# Determination of the Naturally Derived Insect Control Agent Spinosad and Its Metabolites in Soil, Sediment, and Water by HPLC with UV Detection

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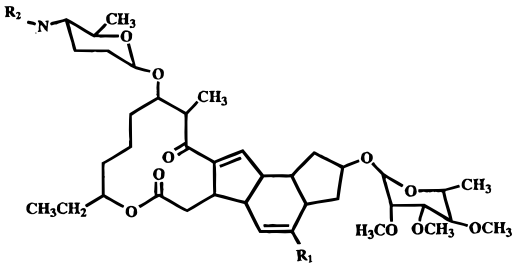
A method is described for the determination of the naturally derived insect control agent spinosad and its metabolites in soil, sediment, and water. The method determines residues of the active ingredients in spinosad (spinosyns A and D) and two metabolites (spinosyn B and *N*-demethylspinosyn D). For soil and sediment, the method has a limit of quantitation of 0.01 $\mu$g/g and a limit of detection of 0.003 $\mu$g/g. For water, the method has a limit of quantitation of 0.001 $\mu$g/mL and a limit of detection of 0.0003 $\mu$g/mL. The analytes are extracted from water, soil, or sediment using appropriate solvents, and the extracts are purified by liquid−liquid partitioning and silica solid phase extraction. All four analytes are determined simultaneously in the purified extracts by reversed-phase high performance liquid chromatography with ultraviolet detection at 250 nm.

**Keywords:** *Spinosad; spinosyn A; spinosyn D; spinosad B; N-demethylspinosyn D; soil; sediment; water; quantitation; HPLC-UV*

## INTRODUCTION

The spinosyns are a naturally derived group of insect control agents that possess activity against several classes of insects but are especially active on species of Lepidoptera. The spinosyns are derived from a newly discovered species of Actinomycetes bacteria, *Saccharopolyspora spinosa*. The common name of the product is spinosad, which is comprised of a mixture of spinosyns A and D. Spinosad has activity in the range of some



Spinosyn A, $R_1 = N(CH_3)_2$ and $R_2 = H$
Spinosyn D, $R_1 = N(CH_3)_2$ and $R_2 = CH_3$
Spinosyn B, $R_1 = NH(CH_3)$ and $R_2 = H$
*N*-Demethyl spinosyn D, $R_1 = NH(CH_3)$ and $R_2 = CH_3$

pyrethroids but is also effective on a variety of insecticide-resistant strains of insects, with no evidence of cross-resistance to date. Spinosad has a low order of toxicity to mammals, birds, and fish, and it is being developed for the management of insect pests in cotton and a variety of other crops (Sparks et al., 1995; Thompson et al., 1995).

Residue methods for water, soil, and sediment were needed for environmental fate monitoring studies (McGibbon et al., 1996). Previous studies using radiolabeled ($^{14}$C) material demonstrated that spinosyns A and D degraded to spinosyn B and *N*-demethylspinosyn D, respectively, in soil (Rainey et al., 1996; Peacock et al., 1996; Reeves et al., 1996) and in water (Saunders et al., 1996). Thus, the following methods are presented for the determination of all four analytes in water, soil, and sediment by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The chemical names and CAS Registry Numbers for the analytes are included in Table 1.

## EXPERIMENTAL PROCEDURES

**Apparatus.** (*a*) *HPLC with a UV Detector.* A Hewlett-Packard Model 1050 HPLC with a UV detector was used in combination with a Hewlett-Packard Model 3396 Series II recording integrator for the measurement of peak height responses. The primary HPLC column was an ODS-AQ [5-$\mu$m particle size, 120 Å, 150 × 4.6 mm i.d. (YMC)], maintained at an oven temperature of 30 °C. The mobile phase consisted of 44% reservoir A/44% reservoir B/12% reservoir C (isocratic), with reservoir A containing methanol, reservoir B containing acetonitrile, and reservoir C containing 2% aqueous ammonium acetate in acetonitrile. The flow rate was 0.8 mL/min. The injection volume was 175 $\mu$L, and the integrator attenuation was $2^3$. The chart speed was 0.2 cm/min. Under these conditions, the four analytes eluted with retention times ranging from approximately 5 to 12 min.

The confirmatory HPLC column was a $C_{18}$/cation mixed mode [5-$\mu$m particle size, 150 mm × 4.6 mm i.d. (Alltech/Applied Science)]. The mobile phase was 40% reservoir A/40% reservoir B/20% reservoir C (isocratic), with reservoir A containing methanol, reservoir B containing acetonitrile, and reservoir C containing 2% aqueous ammonium acetate in acetonitrile. The flow rate was 1.0 mL/min, and all of the other parameters were the same as those just listed for the primary column. Under these conditions, the four analytes eluted with retention times ranging from approximately 5 to 12 min.

*(b) Solid Phase Extraction (SPE) Column and Vacuum Manifold.* The SPE column was a Waters Silica Sep-Pak Plus (690 mg), which was used in conjunction with 25-mL reservoirs (Waters) and an Alltech Associates vacuum manifold.

*(c) Centrifuge.* The centrifuge was an International Equipment Co. Model CU-5000.

*(d) Rotary Vacuum Evaporator.* The rotary vacuum evaporator was a Rinco Instrument Co. Model 1007-4 IN.

*(e) Orbital Shaker.* The orbital shaker was a New Brunswick Model G-33.

