Determination of the Naturally Derived Insect Control Agent Spinosad in Cottonseed and Processed Commodities by High-Performance Liquid Chromatography with Ultraviolet Detection

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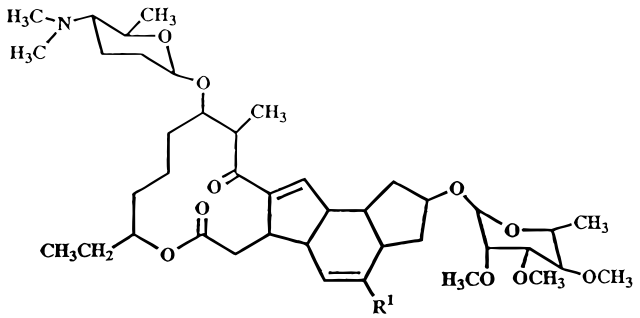
A method is described for the determination of the naturally derived insect control agent spinosad in cottonseed and cottonseed processed commodities (meal, hulls, crude oil, refined oil, and soapstock). The method was validated over the concentration range 0.01−0.1 μg/g, with a limit of quantitation of 0.01 μg/g and a limit of detection of 0.003 μg/g. Residues of the active ingredients in spinosad (spinosyns A and D) were extracted from samples with appropriate organic solvents. The extracting solvents were hexane for cottonseed oil, methylene chloride for soapstock, and 60% hexane/40% acetone for cottonseed, meal, or hulls. An aliquot of the extract was purified by liquid−liquid partitioning and silica solid phase extraction. Spinosyns A and D were determined simultaneously in the purified extracts by reversed-phase high-performance liquid chromatography with ultraviolet detection at 250 nm. Confirmation of residue identity was accomplished by reinjecting the same final solution into the chromatograph under different chromatographic conditions.

Keywords: *Spinosad; spinosyn A; spinosyn D; cottonseed; 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 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 were needed to generate field residue data for the establishment of a residue tolerance for cottonseed and for determining if residues would concentrate in processed products prepared from cottonseed. Previous translocation and metabolism studies using foliar applications of radiolabeled (^{14}C) spinosyns A and D demonstrated that the compounds were extensively degraded and incorporated into the natural components of cottonseed, such that no major spinosyn-related residues were identifiable (Magnussen et al., 1994). Thus, it was necessary to develop methods only for the parent compounds, spinosyns A and D. The following method is presented for the determination of the two analytes in the raw agricultural commodity (cottonseed) and in the processed commodities (cottonseed meal, hulls, crude oil, refined oil, and soapstock) by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (HPLC-UV). The chemical names and CAS Registry Numbers for spinosyns A and D are included in Table 1.



Spinosyn A, R_1 = H
Spinosyn D, R_1 = CH_3

EXPERIMENTAL PROCEDURES

Apparatus. *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-μm particle size, 120 Å, 100 × 2.0 mm i.d. (YMC)], maintained at an oven temperature of 30 °C. The mobile phase consisted of 41% reservoir A/41% reservoir B/18% reservoir C (isocratic), with reservoir A containing methanol, reservoir B containing acetonitrile, and reservoir C containing 2% aqueous ammonium acetate/acetonitrile (67:33, v/v). The flow rate was 0.4 mL/min. The injection volume was 100 μL, and the integrator attenuation was 2^3. The chart speed was 0.2 cm/min. Under these conditions, the retention times for spinosyns A and D were ≈11.5 min and ≈14.5 min, respectively.

The confirmatory HPLC column was an RP-8 Cation, mixed mode, with 5-μm particle size and 150 × 2.1 mm i.d. (Alltech/Applied Science). The mobile phase was 33% reservoir A/33% reservoir B/20% reservoir C/14% reservoir D (isocratic), with reservoir A containing methanol, reservoir B containing acetonitrile, reservoir C containing 2% aqueous ammonium acetate/acetonitrile (67:33, v/v), and reservoir D containing 0.1 N acetic acid. All of the other parameters were the same as those just listed for the primary column. Under these conditions, the retention times for spinosyns A and D were ≈12 and ≈13 min, respectively.

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

spinosyn	CAS Registry No.[a]	chemical name
A	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
D	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

[a] Supplied by the author.

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.

Centrifuge. The centrifuge was an International Equipment Company model CU-5000.

Sample Grinder. The sample grinder was a Fitzpatrick Company Homoloid model J, with a screen size of 3−5 mm.

Rotary Vacuum Evaporator. The rotary vacuum evaporator was a Rinco Instrument Company model 1007−4 IN.

Orbital Shaker. The orbital shaker was a New Brunswick model G-33.

Water Purification System. The water purification system was a Millipore Corporation Milli-Q UV Plus.

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

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

Filter Paper. The filter paper was Schleicher and Schuell Number 588, 15-cm, pre-pleated (0.19-mm thickness).

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, and then 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.

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

Evaporator. The evaporator was a Zymark Corporation TurboVap LV.

Reagents. Solvents (acetone, acetonitrile, dichloromethane, hexane, and methanol) were HPLC grade. Water was purified using a Milli-Q UV Plus purification system. Ammonium acetate was HPLC grade. Glacial acetic acid, hydrochloric acid, sodium chloride, sodium hydroxide, and granular anhydrous sodium sulfate were analytical grade. The sodium sulfate (Fisher catalog number 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 mostly evaporated. (Long 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 during the analysis procedure.) The purified active ingredients used for analytical standards were obtained from the Test Substance Coordinator, DowElanco, 9330 Zionsville Road, Building 306/A1, Indianapolis, IN 46268-1053.

Safety Precautions. Safety information on the reagents and chemicals listed in this method was obtained from the container labels or from the suppliers. 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.

