Rapid and Sensitive High-Performance Liquid Chromatographic Method for the Quantitation of Abamectin and Its Delta 8,9-Isomer

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A rapid, specific, and sensitive method has been developed for the determination of abamectin and its delta 8,9-isomer in tomatoes. Sample homogenization and extraction are performed simultaneously with acetonitrile/water/hexane (1:1:5). Abamectin and its delta 8,9-isomer are selectively concentrated by passing the hexane layer through an aminopropyl solid-phase extraction cartridge. The abamectin and delta 8,9-isomer are then eluted from the cartridge and derivatized by an instantaneous reaction with trifluoroacetic anhydride (TFAA)/1-methylimidazole. Quantitation is achieved by HPLC with fluorescence detection. A set of 12 samples can be analyzed by this rapid method in 4 h; conventional fluorescence methods require a much longer time. The recovery of abamectin at levels between 5 and 70 ppb averaged 95%, and the recovery of delta 8,9-isomer at levels between 5 and 67 ppb averaged 84%. For each analyte a quantitation limit of 5 ppb and a limit of detection of 2 ppb were obtained. The method was used to quantitate the incurred residue in the tomato samples from a field trial. Very good agreement was observed between this method and the conventional fluorescence method.

INTRODUCTION

The avermectins are macrocyclic lactones produced by the soil microorganism *Streptomyces avermitilis* (Burg et al., 1979). Two avermectins have been commercialized to date (Fisher, 1990). The semisynthetic 22,23-dihydroavermectin B_1 known as ivermectin is used as an antiparasitic drug in animals and man; avermectin B_1 known as abamectin is widely used as an insecticide and miticide for agricultural crops.

The structure of abamectin, which is a mixture of two homologues, containing not less than 80% avermectin B_{1a} and not more than 20% avermectin B_{1b}, is shown in Figure 1. These homologues differ by only one methylene group in the C-25 side chain. B_{1a} contains a *sec*-butyl group, and B_{1b} contains an isopropyl group. Abamectin has been shown to have excellent activity against phytophagous mites and selected insect species of economic importance in agriculture (Putter et al., 1981; Dybas, 1983). In both laboratory and field studies, abamectin is rapidly degraded by exposure to light and air (Mrozik et al., 1988; Bull et al., 1984) to a more stable compound—the geometrical delta 8,9-isomer (Figure 1), which is the major photodegradate of abamectin on a variety of agricultural matrices. To establish a tolerance for the registered use of abamectin on agricultural crops, the U.S. Environmental Protection Agency requires residue data on the total toxic residue which includes parent abamectin and its delta 8,9-isomer.

Several analytical methods have been reported in the literature to quantitate avermectins by HPLC, with various detection techniques (Tolan et al., 1980; Tway et al., 1984; Chiu et al., 1985; Fox and Fink, 1985; Demchak and MacConnell, 1990). The fluorescence detection methods involve formation of a highly fluorescent derivative by the reaction of avermectins with trifluoroacetic anhydride (TFAA) in the presence of catalytic amounts of *N*-methylimidazole. When this reaction is performed in DMF, the reaction is slow, requiring 1.5 h for the derivatization and further silica column cleanup.

Recently, a more rapid HPLC method has been described (Vuik, 1991) for the detection of abamectin in cucumber. The method employs UV detection based on

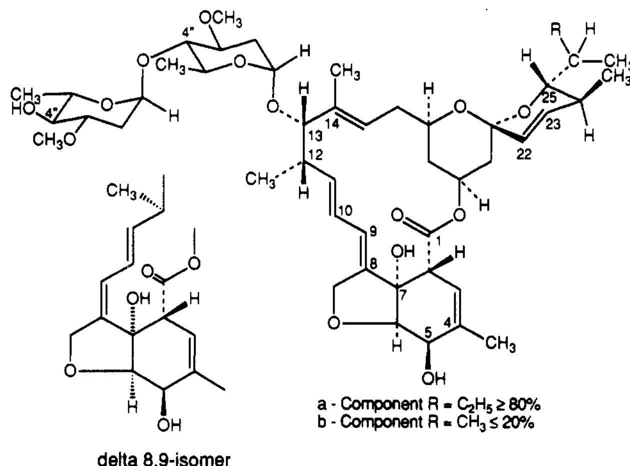

Figure 1. Structures of abamectin and its delta 8,9-isomer.

the conjugated-diene chromophore of abamectin which has an absorbance maxima at 245 nm. This method, however, lacks sensitivity (the detection limit is 40 ng/g) and specificity in that it does not measure the delta 8,9-isomer. In addition, Vuik's method, when employed with tomato samples, showed matrix peaks that interfered with and prevented quantitation of abamectin. The quantitative determination of abamectin in tomatoes is important to establish a tolerance for the registered use of the abamectin on tomatoes, but an improved method was of particular interest to us to save labor and materials compared to the fluorescence derivatization LC method previously used in our laboratory (Analytical Research, MSDRL, 1988).