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**Table 1. Chemical Names and CAS Registry Numbers[a] for Spinosyns**

| | |
|---|---|
| spinosyn A (CAS Registry No. 131929-60-7) | 2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[(5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl)oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradecahydro-14-methyl-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione |
| spinosyn D (CAS Registry No. 131929-63-0) | 2-[(6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranosyl)oxy]-13-[(5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl)oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradecahydro-4,14-dimethyl-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione |
| spinosyn B (CAS Registry No. 131929-61-8) | 2-[(6-deoxy-2,3,4-tri-*O*-methyl-L-mannopyranosyl)oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradecahydro-14-methyl-13-[(tetrahydro-6-methyl-5-(methylamino)-2*H*-pyran-2-yl)oxy]-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione |
| *N*-demethylspinosyn D (CAS Registry No. 149439-70-3) | 2-[(6-deoxy-2,3,4-tri-*O*-methyl-L-mannopyranosyl)oxy]-9-ethyl-2,3,3*a*,5*a*,5*b*,6,9,10,11,12,13,14,16*a*,16*b*-tetradecahydro-4,14-dimethyl-13-[(tetrahydro-6-methyl-5-(methylamino)-2*H*-pyran-2-yl)oxy]-1*H*-*as*-indaceno(3,2-*d*)oxacyclododecin-7,15-dione |

[a] Supplied by the author.

**Table 2. Chemical and Physical Properties of Soil and Sediment Samples Used in the Method Validation Study**

| description | soil type[a] | pH | CEC[b] | OM[c] |
|---|---|---|---|---|
| terrestrial soil | sandy loam | 5.7 | 1.68 | 0.88 |
| terrestrial soil | clay loam | 5.1 | 16.2 | 1.3 |
| pond sediment | clay loam | 7.6 | 15.9 | 3.9 |

[a] USDA classification. [b] Cation exchange capacity, mequiv/100 g. [c] Organic matter, %.

*(f) Water Purification System*. The water purification system was a Millipore Corp. Milli-Q UV Plus.

*(g) Sample Extraction Bottles*. The sample extraction bottles were 8-oz (237-mL) Qorpak glass bottles with PTFE-lined lids (Fisher Scientific).

*(h) Glass Vials*. The vials were 9.5-dram (35-mL), clear glass vials (Fisher Scientific).

*(i) Glass Wool*. The glass wool was Pyrex fiberglass (Fisher Scientific) that was purified by completely submerging 100 g in 400 mL of methanol for 5 min and vacuum filtering, followed by submerging in 400 mL of dichloromethane for 5 min and vacuum filtering. The glass wool was then dried in a fume hood for 2 h.

*(j) Membrane Filters*. The membrane filters for filtering HPLC solvents were Nylon 66, 47-mm i.d., 0.45-μm pore size (Supelco, Inc.).

*(k) Evaporator*. The evaporator was a Zymark Corp. TurboVap LV.

**Reagents.** Solvents (acetone, acetonitrile, dichloromethane, hexane, and methanol) were of HPLC grade. Water was purified using a Milli-Q UV Plus purification system. Ammonium acetate was of HPLC grade. Hydrochloric acid, sodium chloride, sodium hydroxide, and granular anhydrous sodium sulfate were of analytical grade. The sodium sulfate (Fisher catalog no. S421-3) was purified in a Büchner funnel by rinsing 800 g with 1000 mL of hexane under gravity flow. After the hexane had passed through the sodium sulfate, the vacuum was turned on briefly to remove excess solvent, and the sodium sulfate was dried for 5 min in a fume hood with stirring until the solvent had largely evaporated. Longer drying times were avoided to prevent the adsorption of moisture. The sodium sulfate was stored in a sealed glass container. (Sodium sulfate from a different supplier resulted in recoveries of spinosyns A and D that were as low as 27% due to adsorption of the analytes.) The purified active ingredients used for analytical standards were obtained from the Test Substance Coordinator, DowElanco, 9330 Zionsville Road, Building 304, Indianapolis, IN.

**Safety Precautions.** Proper eye protection and protective clothing were worn during all procedures. Volatile and flammable organic solvents were used in fume hoods, away from ignition sources. To avoid the possibility of implosion, polypropylene Erlenmeyer flasks or glass flasks covered with electrical tape were used for evaporations conducted under reduced pressure.

**Water and Soil Samples.** Three different types of water samples were used for the method validation study. The samples included tap water (pH 5.4) and well water (pH 6.8), both from Indianapolis, IN, and pond water (pH 7.2) from Greenfield, IN.

One sediment sample and two different types of terrestrial soils were also used for the method validation. The terrestrial soils included a sandy loam soil from Oconee, GA, and a clay loam soil from Tunica, MS. The sediment sample was obtained from a pond near Greenfield, IN. The chemical and physical characteristics of the soil and sediment samples are included in Table 2.

**Standard Preparation.** Analytical standards for the analytes were obtained from the Test Substance Coordinator, DowElanco. The purity of the standards ranged from 95 to 98%. Individual stock solutions of the four analytes were prepared at 100 μg/mL by weighing 10 mg of each standard, quantitatively transferring to separate 100-mL volumetric flasks, dissolving in 50% methanol/50% acetonitrile, and diluting to volume. Aliquots (10.0 mL) of all four stock solutions were then combined in the same 100-mL volumetric

flask and diluted to volume with methanol/acetonitrile/2% aqueous ammonium acetate (1:1:1) to obtain a mixture containing 10.0 μg/mL of all four analytes. Aliquots of this solution were further diluted with methanol/acetonitrile/2% aqueous ammonium acetate (1:1:1) to obtain HPLC calibration standards at concentrations of 0.0, 0.050, 0.10, 0.50, 1.0, and 1.5 μg/mL.