Standard Preparation. Individual stock solutions of spinosyns A and D were prepared at 50 μg/mL by weighing 10 mg of both standards, quantitatively transferring them to separate 200-mL volumetric flasks, dissolving in 50% methanol/50% acetonitrile, and diluting to volume. Aliquots (20 mL) of both 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 both analytes at 10.0 μg/mL. 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, 0.05, 0.1, 0.5, 1.0, and 1.5 μg/mL.

Solutions for fortifying control samples for the determination of recovery were prepared by combining 10.0-mL aliquots of the two 50.0-μg/mL stock solutions in a 50-mL volumetric flask and diluting to volume with the appropriate solution to obtain a mixture containing each of spinosyns A and D at 10.0 μg/mL. For cottonseed, meal, hulls, and oil, the diluting solvent for this intermediate solution was 60% hexane/40% acetone. For soapstock, the diluting solvent was dichloromethane (DCM). Aliquots of this solution were further diluted with the appropriate solvent to obtain fortification standards at concentrations of 0.05, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, and 1.0 μg/mL. The diluting solvent for these fortification solutions was hexane for crude or refined oil, DCM for soapstock, and 60% hexane/40% acetone for seed, meal, and hulls.

Initial Sample Preparation. Samples of cottonseed, meal, and hulls were frozen with liquid nitrogen and prepared for analysis by grinding through a Homoloid model J grinder with a screen size of 3−5 mm. After grinding, the samples were mixed for homogeneity. (Oil and soapstock samples did not require any initial preparation prior to analysis.)

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 sample analysis) and placing the samples in the dark for any short interruptions during sample processing.

Sample Extraction. *Crude and Refined Cottonseed Oil.* Samples (5 g) were weighed into 8-oz. (237-mL) glass bottles. Fortified recovery samples were prepared from untreated control samples by adding 1.0 mL of the appropriate fortification standard solution in hexane. Hexane (50 mL) was added, and the analysis of the oil samples was then continued as described later under *Sample Extract Purification.*

Solid Cottonseed Soapstock. Solid soapstock samples (10 g) were weighed into 8-oz. (237-mL) glass bottles. Fortified recovery samples were prepared from untreated control samples by adding 1.0 mL of the appropriate fortification standard solution in DCM. DCM (100 mL) was added, the bottle was capped with a PTFE-lined lid, and the sample was shaken on an orbital shaker at 250 rpm for 30 min. A 50-mL aliquot of the DCM extract was filtered through pre-pleated filter paper. The 50-mL aliquot was transferred to a 250-mL evaporating flask by pouring through a long-stemmed funnel. Prior to evaporating the sample, the rotary vacuum evaporator was rinsed with hexane and then methanol to prevent sample contamination. The solvent was evaporated with the rotary vacuum evaporator and a water bath heated to 35−50 °C. Hexane (50 mL) was added, and the analysis of the sample was then continued as described later under *Sample Extract Purification.*

Liquid Cottonseed Soapstock. Liquid soapstock samples (10 g) were weighed into 8-oz. (237-mL) glass bottles. Fortified recovery samples were prepared from untreated control samples by adding 1.0 mL of the appropriate fortification standard solution in DCM. DCM (100 mL) was added, the bottle was

Table 2. Recovery of Spinosyns A and D from Cottonseed and Processed Commodities

			spinosyn A		spinosyn D	
commodity	added (μg/g)	*n*	range	mean	range	mean
cottonseed	0.00	3	ND[a]	ND	ND	ND
	0.003	2	NA[b]	NA	NA	NA
	0.01	8	99−119	109	82−117	99
	0.02	2	74−78	76	78−83	81
	0.04	2	79−81	80	82−82	82
	0.06	2	102−103	103	101−103	102
	0.08	2	93−93	93	92−93	93
	0.1	2	102−104	103	102−104	103
overall		18	74−119	99	78−117	95
meal	0.0	2	ND	ND	ND	ND
	0.003	2	NA	NA	NA	NA
	0.01	8	81−100	91	73−96	85
	0.1	2	80−95	88	80−94	87
overall		10	80−100	90	73-96	85
hulls	0.0	2	ND	ND	ND	ND
	0.003	2	NA	NA	NA	NA
	0.01	8	91−110	99	87−110	100
	0.1	2	89−116	103	87−108	98
overall		10	89−116	100	87−110	100
crude oil	0.00	3	ND	ND	ND	ND
	0.003	2	NA	NA	NA	NA
	0.01	8	86−96	92	85−96	92
	0.02	2	92−92	92	86−92	89
	0.04	2	102−113	108	97−97	97
	0.06	2	91−100	96	88−97	93
	0.08	2	103−103	103	99−99	99
	0.1	2	94−95	95	92−92	92
overall		18	86−113	96	85-99	93
refined oil	0.0	2	ND	ND	ND	ND
	0.003	2	NA	NA	NA	NA
	0.01	8	88−98	97	80−103	90
	0.1	2	70−77	74	68−73	71
overall		10	70−98	92	68−103	86
soapstock	0.00	3	ND	ND	ND	ND
	0.003	2	NA	NA	NA	NA
	0.01	8	93−102	98	100−110	103
	0.02	2	101−101	101	98−109	104
	0.04	2	99−99	99	98−98	98
	0.06	2	99−100	100	100−100	100
	0.08	2	98−98	98	99−99	99
	0.1	2	103−104	104	103−103	103
overall		18	93−104	99	98−110	102

[a] None detected at a detection limit of 0.003 μg/g. [b] Not applicable (the observed residues were below the 0.01 μg/g LOQ.)

