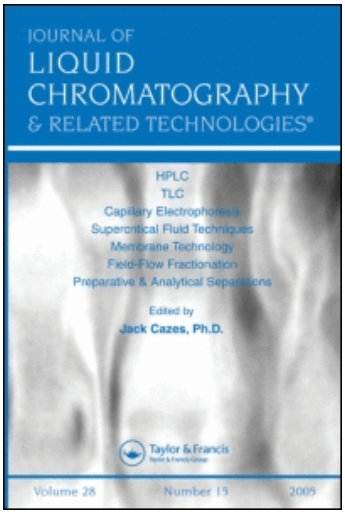

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Determination of Abamectin in Some Forest Matrices by Liquid Chromatography with Fluorescence Detection

K. M. S. Sundaram[a]; J. Curry[a]
[a] Natural Resources Canada, Canadian Forest Service, Marie, ON, Canada

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DETERMINATION OF ABAMECTIN IN SOME FOREST MATRICES BY LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

K. M. S. Sundaram, J. Curry

Natural Resources Canada
Canadian Forest Service
1219 Queen Street East
P.O. Box 490
Sault Ste. Marie, ON, P6A 5M7, Canada

ABSTRACT

A liquid chromatographic method was developed and validated for the determination of abamectin B_{1a} residues in some terrestrial (balsam fir and oak foliage, sandy and clay loam soils and leaf litter) and aquatic (stream water and sediment) forest matrices. The processed foliage, soil, litter, and sediment were fortified with abamectin and extracted with ethyl acetate. The fortified steam water was extracted with dichloromethane. Aliquots of crude extracts were cleaned with Florisil® column chromatography and the purified extracts were derivatized using 1-methylimidazole and trifluoroacetic anhydride. The derivatized abamectin was analysed by reverse phase liquid chromatography, with a fluorescence detector set at 232 nm excitation and 461 nm emission wavelengths. A Spherisorb® ODS2, 5μm, 250 × 4 mm column was used. The samples were run isocratically using methanol-water as the mobile phase. Mean recoveries for the analyte ranged from 83.0 to 93.0%, with a coefficient of variation from 6.3 to 12.4%. Limits of detection and

limits of quantitation for solid matrices ranged from 0.10 to 0.20 and from 0.30 to 0.60 ng/g, respectively, and for stream water the corresponding values were 0.003 and 0.009 ng/mL. The procedure provides a reliable and sensitive method for determining abamectin B_{1a} residues in forest matrices.

INTRODUCTION

Environmental and ecological concerns regarding the use of synthetic chemical pesticides coupled with increasing public pressure have resulted in the use of microbials to combat several defoliating lepidopterous pests in Canadian forestry. However, apart from the bacterium, *Bacillus thuringiensis (B.t.)*, the control options available at present, for the forest defoliators, are very limited. Avermectin B_1, a mixture of two homologous components [avermectin B_{1a} ($\geq$ 80%) and avermectin B_{1b} ($\leq$ 20%)], (Figure 1), is a natural macrocyclic lactone produced by the soil microorganism actinomycete, *Streptomyces avermitilis*.[1] The major component B_{1a} differs from the minor component B_{1b} by a single methylene group, B_{1a} contains a secondary butyl substituent at the C-25 position, whereas B_{1b} has an isopropyl substituent at that carbon. Because it is not economical to separate the two avermectin components on a large scale, the mixture is marketed commercially as abamectin.[2] Abamectin is found to be toxic to different species of insects and mites.[3] It has high activity against a broad range of lepidopterous larvae including the spruce budworm (*Choristoneura sp.*) and gypsy moth [*Lymantria dispar* (L.)], the two leading destructive defoliators of Canadian forests.[4] Field application rates to control various lepidoptera were estimated to be in the order of 15 g/ha.[2] It is a very low dosage compared to the synthetic insecticides used before.