Solutions for fortifying control soil samples for the determination of recovery were prepared by combining 20.0-mL aliquots of the four 100 μg/mL stock solutions in a 100-mL volumetric flask and diluting to volume with 50% methanol/50% acetonitrile to obtain a mixture containing 20.0 μg/mL of the analytes. Aliquots of this solution were further diluted with 50% methanol/50% acetonitrile to obtain fortification standards at concentrations of 0.20, 0.50, 1.0, 1.5, and 2.0 μg/mL.

Solutions for fortifying control water samples for the determination of recovery were likewise prepared in 50% methanol/50% acetonitrile at concentrations of 0.20, 2.0, 5.0, 10, 15, and 20 μg/mL.

**Precautionary Protection from Light.** During the following sample extraction and purification steps, the analytes were protected from photolysis that can occur under normal lighting conditions. Protective measures included working under reduced lighting conditions (e.g., turning off the lights in fume hoods during liquid−liquid partitioning and SPE cleanup steps) and placing the samples in the dark for any short interruptions during sample processing.

**Sample Extraction.** *(a) Water.* A 200-mL aliquot of the water sample was measured in a 250-mL graduated cylinder, and fortified recovery samples were prepared by adding 1.0 mL of the appropriate fortification standard solution to the appropriate control sample. The sample was transferred to a 500-mL separatory funnel. If the 200-mL water sample had been collected or stored in a glass container, 20 mL of methanol was added to the empty container, which was capped, shaken, and rotated in a horizontal position to dissolve residues of the analytes that had adsorbed onto the glass. The methanol rinse was transferred to the separatory funnel. The aqueous phase was made basic by the addition of 4.0 mL of aqueous 1.0 N sodium hydroxide solution. The water sample was checked with pH paper to ensure that the pH was ≥12. If necessary, additional 1.0 N sodium hydroxide solution was added to adjust the pH to ≥12.

A 50-mL aliquot of dichloromethane (DCM) was added to the separatory funnel, which was shaken vigorously for 30 s. (If the water sample had been collected or stored in a glass container, the 50 mL of DCM was first added to the glass container to rinse it in the manner that was just described, and the DCM rinse was then added to the separatory funnel.) After shaking, the aqueous and organic layers were allowed to separate for 5 min. If necessary, a stirring rod was used to help break up the emulsion between the layers. The DCM (lower) layer was drained nearly down to the emulsified layer, collecting the DCM in a 500-mL boiling flask. The emulsion was retained with the aqueous phase in the separatory funnel. The extraction procedure was repeated with two additional 50-mL aliquots of DCM, combining the three DCM extracts in the same 500-mL flask.

Prior to evaporating the sample, the rotary vacuum evaporator was rinsed under vacuum with hexane and then methanol to prevent sample contamination. The DCM was evaporated with the rotary vacuum evaporator and a water bath heated to 35−50 °C. If traces of water remained in the flask upon evaporation of the DCM, 20 mL of methanol was added and the evaporation was repeated.

The water samples used in the method validation study did not require further purification by silica SPE. As a guideline, if the DCM extracts of the water samples are essentially free of color when nearly concentrated to dryness, dissolve the residue remaining in the boiling flask upon evaporation in 2.0 mL of methanol/acetonitrile/2% aqueous ammonium acetate (1:1:1) and continue the analysis of the samples as described later under HPLC. However, if the DCM extracts contain color, or if additional cleanup is known to be needed on the basis of a previous analysis, continue the sample analysis as described later under Purification by Silica SPE.

*(b) Soil or Sediment.* Samples (20 g) were weighed into 8-oz (237-mL) glass bottles. Fortified recovery samples were prepared by adding 1.0 mL of the appropriate fortification standard solution to the appropriate control sample. After the solvent had evaporated, an extraction solution consisting of 60 mL of methanol/5% sodium chloride/1 N sodium hydroxide (65:27:8) was added. If necessary, a spatula was used to break up clumps of soil or sediment so that the particles would readily suspend in the extraction solvent. The jar was sealed with a PTFE-lined lid.

The sample was sonicated in an ultrasonic cleaner for 5 min, shaken on an orbital shaker at 275 rpm for 30 min, and then centrifuged at 2250 rpm for 10 min. The supernatant liquid was decanted into a 250-mL graduated cylinder that was wrapped in aluminum foil to protect against photodegradation of the analytes while a second soil extraction was being conducted. The soil extraction was repeated with a second 60-mL aliquot of methanol/5% sodium chloride/1 N sodium hydroxide (65:27:8), combining the two soil extracts in the same 250-mL graduated cylinder. The combined sample extract was diluted to 130 mL with methanol/5% sodium chloride/1 N sodium hydroxide (65:27:8).

The diluted solution was stirred or shaken to mix. A 65-mL (half) aliquot of the extract was transferred to a 250-mL separatory funnel, and the analysis of the sample was then continued as described next.