In the present work a specific, sensitive, and rapid method is described for the determination of abamectin and its delta 8,9-isomer in tomatoes. The method uses a novel homogenization/extraction scheme, along with NH_2-propyl solid-phase extraction cleanup and instantaneous fluorescence derivatization. The derivatization step uses the trifluoroacetic anhydride (TFAA)/1-methylimidazole (NMIM)/acetonitrile (ACN) reagent system used previously for the derivatization of ivermectin (deMontigny et al., 1990) and 4″-deoxy-4″-(epimethylamino)avermectin

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B_1 benzoate (Prabhu et al., 1991). The method requires no more than 4 h for analysis of 12 samples (from the initial extractions to loading an autoinjector for the HPLC analysis). This method has been validated by analysis of control, fortified, and incurred residue samples. Good agreement was observed between this rapid method and the conventional tomato method tested on field trial samples.

EXPERIMENTAL PROCEDURES

Apparatus and Reagents. (*a*) *Liquid Chromatograph.* A Spectra-Physics (SP) Model SP8700XR solvent delivery pump, SP Model SP8780XR autosampler, and Kratos-Schoeffel Instruments Model FS950 fluorescence detector with a SP Model 4200 integrator were used.

(*b*) *Analytical Column.* A 15 cm length × 4.6 mm i.d. ES Industries Chromega-bond, C_{18} column, 3-μm particle size, was used. A column heater such as the FiAtron CH-30 with a TC-55 controller was used to maintain 30 °C column temperature. A 7 μm, 15 mm length, 3.2 mm i.d., C_{18} standard Brownlee Labs guard column (RP-18 OD-GU) obtained from Rainin Instruments Co., or equivalent, was used before the analytical column.

(*c*) *Detector settings* used were as follows: Kratos Instruments FS950 with excitation lamp, FSA110; blue phosphor coated Hg vapor lamp; standard Kratos flow cell, FSA210; excitation filter, FSA403, 365-nm bandpass filters with emission filters, FSA426, 418-nm cutoff filters; sensitivity range, 0.02 μA or higher; time constant, 6.

(*d*) *Solvents* were of HPLC grade (Burdick & Jackson), distilled in glass.

(*e*) *Mobile phase* used was 5% H_2O in methanol (v/v), isocratic at flow rate of 1.5 mL/min.

(*f*) *Derivatization Reagents.* Trifluoroacetic anhydride (TFAA) was obtained from Pierce and Co. and *N*-methylimidazole (NMIM) from Aldrich Chemical Co.

(*g*) *Both the avermectin B_{1a} delta 8,9-isomer and avermectin B_1* reference standards are in glycerol formal solution. The avermectin B_{1a} delta 8,9 reference standard solution contains 0.38% (w/w) of the delta 8,9-isomer. The avermectin B_1 reference standard solution contains 0.956% (w/w) avermectin B_{1a} and 0.071% (w/w) avermectin B_{1b}. The reference standards were obtained from Merck Sharp & Dohme Research Laboratories (MSDRL).

(*h*) *Solid-Phase Extraction Cartridges.* Aminopropyl (NH_2), 500 mg/3 mL, cartridges from Analytichem International were used.

Extraction and Cleanup Procedure. A flow diagram for the assay procedure is shown in Figure 2. The avermectin B_1 and its delta 8,9-isomer residues are extracted by blending with a Polytron (Brinkman) a 10-g (previously processed) tomato sample with 14 mL of a acetonitrile/water/hexane (1:1:5) mixed solvent for a minimum of 30 s in a 50-mL centrifuge tube. The Polytron probe is subsequently rinsed by blending with 12 mL of hexane/water (5:1) in another 50-mL tube. The rinse is subsequently added to the initial sample tube. To the empty tube is added an additional 10 mL of hexane, and once again the probe is rinsed by blending and saved for a later step. The samples are shaken for 1.5 min and then centrifuged for 3 min. After centrifugation, the hexane (upper) layer is separated and transferred to a clean 50-mL centrifuge tube with the help of a disposable pipet. The sample is reextracted with the 10 mL of hexane which was previously used to rinse the Polytron probe; the solids are resuspended prior to the second extraction. The hexane extracts are combined (approximately 30 mL from each sample) and loaded onto an aminopropyl solid-phase extraction cartridge, previously conditioned with hexane, to retain the avermectins. The hexane eluant is discarded. The column is washed sequentially with hexane, toluene, and methylene chloride and is eluted with 10 mL of 50% acetone in methylene chloride into a 15-mL graduated silanized centrifuge tube. This eluant is evaporated to dryness using nitrogen purging at 70 °C. To each sample tube is added approximately 1 mL of acetonitrile, the mixture is vortexed, and the tubes are sonicated.