Aerial application of insecticides is a favorable method used in forestry to cover vast areas of infested forests. Abamectin has many desirable properties to become a choice insecticide in forestry, *viz.*, its broad spectrum of activity at low dosage levels, its natural origin compared to the synthetic neurotoxic pesticides, and short environmental persistence. If it is chosen as a candidate material to control insect pests in forestry and sprayed aerially, different matrices such as foliage, soil, litter, surface water, sediment, etc. would be exposed and contaminated by the sprayed chemical. Exposed matrices have to be analyzed to determine the initial deposition and persistence of the material in order to assess its biological effectiveness, fate and mobility patterns, and overall environmental safety. Until now, no attempt has been made to analyze the abamectin residues in forest matrices and no published method has been reported for them. Analysis of abamectin and its dihydroderivative (ivermectin) at residue levels is an involved process and a number of high performance liquid chromatographic (HPLC) methods using UV or fluorescence detection have been reported for

agricultural products, animal tissues, milk, plasma, feces, wine, and other biological matrices.[2,5-18] The principal steps involved in these methods are the solvent extraction of the marker analyte (B_{1a} for abamectin) using suitable solvent or solvent mixtures, followed by partition and cleanup steps, derivatization, and eventual quantification by HPLC. Forest matrices, such as conifer needles, soil, litter, and sediment, are complex in nature and are associated with coextractive impurities, such as lipids, pigments, proteins, carbohydrates, etc., compared to agricultural and biological samples. Residue isolation and cleanup techniques must be such that they give high percentage recovery of the target analyte while simultaneously minimizing interferences, which may contribute to high background in the analysis. With this objective in mind, we have developed a reliable and sensitive HPLC method using fluorescence detection to isolate and quantify abamectin in forest matrices using B_{1a} as the marker for the analyte. Details of the method are reported in this paper.

MATERIALS AND METHODS

Analytical Standards

Abamectin standard containing 0.893% B_{1a} (w/w) and 0.044% B_{1b} (w/w) components dissolved in glycerol formal was kindly supplied by Ms. Boutin-Muma of Agriculture Canada, Ottawa, ON. Standard stock solution of B_{1a} containing 25 μg/mL was prepared by transferring exactly 140 mg of the standard glycerol formal solution (containing 1250 μg of avermectin B_{1a}) to a 50.0-mL amber volumetric flask and diluted to the mark with acetonitrile. This standard stock solution also contained 61.6 μg or 1.23 μg/mL B_{1b}. Intermediate standard solution (1 μg/mL) was prepared by transferring 2.0 mL avermectin B_{1a} stock solution to a 50.0-mL amber volumetric flask and diluting to the mark with acetonitrile. Working standards (0.5 to 25 ng/mL) for instrument calibration and fortification of substrates were prepared by the serial dilution of the intermediate standard solution using acetonitrile. All standards were kept in sealed amber volumetric flasks and stored at -10°C when not in use. Avermectin solutions were quite stable for six weeks under these conditions and fresh standards were prepared afterwards.

Reagents

Acetone (AT), acetonitrile (ACN), dichloromethane (DCM), ethyl acetate (EA), hexane (HX), methanol (MT), and tetrahydrofuran (THF) were HPLC grade obtained from VWR Canlab (Mississauga, ON); water was purified with a Millipore Milli-Q® water purification system (Millipore Corp., Milford, MA); trifluoroacetic anhydride (TFAA) and 1-methylimidazole (MIZ) were from Fisher Scientific

(Unionville, ON) and Aldrich Chem. Co. (Milwaukee, WI), respectively, Florisil® [PR grade, activated, solvent (MT and ether) washed and dried at 150°C for 24 h and stored in sealed bottles] was from Floridin Co. (Pittsburgh, PA); Nuchar® SN charcoal (acid washed and dried), silated Pyrex brand glass wool, ammonium hydroxide (30%), sodium sulphate (Na_2SO_4, anhydrous), and Whatman CF-11® cellulose powder were supplied by Fisher Scientific; and the HPLC mobile phase used in the study was prepared by mixing in the ratio of 95:5 (v/v), MT and water, filtering (0.45-μm Acrodisc® filter) and degassing the mixture prior to use.