**Purification by Liquid−Liquid Partitioning.** Soil sample extracts were purified by liquid−liquid partitioning. A 65-mL aliquot of an acidic salt solution consisting of 0.16 N hydrochloric acid in 5% aqueous sodium chloride was added to the separatory funnel containing the 65-mL aliquot of the soil extract solution. The pH of the solution in the separatory funnel was checked with pH paper to ensure that the pH was ≤2. If necessary, a sufficient volume of 1.0 N hydrochloric acid was added to adjust the pH to ≤2.

A 50-mL aliquot of hexane was added, the separatory funnel was vigorously shaken for 20−30 s, and the aqueous and organic layers were allowed to separate for 2 min. If necessary, a stirring rod was used to help break up the emulsion between the layers. Using a 250-mL beaker, the aqueous (lower) layer was drained nearly down to the emulsified layer. After a 2-min wait for the layers to further separate, the remaining aqueous layer was drained into the beaker, leaving the emulsion in the separatory funnel. If the emulsion was adhering to the glass, the separatory funnel was briefly shaken to dislodge the emulsion, and the hexane (upper) layer and the emulsion were then discarded.

After the acidic partitioning was completed, the aqueous phase in the 250-mL beaker was returned to the separatory funnel. The aqueous phase was made basic (pH 10−12) by the addition of 10.0 mL of 1.0 N aqueous sodium hydroxide. The separatory funnel was shaken briefly, and the pH of the aqueous solution was checked with pH paper to ensure that the pH was ≥10. If necessary, additional sodium hydroxide solution was added to obtain the desired pH.

The analytes were then extracted from the aqueous phase by shaking with three 50-mL aliquots of hexane for 20−30 s each. After a 2-min wait for the layers to separate after each partitioning, a stirring rod was used to further aid the separation of the layers.

Using a 250-mL beaker, the aqueous (lower) phase was drained nearly down to the emulsion, leaving the emulsion and approximately 1 mL of aqueous phase in the separatory funnel. After a 2-min wait for the layers to further separate, the remaining aqueous layer, the emulsion, and approximately 1 mL of the hexane (upper) layer was drained into the beaker. The hexane remaining in the separatory funnel was then drained through a funnel containing a small plug of hexane-washed glass wool and 10 mL (16.5 g) of hexane-washed sodium sulfate into a 500-mL boiling flask. The aqueous layer in the beaker was returned to the separatory funnel. The partitioning step was repeated with two additional 50-mL aliquots of hexane. After the hexanes from the second and third partitioning steps were combined into the same 500-mL

boiling flask, the sodium sulfate was rinsed with 15 mL of hexane, which was also added to the boiling flask.

Prior to evaporation of the hexane extract, the rotary vacuum evaporator was rinsed under vacuum with hexane and then methanol. The sample was evaporated to dryness using the rotary vacuum evaporator with the water bath heated to 35−50 °C. The residue was dissolved in 10 mL of hexane for further purification as described next.

**Purification by Silica SPE.** Prior to using each new lot of silica SPE columns, the elution profile was determined to ensure that the appropriate volumes of solvents were discarded and collected in the following procedure. The elution profile described below was determined using a standard solution containing all four analytes at 2.0 μg each in 10 mL of hexane.

An SPE column reservoir was attached to a silica SPE cartridge, and the cartridge was attached to the vacuum manifold. Prior to addition of the sample, the column was conditioned by adding the following sequence of eluants: 10 mL of 75% DCM/25% methanol, then 10 mL of acetonitrile, followed by 20 mL of hexane. Except where dropwise elution is indicated, the solvents were passed through the column using full vacuum (−24 psi).

The following volumes were typical for the silica SPE procedure, but the volumes might require modification for different lots of silica SPE columns. The sample was added in 10 mL of hexane. The evaporating flask was rinsed with two 10-mL aliquots of hexane, which were separately added to the column and eluted. The flask was rinsed with 40 mL of hexane, which was added to the column and eluted. The flask was rinsed with two 5-mL aliquots of DCM, which were separately added to the column and eluted. The flask was rinsed with two 4-mL aliquots of acetonitrile, which were separately added to the column and eluted. All of the solvent that had eluted thus far was discarded. A precleaned, 35-mL vial was then placed in the vacuum manifold for solvent collection. The evaporating flask was rinsed with two 8-mL aliquots of 75% DCM/25% methanol, which were added to the column and eluted into the vial using reduced vacuum to result in a dropwise rate of elution. The sample solution was immediately evaporated using a TurboVap evaporator set at 60 °C and a nitrogen flow of 8 psi. The sample vial was removed from the evaporator immediately upon evaporation of the solvent, and the residue was dissolved in 1.0 mL of methanol/acetonitrile/2% aqueous ammonium acetate (1/1/1). The vial was swirled to dissolve the residue on the bottom of the vial, then tilted to nearly a horizontal position, and slowly rotated to dissolve the residue on the wall of the vial. Because the analytes adsorb very tightly to glass, the swirling and rotating procedure was repeated one time to ensure that the residue had dissolved. Using a disposable Pasteur pipet, the sample solution was transferred to an HPLC vial and capped. The final solution was not filtered through a 0.45-μm filter, because the filters produced interference peaks in the chromatogram. (The lack of filtration of the final solution did not result in an appreciable decrease in column performance over a period of several weeks.)