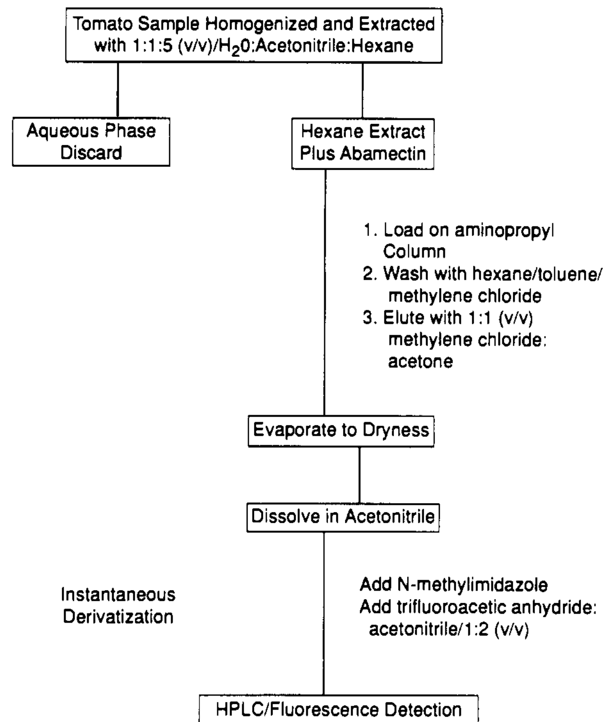

Figure 2. Flow diagram for the analysis of abamectin and its 8,9-isomer in tomato samples.

Derivatization of Samples and Standards. The derivatization procedure is identical to the one used for the derivatization of 4″-deoxy-4″-(epimethylamino)avermectin B_1 benzoate (MK-0244) (Prabhu et al., 1991). The stock and standard solutions are prepared in acetonitrile. To prepare 2.0, 4.0, 6.0, 8.0, and 10.0 ng/mL calibration curve standards for derivatization, 0.2, 0.4, 0.6, 0.8, and 1.0 mL, respectively, of a 50 ng/mL avermectin B_{1a} standard solution are transferred to separate silylated 15-mL tubes. To each sample and standard test tube is added 0.1 mL of the NMIM reagent with a graduated pipet. The tubes are stoppered, vortexed, sonicated, and centrifuged each for 30 s. The sample and standard tubes and a freshly prepared TFAA/ACN (1:2) reagent tube are placed in an ice bath for 5 min. After cooling, the tubes are removed from the ice bath, and 0.3 mL of freshly prepared (TFAA/ACN) reagent is added to each sample and standard tube using a volumetric pipet. Care should be taken not to contaminate tubes with water since the derivatization reaction is very susceptible to water. The tubes are stoppered, vortexed, and centrifuged, and the contents are diluted to 5 mL or an appropriate volume based on the expected residue levels (controls and standards to 5 mL).

Quantitation. Standards are injected at the beginning and at the end of the analysis of a set of samples to evaluate stability of the HPLC system, the standards, and the samples. The avermectin levels are determined by reversed-phase liquid chromatography with fluorescence detection and quantitated by peak height measurement. A new set of standards is included with each sample set because the derivatized standards are not stable under long-term storage, and there are slight differences from day to day in the yield of the fluorescent product. Residues of avermectin B_{1b}, avermectin B_{1a}, and the delta 8,9-isomer are quantitated using the avermectin B_{1a} standard curve. The concentration of avermectin B_{1a}/delta 8,9-isomer or avermectin B_{1b} in a residue sample (unknown) is determined as

$$\text{unknown} = C \times \text{FV}/\text{SW}$$

where C is the concentration of avermectin B_{1a}/avermection delta 8,9-isomer (ng/mL) in the final volume used for HPLC analysis calculated using a least-squares fit to the standards, FV is the final volume for HPLC analysis, and SW is the sample weight.