Apparatus

A chopper (Hobart) for the initial maceration of foliage, litter, and soil, and a Kendall Mixer (Fisher) for the extraction of analyte from different matrices, were used. Centrifugations were achieved with a bench top Centra-8R® centrifuge from Damon, MA (USA) and filtrations were done using Acrodisc®-3 PTFE membrane filters. The solvents were evaporated using a Flash-Evaporator from Buchler Instruments (Fort Lee, NJ) and a Meyer N-Evap® (Organomation). Purification of the extracts were done using Florisil, PrepSep®-N_2 (Fisher) and Nuchar minicolumn chromatography. Solvent extraction and partition of the analyte from different solid matrices and subsequent derivatization after necessary cleanups were all done in Teflon® (VWR Canlab) wares (separatory funnels and centrifuge tubes) to minimize loss of the analyte by adsorption. HPLC analyses were performed by a computer controlled Hewlett-Packard (HP) 1090M liquid chromatograph fitted with an automatic sampler, a variable volume auto-injector, a binary solvent delivery system with helium degasser and a dual-syringe metering pump assembly to give consistent flows. An HP 1064 A fluorescence detector equipped with a 5-μL flow-cell and variable excitation and emission wavelengths, both scannable from 190 to 800 nm, was used to detect the abamectin derivative. The excitation and emission wavelengths were optimized and set at 232 and 461 nm, respectively. The computer work station (HP-9000/310), operated by the HP-7995R software, processed the peak area of each chromatogram obtained. The pre- and analytical columns used were HP-Spherisorb ODS2, 5 μm, 20 × 4 mm and 5 μm, 250 × 4 mm, respectively. They were thermostated at 25°C in the HPLC column compartment. A 100-μL portion of the derivatized extract was injected into the HPLC column and eluted using the mobile phase. The samples were run isocratically at 1.5 mL/min. Under these conditions, the larger B_{1a} component of abamectin eluted at 9.5 ± 0.2 min as a sharp, narrow peak and the smaller B_{1b} component eluted at 8.3 ± 0.5 min. However in the present study, because of its consistency in response, the larger B_{1a} component was taken as the standard to validate the method for abamectin from different forest matrices.

Forest Matrices

Red oak (*Quercus rubra* L.) and balsam fir [*Abies balsamea* (L.) Mill.] foliage were collected from a mixed forest north of Sault Ste. Marie (ON, Canada). Forest litter [pH 5.4; organic matter (OM) > 88%] and soils (sandy loam, pH 5.6, OM 5.4%, sand 56%, clay 6% and silt 38%; clay loam, pH 5.1, OM 11.7%, sand 38%, clay 16% and silt 46%) were collected from forested areas around Timmins (ON, Canada). Water [pH 6.1; alkalinity and hardness (mg/L of $CaCO_3$) 16.2 and 14.3, respectively; turbidity 0.31 JTU] was collected in 1-L Teflon bottles from a slow-flowing stream near Searchmont (ON, Canada) and stored at 4°C in the dark. Sediment (sand 78%, silt 19%, clay 3%, OM 1.4%) was also collected from the stream by gently lowering a capped glass jar to the stream bottom, scooping the sediment after removing the cap, and closing the jar after sufficient amount was collected. Standard procedures required for the collection of various matrices, their transportation, storage, and processing prior to fortification with the analyte are described elsewhere[19] and were followed in this study.

Extraction Procedure

Foliage

Five-gram aliquots of processed[19] fir needles and oak foliage were taken in separate 50-mL Teflon centrifuge tubes and mixed with 5 g Na_2SO_4. Each sample was fortified in quintuplicate with abamectin standard ranging from 0.60 to 5.0 ng/g. The tubes were vortexed for 5 min to mix the analyte uniformly with the matrix and allowed to equilibrate for 30 min. Twenty mL of EA was added to each sample and shaken for 30 min in a Kendall mixer to extract the material. After centrifugation at 3000 rpm for 5 min, the supernatant was filtered through a glass funnel using the EA washed Whatman #4 filter paper into a 250-mL round bottom flask (RBF). The residue in the tube was reextracted twice using 20 mL of the extractant each time and filtered through the same funnel into the RBF. The residue was then transferred carefully to the filter paper. The centrifuge tube and the residue on the filter were then rinsed with 3 x 10 mL of EA and the rinses were collected in the same RBF. The residue was discarded.

The pooled filtrate and rinses were flash evaporated to dryness at 30°C and under low pressure in a rotary evaporator. The residue was dissolved in 20% AT in HX and transferred quantitatively to a graduated Teflon tube, evaporated under nitrogen atmosphere and the volume adjusted to 1.0 mL for column cleanup. Care was taken to rinse the RBF with AT-HX mixture thoroughly, so that no analyte was lost through irreversible adsorption.