**HPLC.** Standard and sample solutions were analyzed by HPLC using the previously described conditions. The suitability of the chromatographic system was determined using the following performance criteria: (a) It was determined that the correlation coefficient ($r^2$) equaled or exceeded 0.995 for the least-squares equation that described the detector response as a function of the concentration of the calibration standards. (b) It was visually determined that baseline resolution was achieved for all four analytes. (c) It was visually determined that a signal-to-noise ratio of approximately 5:1 to 10:1 was achievable for the 0.1 μg/mL calibration standard. If the peak height for any of the samples exceeded the range of the calibration curve, the samples were diluted with methanol/acetonitrile/2% aqueous ammonium acetate (1:1:1) to yield a response within the range of the calibration curve.

**Calculation of Results.** Separate calibration curves were prepared for all four analytes by plotting the concentration of the calibration standards on the abscissa (*x*-axis) and the resulting peak heights on the ordinate (*y*-axis). Using regression analysis, the equation for the calibration curve was determined with respect to the abscissa. The concentration (*C*) of the analyte in the final solution was calculated from the measured peak height response (PR) and the least-squares coefficients for the slope (*m*) and *y*-axis intercept (*b*) as follows:

$$C = (\mathrm{PR} - b)/m \quad (1)$$

The concentration (μg/g or μg/mL) of the analytes in the samples was calculated from the concentration in the final solution (*C*), the aliquot factor (AF = 1 for water or AF = 130 mL/65 mL = 2 for soil), the final volume (*V*), and the weight of the soil sample or the volume of the water sample that was extracted (*W*) using the following equation:

$$\mu\mathrm{g/g\ or\ \mu g/mL} = (C \times \mathrm{AF} \times V)/W \quad (2)$$

The net percent recovery (*R*) was calculated from the net concentration (μg/g or μg/mL) in fortified recovery samples (corrected for any background in the unfortified control sample) using the following equation:

$$R = [(\text{net } \mu\text{g/g})/(\text{added } \mu\text{g/g})] \times 100\% \quad (3)$$

For any sample results requiring correction for recovery, the corrected results were calculated using the average recovery ($R_a$) as follows:

$$\text{corrected residue } (\mu\text{g/g}) = \text{uncorrected residue } (\mu\text{g/g}) \times (100\%/R_a) \quad (4)$$

**Table 3. Recovery of Spinosyns A, D, and B and *N*-Demethylspinosyn D (NDSD) from Pond Water, Tap Water, and Well Water without Silica SPE Cleanup**

| added, μg/mL | sample type | *n* | % recovery (mean ± SD) A | D | B | NDSD |
|---|---|---|---|---|---|---|
| 0.0 | pond water | 3 | ND[a] | ND | ND | ND |
| | tap water | 2 | ND | ND | ND | ND |
| | well water | 4 | ND | ND | ND | ND |
| 0.0003 | pond water | 1 | <LOQ[b] | <LOQ | <LOQ | <LOQ |
| | tap water | 1 | <LOQ | <LOQ | <LOQ | <LOQ |
| | well water | 1 | <LOQ | <LOQ | <LOQ | <LOQ |
| 0.001 | pond water | 8 | 99 ± 10 | 93 ± 10 | 88 ± 8 | 99 ± 7 |
| | tap water | 3 | 97 ± 6 | 95 ± 7 | 79 ± 8 | 92 ± 5 |
| | well water | 3 | 100 ± 6 | 96 ± 8 | 85 ± 5 | 96 ± 5 |
| 0.010 | pond water | 3 | 90 ± 7 | 87 ± 8 | 91 ± 5 | 90 ± 7 |
| 0.025 | pond water | 3 | 90 ± 8 | 88 ± 9 | 86 ± 9 | 82 ± 10 |
| 0.050 | pond water | 3 | 88 ± 2 | 92 ± 10 | 88 ± 2 | 82 ± 2 |
| 0.075 | pond water | 3 | 91 ± 3 | 88 ± 4 | 91 ± 1 | 86 ± 1 |
| 0.10 | pond water | 3 | 86 ± 8 | 83 ± 9 | 89 ± 3 | 86 ± 5 |
| | tap water | 3 | 91 ± 3 | 95 ±7 | 79 ± 8 | 92 ± 5 |
| | well water | 3 | 86 ± 6 | 86 ± 6 | 86 ± 4 | 84 ± 6 |
| | overall | 35 | 93 ± 8 | 90 ± 8 | 87± 6 | 90 ± 8 |

[a] None detected at a detection limit of 0.0003 μg/mL. [b] The peak was detectable, but it was below the 0.001 μg/mL limit of quantitation.

**Table 4. Recovery of Spinosyns A, D, and B and *N*-Demethylspinosyn D (NDSD) from Pond Water with Silica SPE Cleanup**

| added, μg/mL | sample type | *n* | % recovery (mean ± SD) A | D | B | NDSD |
|---|---|---|---|---|---|---|
| 0.0 | pond water | 1 | ND[a] | ND | ND | ND |
| 0.001 | pond water | 8 | 85 ± 11 | 77 ± 6 | 90 ± 14 | 82 ± 10 |
| 0.010 | pond water | 3 | 81 ± 8 | 81 ± 6 | 82 ± 9 | 78 ± 5 |
| | overall | 11 | 84 ± 10 | 78 ± 6 | 87 ± 13 | 81 ± 9 |

[a] None detected at a detection limit of 0.0003 μg/mL.