RESULTS AND DISCUSSION

Typical HPLC fluorescence chromatograms of an abamectin standard and control tomato sample are shown

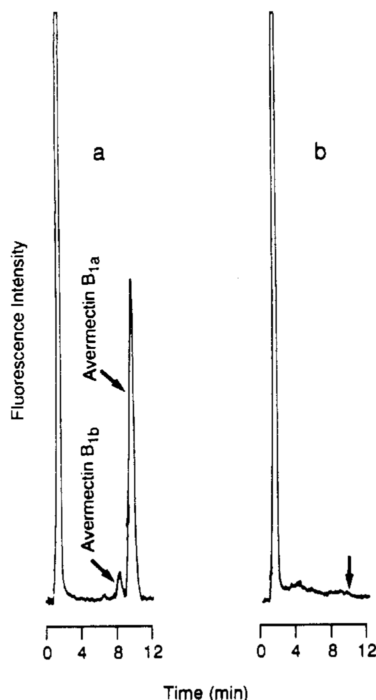

Figure 3. Typical chromatograms: (a) abamectin standard, 10 ng/mL; (b) control tomato sample.

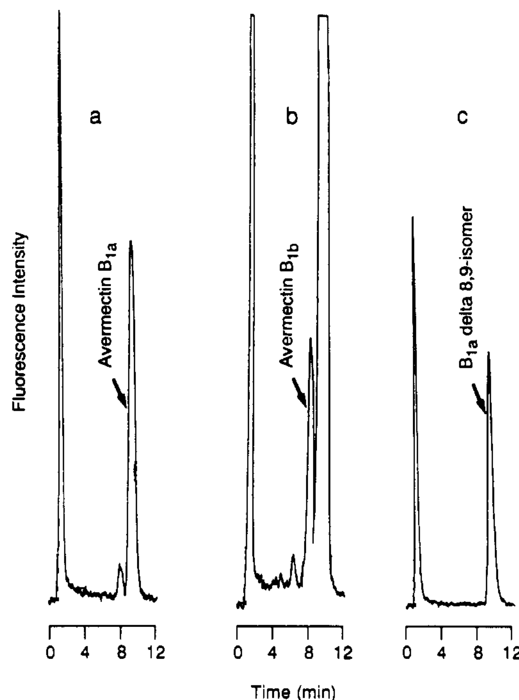

Figure 4. Typical chromatograms of control tomato fortified with (a) 28 ng/g avermectin B_{1a}, (b) 5.2 ng/g avermectin B_{1b}, and (c) 27 ng/g avermectin B_{1a} delta 8,9-isomer.

in Figure 3. For the abamectin standard two homologue peaks were observed; the first peak corresponds to avermectin B_{1b} and the second (larger) peak to avermectin B_{1a}. When the derivative from a standard B_{1a} delta 8,9-isomer is chromatographed (not shown in Figure 3), a single peak is observed which elutes at the same retention time as the B_{1a} component of abamectin. The derivative formed from a standard of B_{1a} delta 8,9-isomer has been shown to be structurally identical to that formed from avermectin B_{1a} by TFAA reaction in DMF (B. H. Arison, MSDRL, June 1987, private communication). The retention times for the fluorescent derivatives of avermectin B_{1a}/delta 8,9-isomer and avermectin B_{1b} are approximately 10 and 9 min, respectively. For the control tomato sample, no peaks were present in the chromatograms which might interfere with the determination of abamectin. Figure 4 shows representative chromatograms of control tomato samples fortified with avermectin B_1 (a) and with avermectin B_{1a} delta 8,9-isomer (b) after the cleanup steps were performed. The chromatograms observed for fortified and incurred residue tomato samples were identical to the chromatograms of abamectin standard, showing the good selectivity achieved by the cleanup steps and the fluorescence derivatization. The limit of detection for the method is 2 ng/g (S/N = 10), and the limit of quantitation is 5 ng/g (S/N = 25) for each of these components, the abamectin B_{1a}, the delta 8,9-isomer, or the avermectin B_{1b}.

The analysis of the standards from 2 to 10 ng/mL showed a good correlation between the concentration (X) and peak height (Y), with the coefficient of determination (r^2) averaging 0.995.