Soil and litter

The extraction procedure used for these matrices was similar except that 10.0-g aliquots of the soil samples fortified at 0.30 to 2.50 ng/g were used and the volume of the extractant was adjusted accordingly. The fortification level in litter was the same as in foliage. Care was taken to mix the Na_2SO_4 thoroughly with the matrix to avoid clumping and the resultant slurry after extraction was centrifuged at 5000 rpm for 10 min to completely settle the soil and litter particulates. The extract was further treated as in foliage.

Sediment

The sediment was initially filtered through a Buchner funnel (43 mm ID) using Whatman #4 filter paper under moderate vacuum to remove most of the absorbed water. Ten-g aliquots, mixed thoroughly with an equal amount of Na_2SO_4, were used for fortification (0.30 to 2.50 ng/g) and subsequent extraction studies. The sample was further treated, as above, in soil and litter.

Water

One hundred-mL aliquots of filtered (0.45 μm Acrodisc) stream water, in 250-mL Teflon separatory funnels, were fortified in quintuplicate with abamectin standard ranging from 0.010 to 0.250 ng/mL. Ten mL of 5% aqueous sodium chloride was added to each water sample to minimize emulsion formation and they were extracted with 4 x 30 mL of DCM. The lower organic phase was drained through a column of Na_2SO_4 (3 cm diam. x 3 cm length) into a 250-mL RBF and the column was rinsed with 3 x 10 mL of the extractant. The combined extracts and the column rinses of each sample were then flash evaporated to dryness and the residue was further treated as in foliage.

Chromatographic Column Cleanup

The chromatographic minicolumn (disposable pasteur pipet, 15 cm x 0.8 cm ID) was packed from bottom to top with a small wad of silated glasswool, 5 cm of activated Florisil and 1 cm of Na_2SO_4. After prewashing the column successively with 5 mL of AT and 5 mL of HX, 1.0 mL of the crude extract was loaded onto the column. It was first eluted with 10 mL of 20% AT in HX followed by 15 mL of AT, without allowing the column to run dry. The initial 10 mL of the eluent was discarded. The eluted abamectin in the 15 mL AT fraction was then collected in a 50-mL Teflon centrifuge tube. It was evaporated under nitrogen to dryness and reconstituted in 0.6 mL of ACN for derivatization.

Derivatization of Samples and Standards

The cleaned-up samples of matrices (each in 0.6 mL of ACN) and 0.6-mL aliquots of each abamectin standard used in the preparation of calibration curve, were treated separately with 0.10 mL of MIZ, vortexed gently and cooled in an ice bath for 10 min. Afterwards, 0.3 mL of cooled, fresh TFAA in ACN was added, vortexed gently and incubated at 30°C for 10 min. The derivatized abamectin was then filtered through a 0.45-μm filter (Acrodisc 3 PTFE, 3 mm) and analyzed by HPLC by injecting 100-μL volumes in quadruplicate. Derivatized standard concentrations ranged from 0.5 to 25 ng/mL and the amount injected ranged from 0.05 to 2.5 ng. In a typical working day *ca* three foliage or soil samples could be extracted and analyzed. The derivatives formed were stable for about 12 d at -20°C (in the dark), after which the material gradually degraded and the RT shifted from 9.5 ± 0.2 min to 5.6 ± 0.3 min. Total breakdown occurred after 45 d and the addition of aqueous ammonia was found to facilitate the rapid degradation. The structures of abamectin and the fluorescent derivative formed in the presence of MIZ and TFAA are given in Figure 1.

Calibration Curve Preparation

Calibration samples of abamectin in ACN were prepared to cover the range from 0.50 to 25 ng/mL. After derivatization, 100-μL aliquots were injected (0.05 to 2.5 ng of abamectin) in quadruplicate and a calibration curve was constructed by plotting the average peak area counts against amount of abamectin in ng (Figure 2). The area counts of the four injections agreed within 1.7%, showing good repeatability. A linear regression line fitted over the 50-fold concentration range gave a regression equation of $y = 4.531 \times c$ (y = the peak area count and c = the amount of abamectin injected in ng). The coefficient of determination was 0.9996, thus indicating the excellent linearity in the response of FD to the analyte over the range of concentrations studied. Abamectin concentrations in the fortified forest matrices were computed from the calibration curve using the measured area counts of the derivatized extracts.