capped with a PTFE-lined lid, and the sample was shaken on an orbital shaker at 250 rpm for 5 min. (Shaking the liquid soapstock samples at a higher speed or for a longer period of time was avoided to prevent the formation of an emulsion that would not break during centrifugation.) The sample was centrifuged at 2250 rpm for 5−10 min. More than half (i.e., >50 mL) of the DCM solution was carefully decanted through a funnel into a 250-mL separatory funnel. Because of its viscosity, the soapstock decanted more slowly than the DCM, and care was taken to transfer only a minimal amount of the soapstock along with the DCM. The stopcock on the separatory funnel was opened, and ≈51−55 mL of the DCM (lower phase) was drained into a graduated cylinder. Care was taken to avoid draining the soapstock into the graduated cylinder. The soapstock and any DCM remaining in the separatory funnel were discarded. Using a disposable Pasteur pipet, any soapstock particles were removed from the top of the DCM in the graduated cylinder, and the volume was then reduced to 50 mL by removing additional DCM with the pipet. The 50-mL aliquot was transferred through a long-stemmed funnel into a 250-mL evaporating flask. Prior to evaporating the sample, the rotary vacuum evaporator was rinsed 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. Hexane (50 mL) was added, and the analysis of the sample was then continued as described later under *Sample Extract Purification.*

Cottonseed, Hulls, and Meal. Cottonseed, hull, or meal samples (10 g) were weighed into 8-oz. (237-mL) glass bottles. Fortified recovery samples were prepared from untreated control samples by adding 1.0 mL of the appropriate fortification standard solution in 60% hexane/40% acetone. The extracting solvent (60 mL of 60% hexane/40% acetone) was added, the bottle was sealed with a PTFE-lined lid, and the sample was shaken on an orbital shaker at 250 rpm for 30 min. The samples were centrifuged at 2250 rpm for 5 min. A 30-mL aliquot of the supernatant liquid was decanted through pre-pleated filter paper into a 50-mL graduated cylinder. (If necessary, the sample tissue was pressed with a spatula while decanting to obtain sufficient volume for the 30-mL aliquot.) The 30-mL aliquot was transferred to a 250-mL evaporating flask by pouring through a long-stemmed funnel. Prior to evaporating the sample, the rotary vacuum evaporator was rinsed with hexane and then methanol to prevent sample contamination. The solvent was evaporated with the rotary vacuum evaporator and a water bath heated to 35−50 °C. (A small amount of cottonseed oil remained in the flask.) Hexane (50 mL) was added, and the analysis of the sample was then continued as described later under *Sample Extract Purification.*

Sample Extract Purification. *Liquid−Liquid Partitioning.* The extract in 50 mL of hexane was transferred to a 250-mL separatory funnel. The sample container was rinsed with 25 mL of methanol/5% aqueous sodium chloride (70:30, v/v), which was transferred to the separatory funnel. The container was rinsed with 25 mL of an aqueous solution containing 0.04 N hydrochloric acid and 5% sodium chloride, which was also transferred to the separatory funnel. The separatory funnel was gently shaken for 20−30 s. (Vigorous shaking was avoided to minimize the formation of emulsions.) . After waiting ≈15 min for the layers to separate, the aqueous (lower) layer, including the slight emulsion, was drained into a 250-

Table 3. Calculated Limits of Detection and Quantitation (μg/g) for Spinosyns A and D

		amount (μg/g)					
spinosyn	parameter	cottonseed	meal	hulls	crude oil	refined oil	soapstock
A	$\bar{x}$[a]	0.0109	0.0091	0.0099	0.0092	0.0097	0.0098
	s[b]	0.0008	0.0005	0.0008	0.0005	0.0004	0.0005
	LOD (3*s*)[c]	0.002	0.002	0.002	0.002	0.001	0.002
	LOQ (10*s*)[d]	0.008	0.005	0.008	0.005	0.004	0.005
B	$\bar{x}$	0.0099	0.0085	0.0100	0.0092	0.0090	0.0103
	s	0.0012	0.0009	0.0010	0.0006	0.0007	0.0005
	LOD (3*s*)	0.004	0.003	0.003	0.002	0.002	0.002
	LOQ (10*s*)	0.012	0.009	0.010	0.006	0.007	0.005

[a] Mean value of the μg/g results for the 0.010-μg/g recoveries. [b] Standard deviation of the μg/g results for the 0.010-μg/g recoveries. [c] Calculated LOD, calculated as 3*s*. [d] Calculated LOQ, calculated as 10 *s*.

