentrations. Nevertheless, 2–8 ppm of EL-494 was present in the plant after 4 weeks. The reduction in residue of EL-494 does not necessarily reflect metabolism but includes weathering and dilution by plant growth since only 20-g samples were used for analysis. However, it should be noted that even after 9 weeks enough EL-494 remained to theoretically control insects such as the gypsy moth (Abdel-Monem and Mumma, 1980).

This paper presents a rapid, simple, and sensitive high-pressure LC procedure for the analysis of EL-494. The applicability of this procedure was demonstrated on the analysis of EL-494 residues on alfalfa, but the procedure should be applicable to residues on other plants. A disappearance curve for EL-494 on alfalfa was generated which potentially provides information for making rational decisions on the suitability of using EL-494 in a single application method for control of insect pests on alfalfa.

ACKNOWLEDGMENT

Appreciation is expressed to Dr. Wallace E. Rogers, Dr. Philip Brewer, and Eli Lilly Research Corp. for financial support and for samples of EL-494.

LITERATURE CITED

Abdel-Monem, A. H.; Cameron, E. A.; Mumma, R. O. *J. Econ. Entomol.* **1980**, *73*, 22-26

DiPrima, S. J.; Cannizzaro, R. D.; Roger, J. C.; Ferrell, C. D. *J. Agric. Food Chem.* **1978**, *26*, 968.

Lilly Research Laboratory, Indianapolis, IN 46206, 1977, Technical Report on EL-494, 1-4.

Oehler, D. D.; Holman, G. M. *J. Agric. Food chem.* **1975**, *23*, 590.

Schaefer, C. H.; Dupras, E. F., Jr. *J. Agric. Food Chem.* **1976**, *24*, 733.

Schaefer, C. H.; Dupras, E. F., Jr. *J. Agric. Food Chem.* **1977**, *25*, 1026.

Received for review April 28, 1980. Accepted September 15, 1980. Authorized for publication as paper no. 5959 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Supported in part by Northeastern Regional Research Project NE-115 and Regional Research Funds.

Determination of Mosquito Chemosterilant Recovered from Air during Real and Simulated Use

David A. Carlson* and Donald L. Bailey

Many studies have documented volatilization of pesticides from water and treated surfaces, especially water-insoluble chlorinated hydrocarbons. We report on recovery of a water-soluble chemosterilant, bisazir [*P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide] from the air during simulated use. When samples were obtained by drawing air at 0.5 L/min through porous polymer sampling tubes suspended above pans containing 1% solutions of the material, analysis by GLC–FPD showed concentrations in air of 100–700 ng/L. When this sampling method was used at a remote work station, 9–54 ng/L of the material was recovered from the air during and after working hours in the work area. Installation of a second vent fan reduced quantities found by 50–80% in three tests. The vapor pressure of this chemosterilant was estimated by comparison of GLC retention times of *n*-paraffins to be 5 μm (5×10^{-3} mmHg) at 25 °C, which is considered highly volatile. Vapor pressures of hempa, tepa, and thiotepa were found to be 19.5, 3.5, and 1.5 μm, respectively, at 25 °C by this same method.

Chemosterilants have been used as genetic alkylating agents for a number of years in laboratory and field demonstrations of the sterile male technique. Potential users of chemosterilants should be aware that the physical appearance of chemicals, whether white solid or viscous liquid, does not mean that they are nonvolatile at room temperature. Water solubility does not preclude the loss to the surroundings of a hydrophilic chemical from nonaqueous or aqueous solution. Male stable flies, *Stomoxys calcitrans* (LaBrecque and Meifert, 1975), house flies, *Musca domestica* (Meifert and LaBrecque, 1977), *Culex pipiens quinquefasciatus* mosquitoes (Patterson et al., 1970), and *Anopheles albimanus* (Bailey et al., 1979) mosquitoes have been reared in large numbers and sterilized, the last by immersing pupae for 1 h in a 1.0% solution of an appropriate chemosterilant. Gas chromatographic (GLC) analyses of treated pupae ensured that metabolism had occurred and that residues declined to a minimum level before release of adult insects in the field (Bowman and Beroza, 1966).

Jensen and Schall (1966) determined GLC retention times of 24 herbicides, all esters of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. They used a nonpolar column of 3% SE-30 and found that calculated vapor pressures correlated well with experimentally determined vapor pressures. Since plots of the familiar equation log P vs. $1/T$ showed these data to be linear, extrapolation to 25 °C gave values close to those determined by the Knudsen method of diffusion through a pinhole (Knudsen, 1909; Mullison and Hummer, 1949). Many studies of airborne pesticides and herbicides have been made by trapping vapors in cold traps, ethylene glycol filled impingers, or on various solid substrates including Tenax GC, Porapak Q, and Chromosorb 101 (Seiber et al., 1975) and Chromosorb 102 (Thomas and Seiber, 1974), but there are no previous reports of recovery of chemosterilants from the surroundings during their use.

Our preliminary experiments showed unexpectedly large recoveries of chemosterilant from the air in the laboratory and in a remote working area near aqueous solutions of bisazir. The desire to demonstrate reduced exposure of workers to bisazir prompted confirmation of this phenomenon. For that reason multiple replications for statistical purposes were not considered essential. We report on methods of trapping and quantitation of one such compound recovered from air and its loss to air from aqueous solution. Samples were taken from an actual work site for analysis. Finally, the vapor pressures of several chemosterilants were estimated by gas chromatography.

MATERIALS AND METHODS

A 1% solution in water of a thioaziridine chemosterilant, *P,P*-bis(1-axiridinyl)-*N*-methylphosphinothioic amide (hereafter referred to as bisazir) was used for recovery studies.

Solvents used were reagent-grade chloroform and ethylene glycol (Fisher); *n*-hexane (Phillips) was washed with concentrated sulfuric acid and water and distilled from metallic sodium. Chromosorb 102 (Johns-Manville; 60–80 mesh) was used in glass traps.

Loss Trials Using Ethylene Glycol Traps. A 1-pt paper cup filled with 200 mL of a 1% solution of bisazir

Insects Affecting Man and Animals Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Gainesville, Florida 32604.