RESULTS AND DISCUSSION

Validation of Method

The quantification procedure and conditions (column, mobile phase, flow rate, wavelength, column temperature and pressure, etc.) reported in this paper were

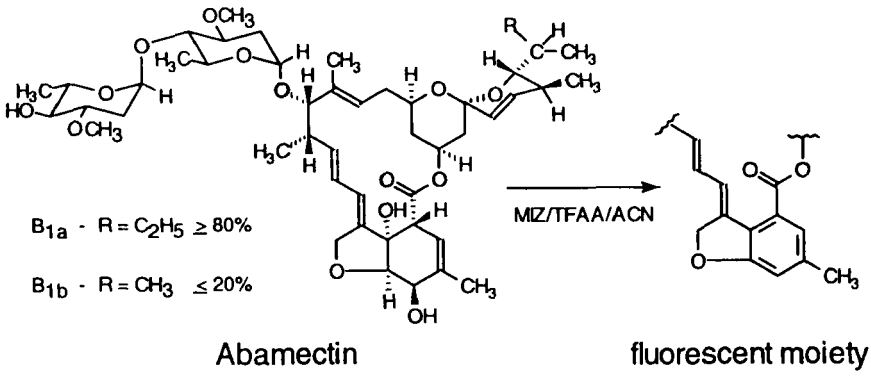

Figure 1. Structure of abamectin and its fluorescent derivative.

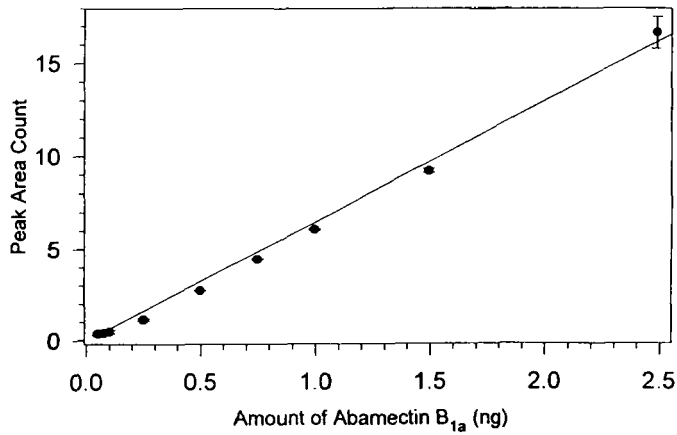

Figure 2. Abamectin standard curve. Amount vs. peak area count.

selected by trial and error and using the earlier publications[3,5-18] as a guideline. They were optimized to avoid interferences from the matrix constituents with the active ingredient, *i.e.*, derivatized abamectin. Repeatability of the experiment was verified by injecting 100 μL of different derivatized abamectin standards in quadruplicate onto the Spherisorb ODS2 column and measuring the peak area. The peak area for the four injections of the same analyte concentration were very similar, deviating only about 1.7% from the mean. The linearity of the detector response was checked

and confirmed by injecting increasing amounts of the derivatized analyte (100-μL quantities) over a concentration range of 0.50 to 25 ng/mL (0.05 to 2.5 ng) and preparing a calibration curve with a correlation coefficient $R^2 = 0.9996$. Different excitation and emission wavelengths were scanned, ranging from 200 to 400 nm for excitation and 400 to 600 nm for emission. The optimum wavelengths chosen for the excitation and emission were 232 and 461 nm, respectively, and this combination produced the best fluorogenic response for the derivatized analyte. Attempts to alter either the reported mobile phase composition (5% water in MT) or to substitute MT with ACN or THF resulted in deficient resolution and stationary phase clogging, consequently leading to tailing of chromatographic peaks.