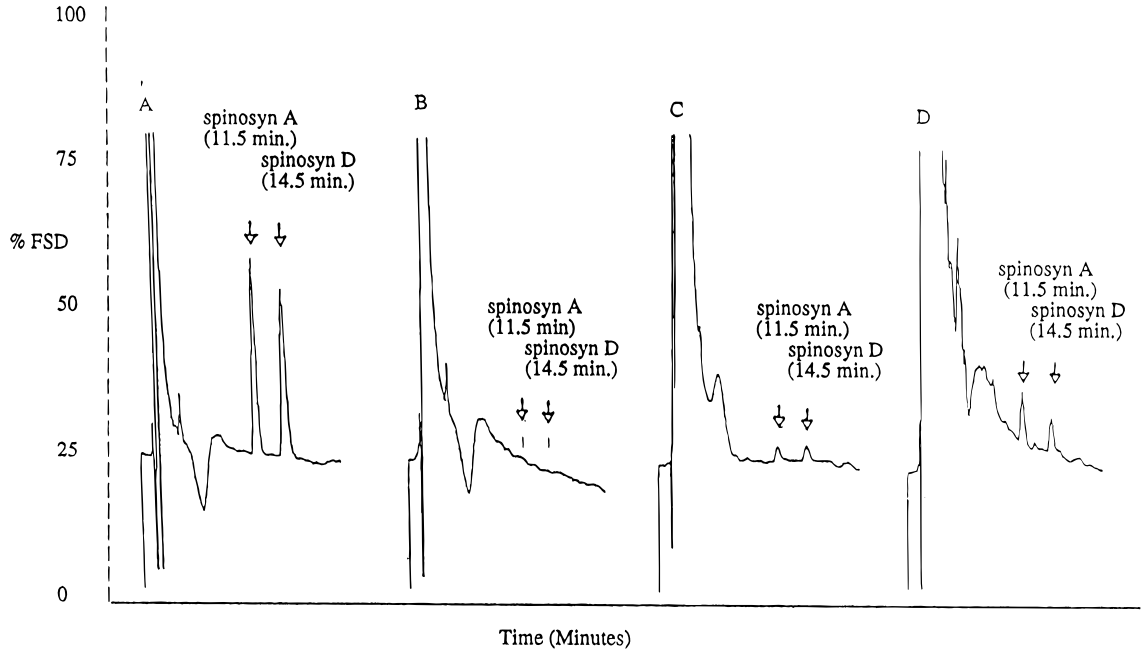

Figure 1. Representative chromatograms of spinosyns A and D: (A) standard, 20 ng of both analytes; (B) untreated control cottonseed containing no detectable residue; (C) control cottonseed fortified with spinosyns A and D at 0.003 μg/g (LOD); (D) control cottonseed fortified with 0.01 μg/g, equivalent to recoveries of 99% for spinosyn A and 82% for spinosyn D.

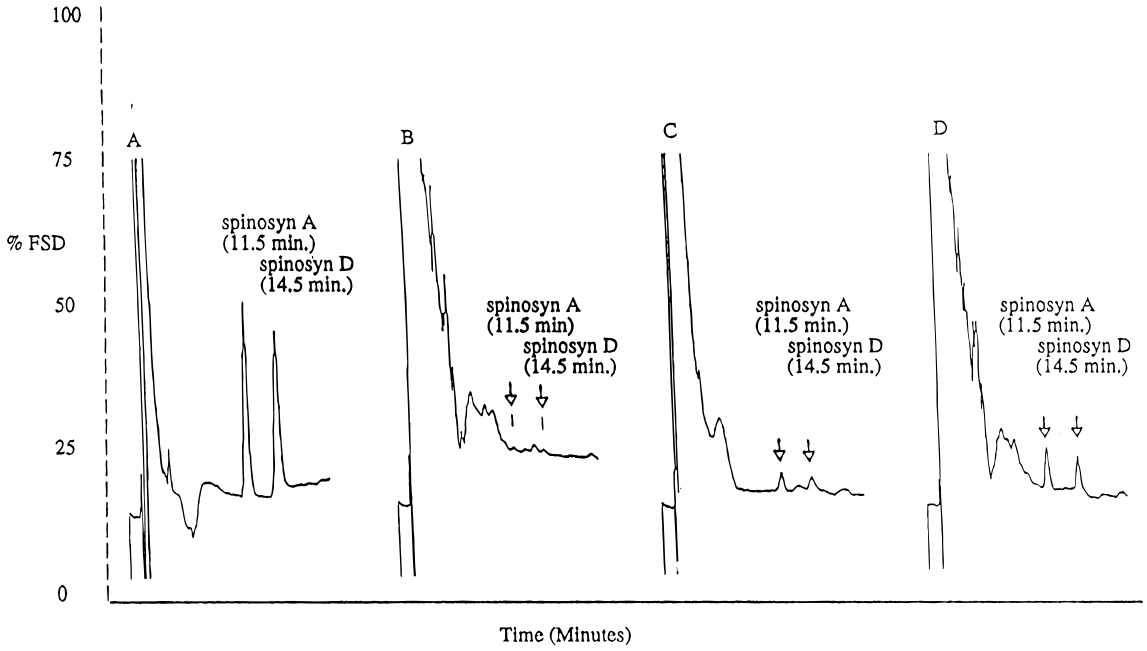

Figure 2. Representative chromatograms of spinosyns A and D: (A) standard, 20 ng of both analytes; (B) untreated control cottonseed meal containing no detectable residue; (C) control meal fortified with spinosyns A and D at 0.003 μg/g (LOD); (D) control meal fortified with 0.01 μg/g, equivalent to recoveries of 91% for spinosyn A and 96% for spinosyn D.

mL beaker. The hexane (upper) layer was discarded. (It was occasionally necessary to use a stirring rod to aid the separation of the aqueous and hexane layers.) Samples of cottonseed, meal, hulls, refined oil, and soapstock required only one hexane partitioning under these acidic conditions. However, for crude cottonseed oil samples, the aqueous phase in the 250-mL beaker was returned to the separatory funnel, 50 mL of hexane was added, and the partitioning step was repeated one time for additional purification.

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 4.0 mL of 1 N aqueous sodium hydroxide. Spinosyns A and D were then extracted from the aqueous phase by shaking with three 50-mL aliquots of hexane for 20−30 s each. After waiting for the layers to separate after each partitioning, the aqueous (lower) phase (including the slight emulsion) was drained into the 250-mL beaker. The three hexane extracts were combined in a 500-mL evaporating flask by draining through a funnel containing a small plug of purified glass wool and 40 mL (approximately 25 g) of hexane-washed sodium sulfate. After draining the hexane from the third partitioning step, the sodium sulfate was rinsed with 15 mL of hexane. Prior to evaporating the sample, the rotary vacuum evaporator was rinsed with hexane and then methanol. The samples were evaporated to dryness with the rotary vacuum evaporator and a water bath heated to 35−50 °C. The residue was dissolved in 10 mL of hexane for further purification by silica SPE as described next.