The rapid method for the determination of abamectin in tomato has been validated by the following evaluations. Table I summarizes recovery studies performed on untreated control samples fortified with abamectin and its delta 8,9-isomer at various levels. In each experiment, a 10-g sample was fortified before the cleanup and extraction steps were performed. Recoveries for abamectin and the delta 8,9-isomer were determined by quantitation vs the B_{1a} component from the abamectin standard. Recoveries were good and ranged from 79 to 104%, with precisions

Table I. Recovery of Avermectin B_{1a}, Avermectin B_{1b}, and the Delta 8,9-Isomer B_{1a} from Tomato

fortified with	fortifn level, ng/g	calcd concn, ng/g	% recov	precision (% RSD)	% av recov
avermectin B_{1a}	5.0	4.4, 4.5, 4.5	88, 90, 90	1.3	89
	28	26, 32, 29	93, 114, 104	10	104
	70	67, 59, 64	96, 84, 91	6.7	90
avermectin B_{1b}	5.2	4.9, 4.8, 5.3	94, 92, 102	5.5	96
delta 8,9-isomer B_{1a}	5.4	4.7, 4.7, 4.7	87, 87, 87	0	87
	27	21.3, 23.1, 23.4	79, 86, 87	5.2	84
	68	54, 54, 53.1	79, 79, 78	0.7	79
control	0	0, 0, 0	0, 0, 0		

that ranged from 0 to 10% RSD. The method can therefore be used for the quantitation of abamectin residues ≥5 ppb in tomatoes.

To demonstrate that the rapid assay method was capable of recovering incurred as well as fortified abamectin residues from tomatoes, samples from field trials were assayed by the rapid method and by the conventional method (Analytical Research, MSDRL, 1988). The concentrations, in nanograms per gram of avermectin B_{1a} and its delta 8,9-isomer, as determined by the two methods are summarized in Table II. Values obtained by a contract laboratory, Analytical Development Corp. (ADC), are indicated. All other assays were done in our laboratory. The results derived from the rapid method compared favorably with those from the conventional method. Figure 5 shows the correlation obtained between two methods. Each point corresponds to an average or a single assay result of a sample. The correlation between values from the methods is 0.97 (r), and the slope of the regression line is 0.82.

In conclusion, a rapid method for the quantitation of abamectin in tomatoes has been developed. The method uses fewer extraction/cleanup steps than the previous HPLC fluorescence method and an instantaneous derivatization to produce a fluorescence compound. The major advantages of this rapid method over a rapid UV detection

Table II. HPLC Fluorescence Assay for Incurred Residue Tomato Samples from the Field Trials

tomato sample	avermectin B_{1a}/delta 8,9-isomer			
	new short method		old established method	
	ng/g	RSD, %	ng/g	RSD, %
control[a] 1	0	NA[b]	0	NA
2	14, 14, 14 (av = 14)	0	14, 13, 13 (av = 13)	4.3
3	15, 13, 12 (av = 13)	11	13, 13, 17 (av = 14)	16
control 4	0	NA	0[c]	NA
control 5	0	NA	0[c]	NA
6	20, 19, 20 (av = 20)	3	16[c]	NA
7	14, 16, 20 (av = 17)	18	12[c]	NA
8	7.5, 6.8, 6.1 (av = 6.8)	10	5.4[c]	NA

[a] Control, untreated control samples assay results based on single assay. [b] NA, not applicable. [c] Results provided by Analytical Development Corp., Colorado Springs, CO; based on single assay.

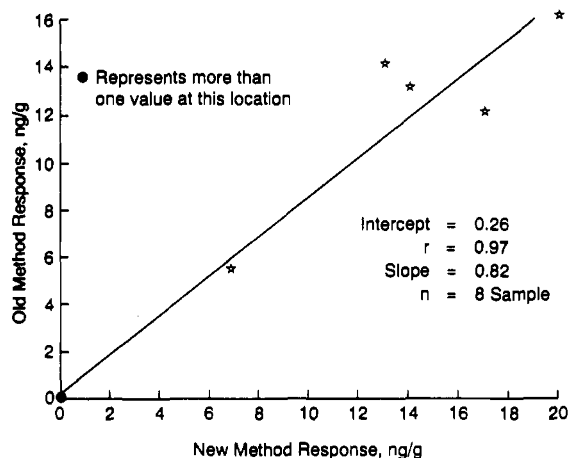

Figure 5. Plot of tomato sample residue levels from field trial samples determined by rapid method vs the conventional method.

method (Vuik, 1991) are increased sensitivity, improved selectivity, and the ability to quantitate both avermectin B_1 and its delta 8,9-isomer. The use of the new instantaneous fluorescence derivatization does not add any appreciable time to the assay performance. The time required for a 12-sample set with the rapid method is significantly reduced from that required with the conventional fluorescence assay method.

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Registry No. Abamectin, 71751-41-2; abamectin $\Delta^{8,9}$ isomer, 113665-89-7.