**Table 5. Recovery of Spinosyns A, D, and B and *N*-Demethylspinosyn D (NDSD) from Sandy Loam Soil, Clay Loam Soil, and Pond Sediment**

| added, μg/g | sample type | n | % recovery (mean ± SD) A | D | B | NDSD |
|---|---|---|---|---|---|---|
| 0.0 | sandy loam | 2 | ND[a] | ND | ND | ND |
| | clay loam | 2 | ND | ND | ND | ND |
| | sediment | 4 | ND | ND | ND | ND |
| 0.003 | sandy loam | 1 | <LOQ[b] | <LOQ | <LOQ | <LOQ |
| | clay loam | 1 | <LOQ | <LOQ | <LOQ | <LOQ |
| | sediment | 1 | <LOQ | <LOQ | <LOQ | <LOQ |
| 0.010 | sandy loam | 3 | 76 ± 0 | 80 ± 9 | 73 ± 6 | 70 ± 3 |
| | clay loam | 3 | 87 ± 3 | 93 ± 4 | 74 ± 0 | 79 ± 3 |
| | sediment | 8 | 80 ± 5 | 80 ± 8 | 78 ± 6 | 73 ± 4 |
| 0.025 | sediment | 3 | 81 ± 8 | 84 ± 7 | 82 ± 6 | 76 ± 8 |
| 0.050 | sediment | 3 | 76 ± 5 | 77 ± 6 | 72 ± 7 | 69 ± 7 |
| 0.075 | sediment | 3 | 82 ± 7 | 83 ± 7 | 80 ± 6 | 78 ± 6 |
| 0.10 | sediment | 3 | 83 ± 4 | 83 ± 4 | 83 ± 4 | 76 ± 2 |
| 1.0 | sandy loam | 3 | 86 ± 2 | 86 ± 1 | 81 ± 2 | 81 ± 2 |
| | clay loam | 3 | 86 ± 1 | 84 ± 0 | 83 ± 1 | 82 ± 2 |
| | sediment | 3 | 84 ± 3 | 82 ± 3 | 78 ± 2 | 76 ± 2 |
| | overall | 35 | 82 ± 5 | 83 ± 6 | 78 ± 6 | 76 ± 6 |

[a] None detected at a detection limit of 0.003 μg/g. [b] The peak was detectable, but it was below the 0.010 μg/g limit of quantitation.

**Calculated Limits of Detection and Quantitation.** For the soil and sediment method, the limits of detection (LOD) and quantitation (LOQ) were calculated using the standard deviation of the μg/g results from the soil samples fortified at 0.01 μg/g. For the water method, the LOD and LOQ were calculated from the results of the water samples fortified at 0.001 μg/mL. Following a technique described previously (Keith et al., 1983), the LOD was calculated as 3 times the standard deviation (3*s*), and the LOQ was calculated as 10 times the standard deviation (10*s*).

**Confirmation of Results.** A technique for the confirmation of residues was developed using the confirmatory HPLC conditions that were previously described under Apparatus. Residues that were detected in some of the sample solutions injected onto the primary HPLC column (ODS-AQ) were confirmed by injecting those same sample solutions onto a different type of HPLC column ($C_{18}$/cation). The residues were considered to be confirmed if the retention times of the analytes in the samples matched those in the standards on both columns and if the $C_{18}$/cation confirmatory column gave results (μg/g or μg/mL) that were within ±20% of the results obtained on the primary column.

**Pesticide Interference Study.** Seventy pesticides commonly used on cotton, fruit, and vegetables have been previously tested for potential interference with the analytes by direct injection into the liquid chromatograph (West, 1996). Any pesticides that produced interference peaks at the retention times of the analytes were carried through the entire analytical procedure and analyzed using the primary HPLC-UV conditions to determine if they would still interfere after going through the sample purification procedures.

## RESULTS AND DISCUSSION

To determine the recovery levels and the precision of the residue method, a method validation study was conducted with the following types of water samples: tap water (pH 5.4), well water (pH 6.8), and pond water (pH 7.2). The validation study also included two types of soil (sandy loam and clay loam) and a pond sediment. These soil and sediment samples possessed pH values ranging from 5.1 to 7.6, cation exchange capacities ranging from 1.68 to 16.2, and organic matter content ranging from 0.88 to 3.9% (Table 2).