Purification by Silica SPE. Prior to using each new lot of silica SPE columns, the elution profile was determined with a standard solution containing 2.0 μg of spinosyns A and D in

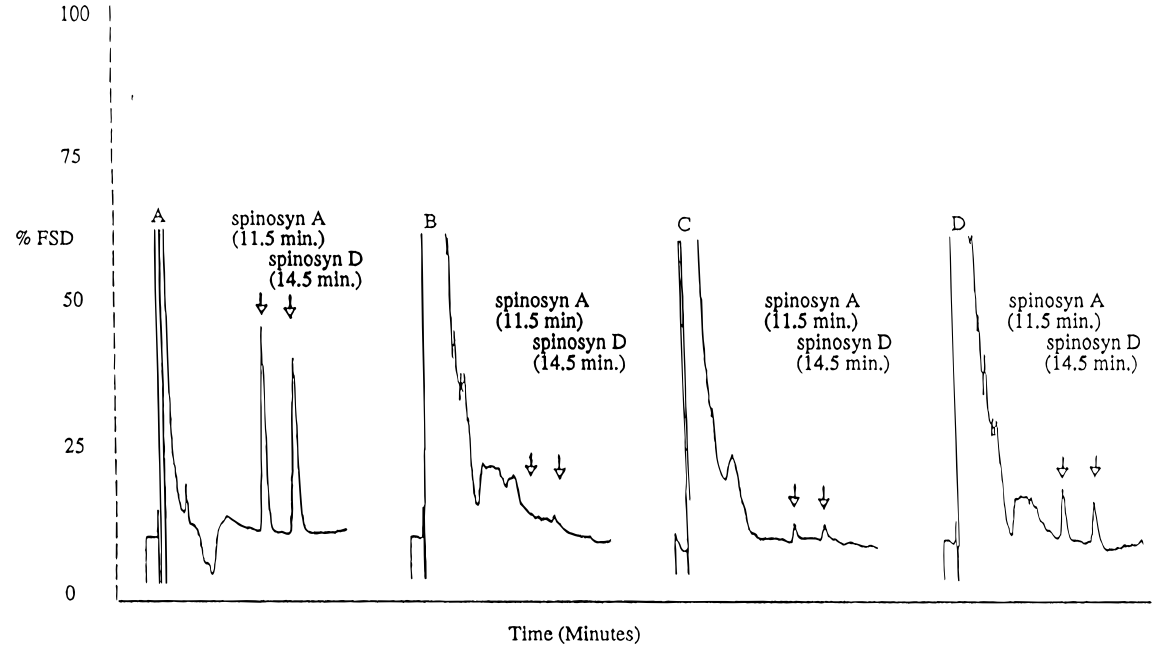

Figure 3. Representative chromatograms of spinosyns A and D: (A) standard, 20 ng of both analytes; (B) untreated control cottonseed hulls containing no detectable residue; (C) control hulls fortified with spinosyns A and D at 0.003 μg/g (LOD); (D) control hulls fortified with 0.01 μg/g, equivalent to recoveries of 100% for spinosyn A and 110% for spinosyn D.

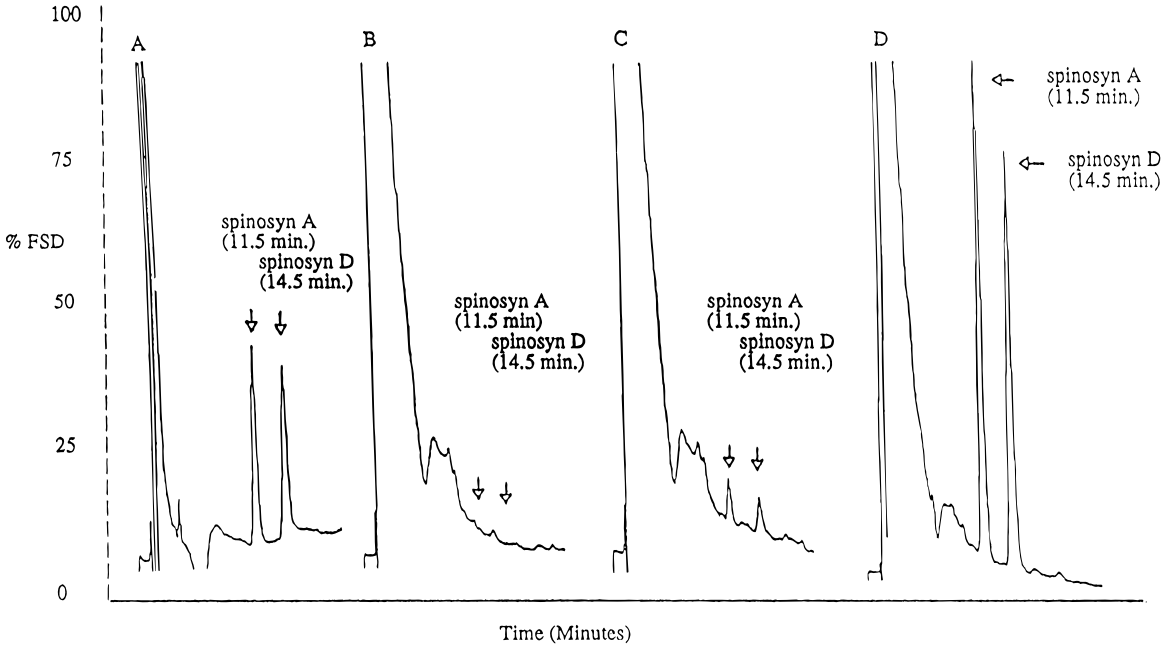

Figure 4. Representative chromatograms of spinosyns A and D: (A) standard, 20 ng of both analytes; (B) untreated control crude cottonseed oil containing no detectable residue; (C) control crude oil fortified with spinosyns A and D at 0.01 μg/g equivalent to recoveries of 86% for spinosyn A and 85% for spinosyn D; (D) control crude oil fortified with 0.1 μg/g, equivalent to recoveries of 95% for spinosyn A and 93% for spinosyn D.