No silylation of glassware (especially the RBF and the mini-chromatographic columns) was done, as required;[8] however the results obtained from recovery studies (Tables 1 and 2) were good and were within the limits of acceptance (80 - 100% of the expected value), presumably due to the use of Teflon ware in the extraction, partition and derivatization steps. Nevertheless, adequate care was necessary in many other areas to obtain good chromatographic profile and recovery levels. The optimum solvent volume (0.6 mL of ACN) used to dissolve the abamectin residue prior to derivatization, appropriate residue concentration required for the complete derivatization, absence of moisture in the reaction mixture, impurity free Na_2SO_4 and Florisil (for purification see ref. 20), as well as the activity level of the latter, were essential for the success of the study. HPLC column overloading, after frequent injections, resulted in clogging of the stationary phase, and consequent build-up of back pressure did occur occasionally, resulting in peak asymmetry. This was avoided by frequent changing of the guard column and reversing the analytical column and flushing it with the mobile phase.

Many extracting solvents were tried for quantitative recovery of the analyte from fortified environmental samples. HX and other nonpolar aliphatic hydrocarbons were unsuitable because of the poor recoveries of abamectin from fortified water samples. Among the chlorinated aliphatics, DCM gave consistently good recoveries and was chosen as the preferred extractant. Use of MT and AT as extracting solvents for solid matrices brought with them considerable amounts of coextractive impurities, necessitating extensive, time-consuming, and costly cleanups, which ultimately resulted in low analyte recoveries. Trial studies showed that EA was appropriate and suitable to extract abamectin from fortified solid matrices. Similarly, different chromatographic column packings (PrepSep-N_2, Nuchar-cellulose and activated Florisil) were tried along with pure and different combinations of eluting solvent systems (MT, ACN, THF, AT, HX, EA, and mixtures of these in different ratios). Among them, the use of Florisil minicolumn, pre-rinsed successively with AT then HX and elution of the analyte first with AT/HX mixture to remove lipids and other coextractives and later with AT, gave

Table 1

Recovery and Intra-Assay Precision in the Analysis of Abamectin (B_{1a} Component) from Fortified Terrestrial Matrices (N = 5)

Matrix	Fortification Value (ng/g)	Average Recovery (% of Fortified Value)	S D (±)	CV (%)
	0.60	78.2	9.8	12.5
	1.00	82.8	7.1	8.6
Fir needles	2.00	85.0	4.1	4.8
	3.00	85.4	4.8	5.6
	5.00	83.7	7.3	8.7
	mean	83.0	6.6	8.0
	0.60	72.6	9.8	13.5
	1.00	88.2	7.7	8.7
Oak foliage	2.00	83.7	9.6	11.5
	3.00	87.4	3.1	3.6
	5.00	86.7	6.0	6.9
	mean	83.7	7.2	8.8
	0.60	89.4	19.5	21.8
	1.00	80.0	12.2	15.3
Litter	2.00	85.6	10.0	11.7
	3.00	85.3	8.3	9.7
	5.00	85.7	3.0	3.5
	mean	85.2	10.6	12.4
	0.30	86.5	11.2	13.0
	0.50	84.4	6.6	7.8
Soil	1.00	88.7	3.1	3.5
(clay loam)	1.50	87.3	5.4	6.2
	2.50	86.7	2.6	3.0
	mean	86.7	5.8	6.7
	0.30	75.0	5.2	6.9
	0.50	89.5	3.7	4.1
Soil	1.00	86.0	7.2	8.4
(sandy loam)	1.50	89.9	6.1	6.8
	2.50	93.2	4.9	5.3
	mean	86.7	5.4	6.3

Table 2

Recovery and Intra-Assay Precision in the Analysis of Abamectin (B_{1a} Component) from Fortified Aquatic Matrices (N = 5)

Matrix	Fortification Value	Average Recovery (% of Fortified Value)	S D (±)	CV (%)
	0.30 ng/g	95.6	16.8	17.6
	0.50	88.3	13.7	15.5
Sediment	1.00	86.5	5.4	6.2
(stream)	1.50	86.0	6.2	7.2
	2.50	91.7	3.3	3.6
	mean	89.6	9.1	10.0
	0.010 ng/mL	76.8	10.2	13.3
	0.050	99.9	6.8	7.8
Water	0.100	96.9	4.7	4.1
(stream)	0.150	95.9	7.6	7.9
	0.250	95.6	6.1	6.4
	mean	93.0	7.1	7.9

cleaner eluates and acceptable recoveries of the material. The use of commercially available PrepSep-N_2 columns were found to be satisfactory, except for their high cost compared to the home-made Florisil columns. The Nuchar-cellulose columns were unsatisfactory due to strong adsorption of the analyte onto the column packing.