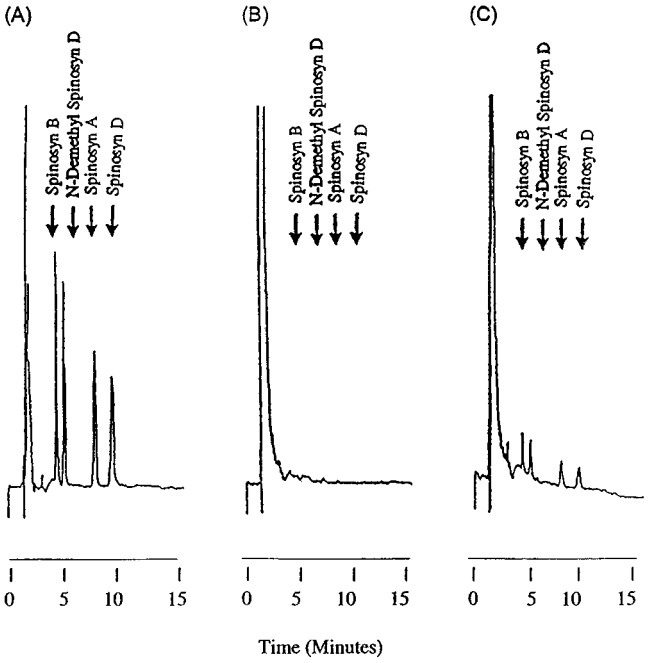


**Figure 1.** Representative chromatograms from the determination of spinosyns A, D, and B and *N*-demethylspinosyn D in pond water: (A) standard, 87.5 ng of each analyte; (B) control water containing no detectable residue; (C) control water fortified with 0.001 μg/mL of all four analytes, equivalent to recoveries of 95% (spinosyn B), 91% (*N*-demethylspinosyn D), 88% (spinosyn A), and 81% (spinosyn D).

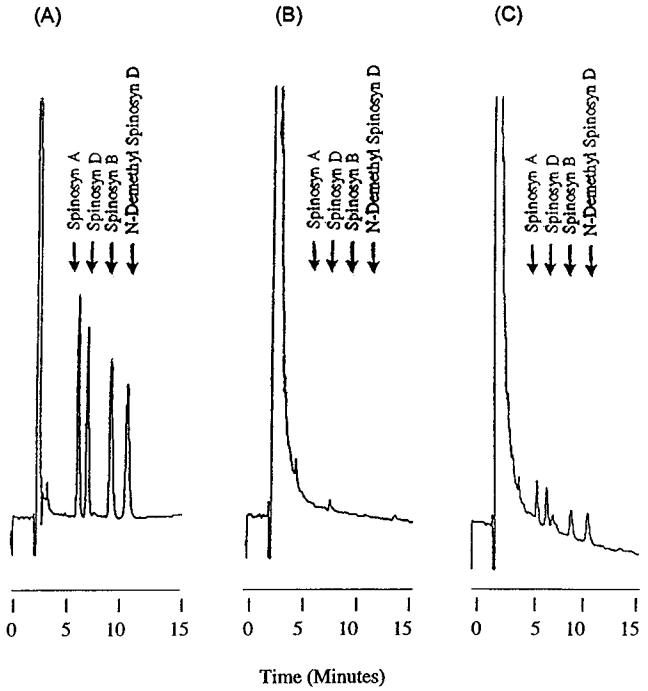


**Figure 2.** Representative chromatograms from the confirmation of residues of spinosyns A, D, and B and *N*-demethylspinosyn D in pond water: (A) standard, 87.5 ng of each analyte; (B) control water containing no detectable residue; (C) control water fortified with 0.001 μg/mL of all four analytes, equivalent to recoveries of 98% (spinosyn A), 108% (spinosyn D), 93% (spinosyn B), and 110% (*N*-demethylspinosyn D).

The results of the validation study are summarized for the various sample types in Tables 3−5. For pond water, the method was validated with and without the optional silica SPE cleanup. Although the use of the

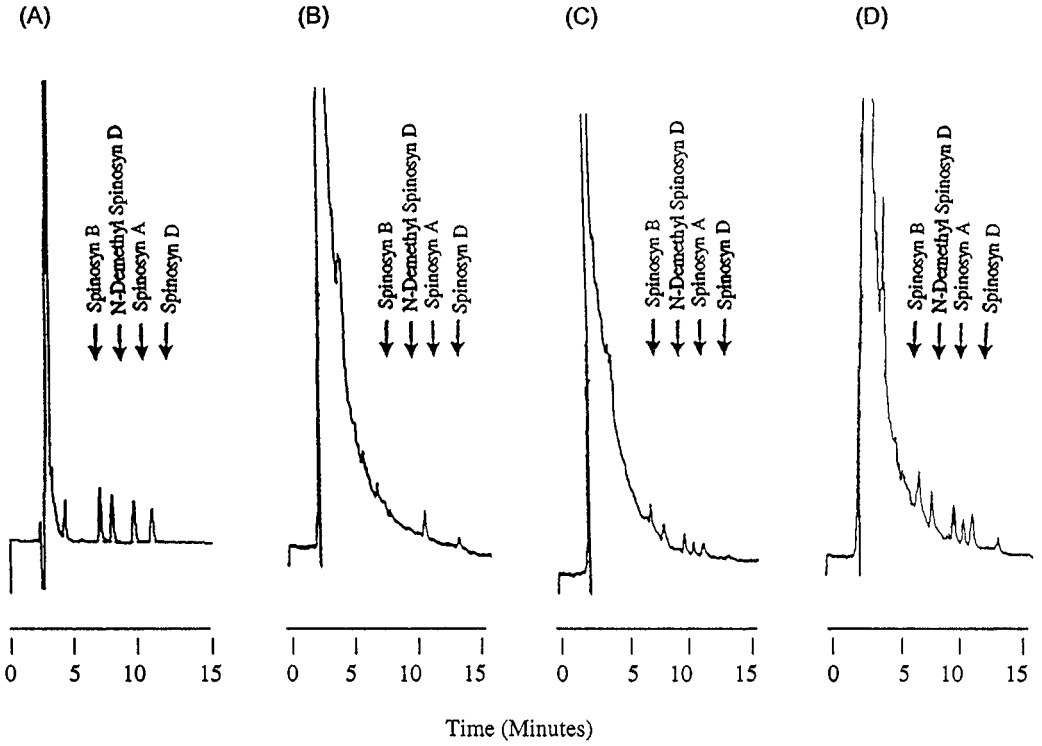


**Figure 3.** Representative chromatograms from the determination of spinosyns A, D, and B and *N*-demethylspinosyn D in pond sediment: (A) standard, 17.5 ng of each analyte; (B) control sediment containing no detectable residue; (C) control sediment fortified with 0.003 μg/g of all four analytes (LOD); (D) control sediment fortified with 0.010 μg/g of all four analytes, equivalent to recoveries of 75% (spinosyn B), 75% (*N*-demethylspinosyn D), 82% (spinosyn A), and 89% (spinosyn D).