10 mL of hexane to ensure that the appropriate volumes of solvents were discarded and collected in the following procedure. The following volumes were typical, but might require modification for different lots of silica SPE columns. An SPE column reservoir was attached to a silica SPE cartridge, and the cartridge was attached to the vacuum manifold. Prior to adding 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.

The sample was added in 10 mL of hexane. The evaporating flask was rinsed with two 10-mL aliquots of hexane that 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 6-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 clean, 35-mL vial was then added to the vacuum manifold for solvent collection. The evaporating flask was rinsed with 5 mL of 75% DCM/25% methanol, which was added to the column and eluted into the vial by dropwise elution. The sample solution was immediately evaporated with a TurboVap evaporator set at 60 °C and a nitrogen flow of 8 psi. 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 spinosyns A and D adsorb very tightly to glass, the swirling and rotating procedure was repeated one time to ensure that

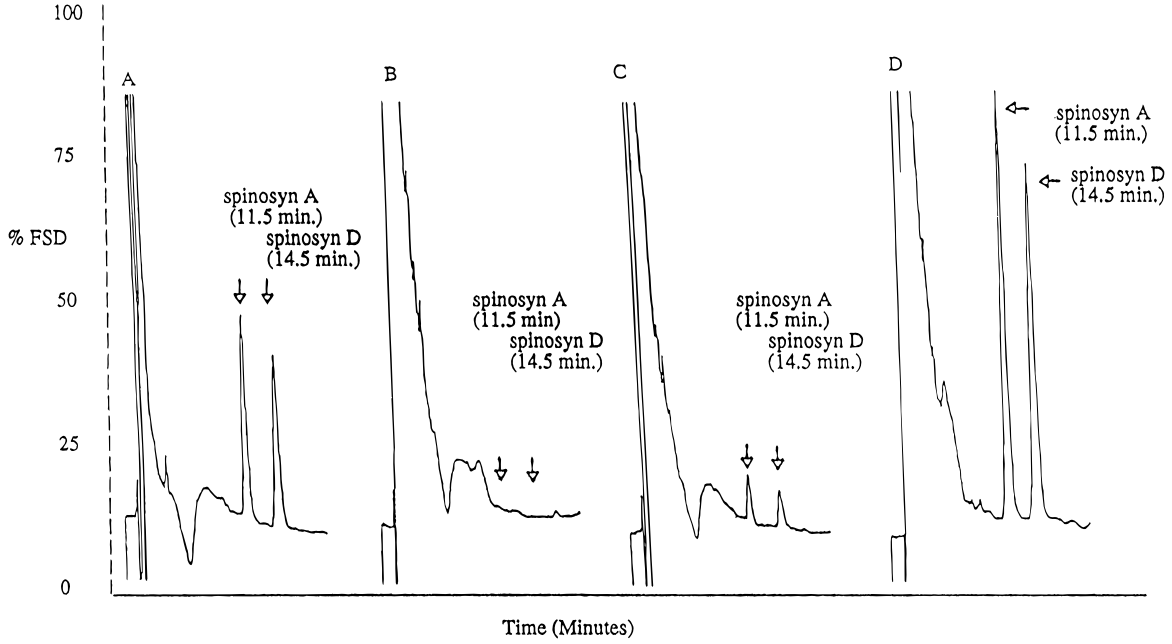

Figure 5. Representative chromatograms of spinosyns A and D: (A) standard, 20 ng of both analytes; (B) untreated control refined cottonseed oil containing no detectable residue; (C) control refined oil fortified with spinosyns A and D at 0.01 μg/g, equivalent to recoveries of 88% for spinosyn A and 80% for spinosyn D; (D) control refined oil fortified with 0.1 μg/g, equivalent to recoveries of 77% for spinosyn A and 33% for spinosyn D.