Limits of Detection and Quantitation

The limits of detection (LOD) of abamectin in different forest matrices were determined as 3 × SD at the lowest fortification level for a particular substrate. The limits of quantitation (LOQ) were expressed arbitrarily as 3 × LOD. The LOD and LOQ values obtained for the different substrates in this study are given below:

	LOD*	LOQ*
Water	0.003	0.009
Foliage	0.20	0.60
Litter	0.20	0.60
Soil	0.10	0.30
Sediment	0.10	0.30

* water, ng/mL; others, ng/g; injection volume 100 μL.

Abamectin Recovery

The recovery of abamectin from various terrestrial matrices fortified at different concentration levels (foliage and litter, 0.60 to 5.00 ng/g; and soils 0.30 to 2.50 ng/g) is given in Table 1. The recovery for aquatic substrates (fortified: sediment 0.30 to 2.50 ng/g and water 0.010 to 0.250 ng/mL) is given in Table 2. The mean percentage recovery for each sample with its standard deviation (SD) and average coefficient of variation (CV) were derived from multiple injections of quintuplicate samples. Generally, as seen in Tables 1 and 2, the recoveries varied from the expected values according to the complexity of the matrix and to some extent, with the level of fortification. The recovery levels were relatively low in foliage and high in water, and often, but not always, the recoveries were better at higher fortification levels.

The mean percent recoveries of fir and oak foliage were 83.0 and 83.7, respectively, with the percent means of the measured values differing from the fortified concentrations by 17.0 and 16.3, respectively. Mean recovery values (%) for litter, clay loam and sandy loam soils, sediment, and stream water were 85.2, 86.7, 86.7, 89.6, and 93.0, respectively (Tables 1 and 2). The percent means of the measured values differed from the fortified concentrations for these matrices by 14.8, 13.3, 13.3, 10.4, and 7.0, respectively. The good percent recoveries of abamectin obtained from the fortified samples of known concentrations show that the accuracy of the method was satisfactory. The inter-assay precision, showing the reproducibility of the abamectin recoveries between each replicate sample, as indicated by the corresponding SD and CV (%) values, varied according to the fortification level. The trend observed was that the precision was low (high SD and CV) at low fortification levels and vice versa. For example, for litter the SD (±) and CV (%) at 0.60 ng/g fortification level were 19.5 and 21.8, respectively, whereas at 5.0 ng/g, the corresponding values were only 3.0 and 3.5 (Table 1). However, the intra-assay precision reported in terms of the mean values of the SD (±) and CV (%) obtained respectively within each substrate, *viz.*, fir needles (6.6 and 8.0), oak foliage (7.2 and 8.8), litter (10.6 and 12.4), clay loam soil (5.8 and 6.7), sandy loam soil (5.4 and 6.3), sediment (9.1 and 10.0), and water (7.1 and 7.9) were rather low indicating good precision of the reported method (Tables 1 and 2).

Chromatograms

A typical chromatogram of the reagent blank and abamectin standard obtained by injecting 5.0 ng in 100 μL onto the HPLC is shown in Figure 3a. The peaks with RT (average) 9.5 ± 0.2 min for B_{1a} (major peak) and 8.3 ± 0.5 min for B_{1b} (minor peak) were symmetrical, with the baseline separated and well removed from the solvent front. Deviation in RTs for each injection was observed, however it was not