**Table 6. Calculated Limits of Detection and Quantitation (μg/g or μg/mL) for Spinosyns A, D, and B and *N*-Demethylspinosyn D (NDSD)**

| | soil or sediment | | | | water | | | |
|---|---|---|---|---|---|---|---|---|
| | A | D | B | NDSD | A | D | B | NDSD |
| $\bar{x}^a$ | 0.0081 | 0.0083 | 0.0076 | 0.0073 | 0.00094 | 0.00088 | 0.00087 | 0.00091 |
| $s^b$ | 0.0005 | 0.0009 | 0.0005 | 0.0005 | 0.00011 | 0.00011 | 0.00010 | 0.00010 |
| LOD (3*s*)[c] | 0.002 | 0.003 | 0.002 | 0.002 | 0.0003 | 0.0003 | 0.0003 | 0.0003 |
| LOQ (10*s*)[d] | 0.005 | 0.009 | 0.005 | 0.005 | 0.001 | 0.001 | 0.001 | 0.001 |

[a] Mean value of the results from the 0.010 μg/g soil recovery samples or the 0.010 μg/mL water samples. [b] Standard deviation of the results from the 0.010 μg/g soil recovery samples or the 0.001 μg/mL water samples. [c] Calculated LOD, calculated as 3*s*. [d] Calculated LOQ, calculated as 10*s*.

silica SPE cleanup procedure was not required for cleanup of the samples used in the method validation study, the procedure was included with some of the pond water samples to determine its effect upon recoveries. The silica SPE column was found to remove some additional (noninterfering) peaks from the chromatogram of the pond water sample, but it reduced average recoveries by approximately 10% (Tables 3 and 4).

The following recovery values (mean ± 1 standard deviation) resulted when water samples were fortified over the concentration range of 0.001−0.1 μg/mL and the silica SPE cleanup was not used: spinosyn A, 93 ± 8%; spinosyn D, 90 ± 8%; spinosyn B, 87 ± 6%; and *N*-demethylspinosyn D, 90 ± 8% (Table 3). The following recovery values resulted when the silica SPE cleanup was used: spinosyn A, 84 ± 10%; spinosyn D, 78 ± 6%; spinosyn B, 87 ± 13%; and *N*-demethylspinosyn D, 81 ± 9% (Table 4).

Typical chromatograms demonstrating the determination and confirmation of the analytes in pond water are included in Figures 1 and 2, respectively. The alternative conditions used for the confirmation (Figure 2) resulted in a different order of elution for the analytes compared to that obtained with the primary HPLC conditions (Figure 1).

The following recovery values (mean ± 1 standard deviation) resulted when the soil and sediment samples were fortified over the concentration range of 0.01−1.0 μg/g: spinosyn A, 82 ± 5%; spinosyn D, 83 ± 6%; spinosyn B, 78 ± 6%; and *N*-demethylspinosyn D, 76 ± 6% (Table 5). Typical chromatograms for the determination of the analytes in pond sediment are included in Figure 3.

The average correlation coefficient ($r^2$) for the least-squares equations describing the detector response as a function of concentration of the standard curve solutions was 0.9999 for all four analytes. Linearity at concentrations exceeding the range of the calibration curve (0.0−1.5 μg/mL) was not investigated.

The calculated LOD and LOQ values are presented in Table 6. For the four analytes, the calculated LOD for the soil and sediment method ranged from 0.002 to 0.003 μg/g, and the calculated LOQ ranged from 0.005 to 0.009 μg/g. These calculated values supported the validated method LOD and LOQ of 0.003 and 0.01 μg/g, respectively. The method LOD was further supported by the presence of detectable peaks in chromatograms resulting from the analysis of control samples fortified at 0.003 μg/g (Figure 3). Quantitative recovery values

were not calculated for the samples at the LOD, because the analytes were present at levels below the LOQ.

Likewise, the calculated LOD for the water method was 0.0003 μg/mL, and the calculated LOQ was 0.001 μg/mL. These calculated values supported the validated method LOD and LOQ of 0.0003 and 0.001 μg/mL, respectively

Pesticides commonly used on cotton and vegetables were previously tested for potential interference with the analytes (West, 1996). Seventy pesticides were tested for interference by direct injection into the liquid chromatograph. Most of the pesticides eluted with the solvent front, and only avermectin $B_{1a}$, dicofol, propargite, thiodicarb, and tralomethrin produced interference peaks that matched the retention times of the analytes. However, none of these five pesticides interfered when carried through the entire analytical procedure and then injected into the liquid chromatograph. Thus, the cleanup procedures described in the method effectively removed the potentially interfering pesticides as well as the interfering coextractives from the samples.

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