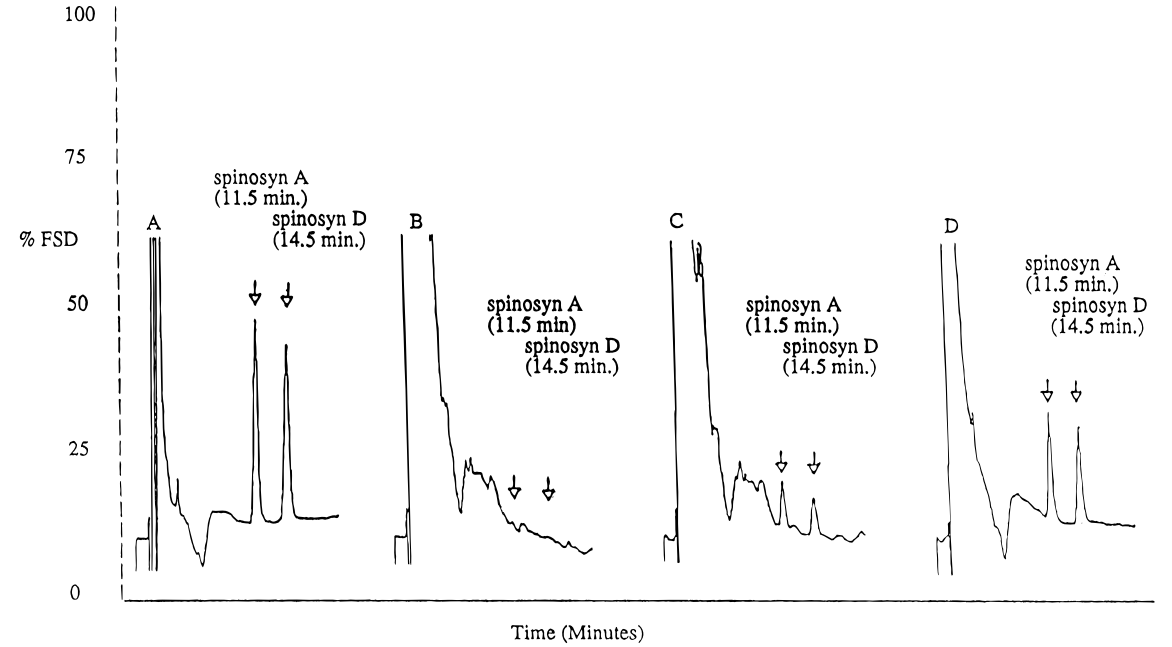

Figure 6. Representative chromatograms of spinosyns A and D: (A) standard, 20 ng of both analytes; (B) untreated control cottonseed soapstock containing no detectable residue; (C) control soapstock fortified with spinosyns A and D at 0.01 μg/g, equivalent to recoveries of 93% for spinosyn A and 100% for spinosyn D; (D) control soapstock fortified with 0.02 μg/g, equivalent to recoveries of 101% for spinosyn A and 109% for spinosyn D.

the residue had dissolved. With 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.

HPLC. Standard and sample solutions were analyzed by HPLC using the previously described conditions. The suitability of the chromatographic system was determined with the following performance criteria: (a) It was determined that the correlation coefficient (r^2) equaled or exceeded 0.997 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 spinosyns A and D. (c) It was visually determined that a signal-to-noise ratio of ≈5:1 to 10:1 was achievable for the 0.05-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 spinosyns A and D by plotting the concentration of the calibration standards on the abscissa (*x*-axis) and the resulting peak heights on the ordinate (*y*-axis). By 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:

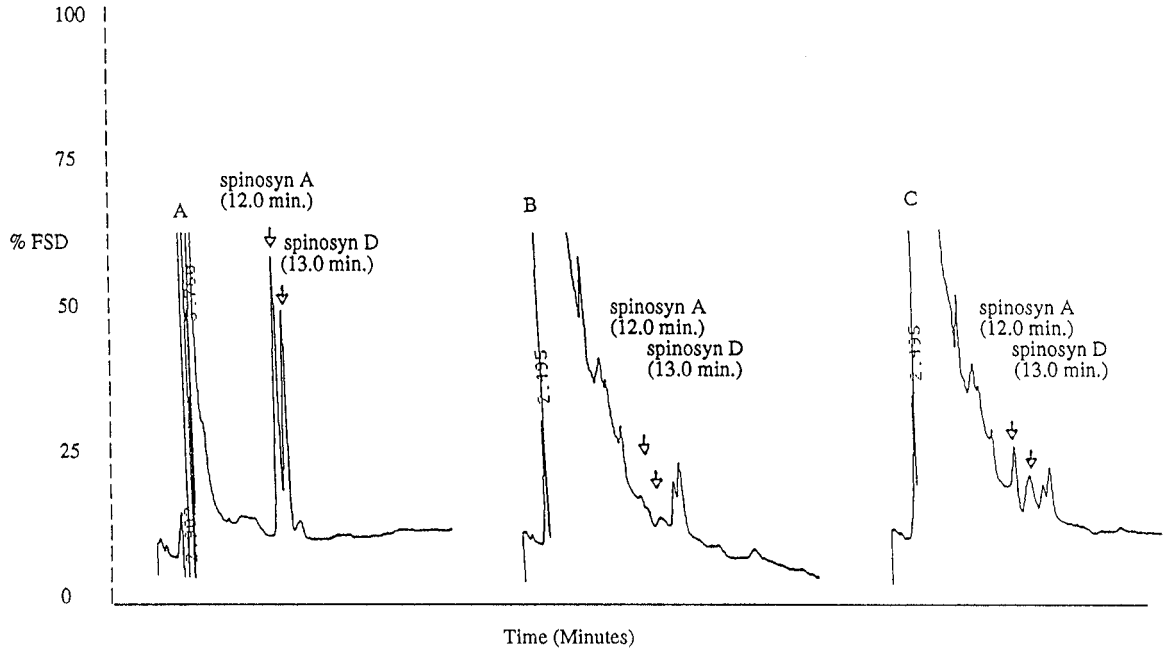

Figure 7. Representative chromatograms for the confirmation of spinosyns A and D with the alternative chromatographic conditions: (A) standard, 20 ng of both analytes; (B) untreated control cottonseed containing no detectable residue; (C) control cottonseed fortified with 0.01 μg/g, equivalent to recoveries of 85% for spinosyn A and 92% for spinosyn D.