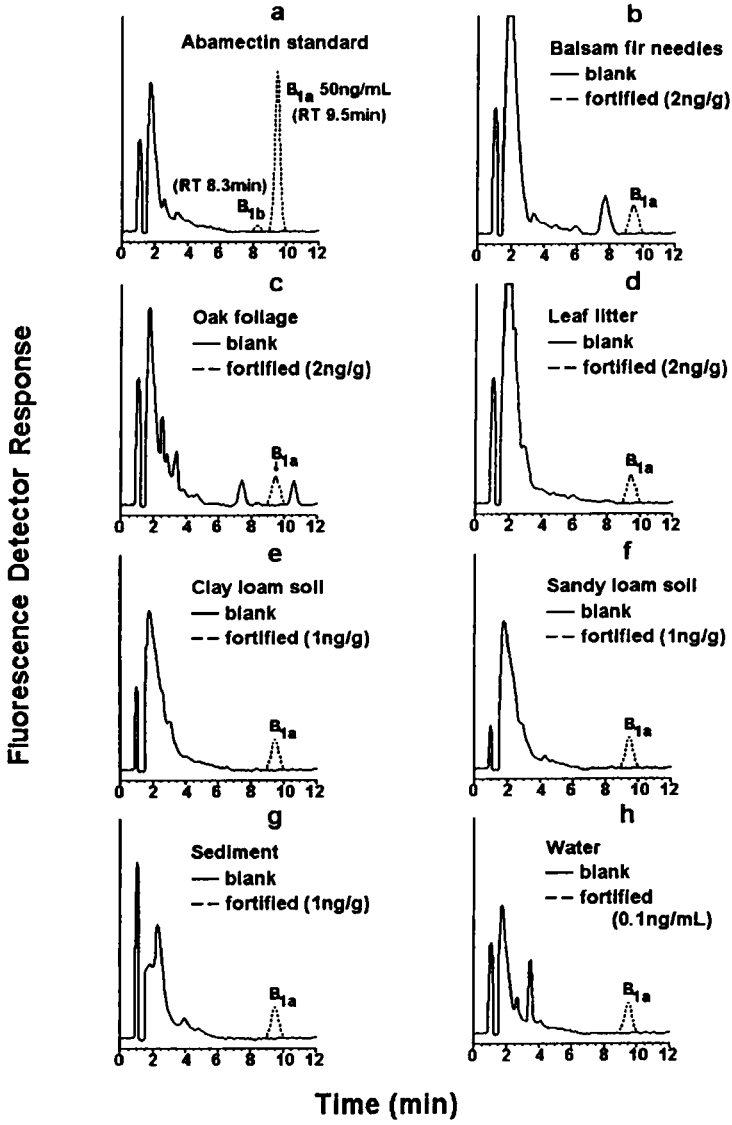

Figure 3. Typical chromatograms of fortified and blank forest substrates (abamectin peak shown as dotted line over blank chromatogram).

significant, as can be ascertained from the recorded SD values. Figure 3b is a typical chromatogram obtained for conifer needles, blank and fortified at 2.00 ng/g and injecting 100-μL volumes (abamectin B_{1a} is shown by dotted line). The B_{1a} analyte peak was narrow and separated well without any interference from the peaks derived from the endogenous materials present in the needle. Similar patterns were also observed in the chromatograms obtained for blank and fortified samples of oak foliage (Figure 3c), leaf litter (Figure 3d), forest soils (fortified at 1.00 ng/g; Figures 3e and 3f), and stream sediment (Figure 3g). The optimal analytical and HPLC conditions used in the study were adequate to quantify the abamectin B_{1a} analyte from forest foliage, soil, litter, and sediment. The chromatograms (blank and fortified at 0.100 ng/mL) from the water extracts (Figure 3h) were cleaner, without the many extraneous peaks found in the other matrices. The total chromatographic analysis time was 20 min. Based on the analysis of 56 fortified samples, comprising different sample types (including water), the average analysis time per sample was found to be about 2.5 h.

CONCLUSIONS

Within the framework of method validation, and using parameters such as consistency in the recovery of the analyte from fortified forest matrices reflecting accuracy, satisfactory intra-assay precision defined in terms of SD, and inter-assay precision reflecting reproducibility, the good recoveries obtained for all the matrices at different fortification levels, clearly show that the method is applicable to determine abamectin residues from a wide range of forest matrices. The method is reliable, robust, and sensitive and will be a useful tool for the routine determination of abamectin concentrations; however, it is somewhat time-consuming. Nevertheless, to ensure good results, it is essential that the various steps reported in this method should be carefully followed. With necessary modifications, this method could find wide applicability in examining the initial deposition and the fate of abamectin in diverse forest matrices. The throughput of the assay is primarily dependent upon the complexity of the matrix and the chromatographic separation. For example, when no column cleanup is required (typically for the analysis of standards), the throughput is limited only by the derivatization step and speed of the autosampler.

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