Table 4. Pesticides Tested for Interference with Spinosyns A and D

acephate	fenamiphos	oryzalin	chlorpyrifos	malathion	simazine
aldicarb	fenvalerate	oxamyl	cyanazine	mancozeb	sulprofos
avermectin B_{1a}	fluazifop-butyl	oxyfluorfen	cyhalothrin	metalyxil	terbicil
azinphos-methyl	flumetsulam	paraquat dichloride	cypermethrin	mepiquat chloride	terbufos
benomyl	fluometuron	PCNB[a]	DCPA[a]	methidathion	thiodicarb
bensulide	fonofos	pendimethalin	diazinon	methomyl	tillam (pebulate)
bifenthrin	glyphosate	permethrin	dicofol	methyl parathion	tralomethrin
bloc (fenarimol)	imidan (phosmet)	profenofos	dimethoate	metribuzin	triadimefon
botran (DCNA)	iprodione	prometryn	disulfoton	MSMA[a]	trifluralin
butifos	isoproturon	pronamide	endosulfan	napropamide	triforine
carbaryl	karmex (DCMU)	propargite	EPTC[a]	norflurazon	ziram
chlorothalonil	kelthane	sethoxydim	ethephon		

[a] DCPA, Dacthal; EPTC, Eptam; MSMA, monosodium methanearsonate; PCNB, pentachloronitrobenzene.

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

The concentration (μg/g) of the analytes in the samples was calculated from the concentration in the final solution (C), the aliquot factor (AF = extraction volume divided by aliquot volume), the final volume (V), and the weight of the sample that was extracted (W) the following equation:

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

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

$$R = \frac{\mu\mathrm{g/g,\ net}}{\mu\mathrm{g/g\ added}} \times 100\% \quad (3)$$

For any sample results requiring correction for recovery, the corrected results can be calculated with the average recovery (R_a) as follows:

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

Calculated Limits of Detection and Quantitation. The limits of detection (LOD) and quantitation (LOQ) were calculated with the standard deviation from the 0.01-μg/g recovery results. Following a technique described previously (Keith et al., 1983), the LOD was calculated as 3 times the standard deviation, and the LOQ was calculated as 10 times the standard deviation.

Confirmation of Results. Confirmation of residues was accomplished with the previously described confirmatory HPLC conditions. The same sample solutions were injected into a different type of HPLC column with a different mobile phase composition. Confirmation of the residues occurred if the retention times of the analytes in the samples matched those in the standards under both sets of chromatographic conditions and if the confirmatory technique gave results that were within ±20% of the original results.

Pesticide Interference Study. Seventy pesticides commonly used on cotton and vegetables were tested for potential interference with spinosyns A and D. At concentrations of 0.4−10 μg/mL, the pesticides were tested for interference by direct injection into the liquid chromatography column. Any pesticides that produced interference peaks at the retention times of the analytes were carried through the entire analytical procedure and analyzed with the primary HPLC-UV conditions to determine if they would still interfere after going through the sample purification procedures.

RESULTS AND DISCUSSION

A method validation study was conducted to determine the recovery levels and the precision of the residue method, and the results are summarized for the various commodities in Table 2. For the six commodities combined, average recoveries ranged from 90 to 100% for spinosyn A and from 85 to 102% for spinosyn D.

The average correlation coefficient (r^2) for the least squares equations describing the detector response as a function of standard curve concentration was 0.9999 for both spinosyns A and D. Linearity at concentrations exceeding the range of the calibration curve (0.0−1.5 μg/mL) was not investigated.

The LOD and LOQ values are presented in Table 3. The calculated LOD ranged from 0.001 to 0.002 μg/g for spinosyn A and from 0.002 to 0.004 μg/g for spinosyn D. These values generally supported an LOD for the method of 0.003 μg/g for both analytes. 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 for the six different commodities. Examples are included in Figures 1−3. No quantitative recovery values are included with the chromatograms verifying the claimed LOD because the analytes were present at levels below the limit of quantitation (i.e., <0.01 μg/g).

Likewise, the calculated LOQ ranged from 0.004 to 0.008 μg/g for spinosyn A and from 0.005 to 0.012 μg/g for spinosyn D in the six different commodities (Table 3). These values supported the validated LOQ of 0.01 μg/g for both analytes in all of the sample types. Chromatograms demonstrating the recovery of spinosyns A and D at the validated LOQ are illustrated in Figures 1−6. Chromatograms demonstrating the determination of spinosyns A and D at other levels of fortification (0.02 or 0.1 μg/g) are contained in Figures 4−6. Chromatograms demonstrating the confirmation of spinosyns A and D in cottonseed using the confirmatory chromatographic conditions are contained in Figure 7.

Pesticides commonly used on cotton and vegetables (Table 4) were tested for potential interference with spinosyns A and D. At concentrations of 0.4−10 μg/mL, 70 pesticides were tested for interference by direct injection into the liquid chromatography column. 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 spinosyns A or D. However, none of these five pesticides interfered when they were carried through the entire analytical procedure and then injected into the liquid chromatography column. Thus, the cleanup procedures described for this method effectively removed the potentially interfering pesticides as well as the interfering coextractives from the samples.

LITERATURE CITED

Keith, L. H.; Crummett, W. B.; Deegan, J.; Libby, R. A.; Taylor, J. Y.; Wentler, G. Principles of environmental analysis. *Anal. Chem.* **1983**, *55*, 2210−2218.

Magnussen, J. D.; Castetter, C. A.; Wooten, H., DowElanco, unpublished results.

Sparks, T. C.; Thompson, G. D.; Larson, L. L.; Kirst, H. A., Jantz, O. K.; Worden, T. V.; Hertlein, M. B.; Busacca, J. D. Biological characteristics of the spinosyns: new naturally derived insect control agents. *Proc. Beltwide Cotton Conf.* **1995**, 903−907.

Thompson, G. D.; Busacca, J. D.; Jantz, O. K.; Borth, P. W.; Nolting, S. P.; Winkle, J. R.; Gantz, R. L.; Huckaba, R. M.; Nead, B. A.; Peterson, L. G.; Porteous, D. J.; Richardson, J. M. Field performance in cotton of Spinosad: a new naturally derived insect control system. *Proc. Beltwide Cotton Conf.* **1995**, 907−910.

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