Determination of Spinosad and Its Metabolites in Food and Environmental Matrices. 2. Liquid Chomatography−Mass Spectrometry

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A selective and sensitive method utilizing liquid chromatography−mass spectrometry (LC-MS) has been developed for determining residues of the natural insect control agent spinosad in several crop matrices that are difficult to analyze by HPLC with UV detection. The method determines the active ingredients (spinosyns A and D) and three minor metabolites (spinosyns B and K and *N*-demethylspinosyn D) in alfalfa hay, wheat hay, wheat straw, sorghum fodder, and corn stover. The analytes are extracted from the samples with an acetonitrile/water solution, and the extracts are purified by solid phase extraction with a C_{18} disk and a silica cartridge. All five analytes are determined simultaneously in a single injection using positive atmospheric pressure chemical ionization LC-MS with selected ion monitoring. The average recoveries ranged from 69 to 96% with standard deviations ranging from 4 to 15%. The method has a validated limit of quantitation of 0.01 μg/g and a limit of detection of 0.003 μg/g. The LC-MS method can also provide residue confirmation in addition to quantitation.

Keywords: *Spinosad; spinosyn A; spinosyn D; spinosyn B; spinosyn K; N-demethylspinosyn D; residues; alfalfa; wheat; sorghum; corn; LC-MS*

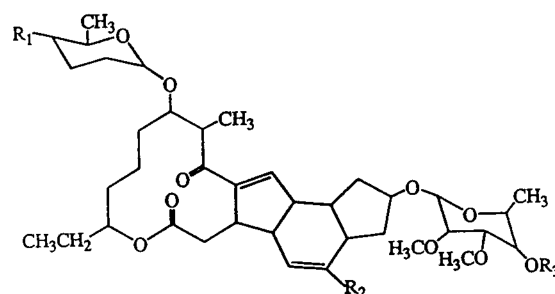
INTRODUCTION

Spinosad is a natural insect control agent that is registered in numerous countries on a variety of food and feed crops (West et al., 2000). Spinosad is comprised of spinosyns A and D (Figure 1), which are the active ingredients in Tracer Naturalyte, Success Naturalyte, SpinTor Naturalyte, and Conserve (all trademarks of Dow AgroSciences LLC) insect control products.

Previous studies using radiolabeled (^{14}C) material demonstrated that spinosyns A and D were metabolized in crops to spinosyns B and K and *N*-demethylspinosyn D (D. P. Rainey, J. D. Magnussen, and D. F. Berard, Dow AgroSciences LLC, personal communication, 1994). The structures of the three metabolites are shown in Figure 1. Thus, chromatographic methods were needed that could determine the total residue of spinosad and its metabolites.

Reliable and rapid methods using a magnetic particle-based immunoassay (IA) test kit have been published for determining spinosad residues in 34 food, feed, and environmental matrices (Young et al., 2000). These methods involved an extraction of the residues from the matrices with suitable solvents. For some matrices, the sample extracts could be diluted and measured directly by IA without any cleanup. For other matrices, sample extracts were purified using liquid−liquid partitioning and/or a solid phase extraction (SPE) cartridge prior to measurement by IA.

In addition, reliable HPLC-UV methodology has been published for the determination of spinosad and its metabolites in soil, sediment, water, animal tissues, and



spinosyn A, $R_1 = N(CH_3)_2$, $R_2 = H$, and $R_3 = CH_3$
spinosyn D, $R_1 = N(CH_3)_2$, $R_2 = CH_3$, and $R_3 = CH_3$
spinosyn K, $R_1 = N(CH_3)_2$, $R_2 = H$, and $R_3 = H$
spinosyn B, $R_1 = NH(CH_3)$, $R_2 = H$, and $R_3 = CH_3$
N-demethyl spinosyn D, $R_1 = NH(CH_3)$, $R_2 = CH_3$, and $R_3 = CH_3$

Figure 1. Structures of spinosad and metabolites.

>50 crops and crop processed commodities (West et al., 2000; West and Turner, 1998; West, 1996, 1997; Yeh et al., 1997). Sample extracts were typically purified by liquid−liquid partitioning or by SPE with C_{18} disks, followed by further purification with silica and/or cyclohexyl SPE cartridges.

Although HPLC-UV worked well for a very wide variety of sample matrices, extracts of five sample types could not be sufficiently purified for determining spinosad and its metabolites by HPLC-UV. The sample types that were difficult to analyze by HPLC-UV included alfalfa hay, wheat hay, wheat straw, sorghum fodder, and corn stover. These crop matrices contained

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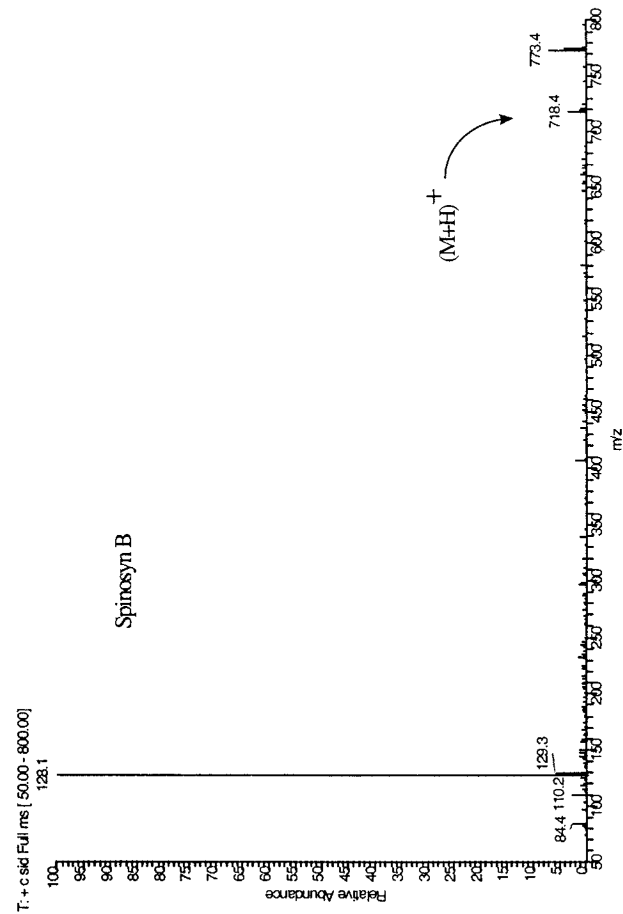
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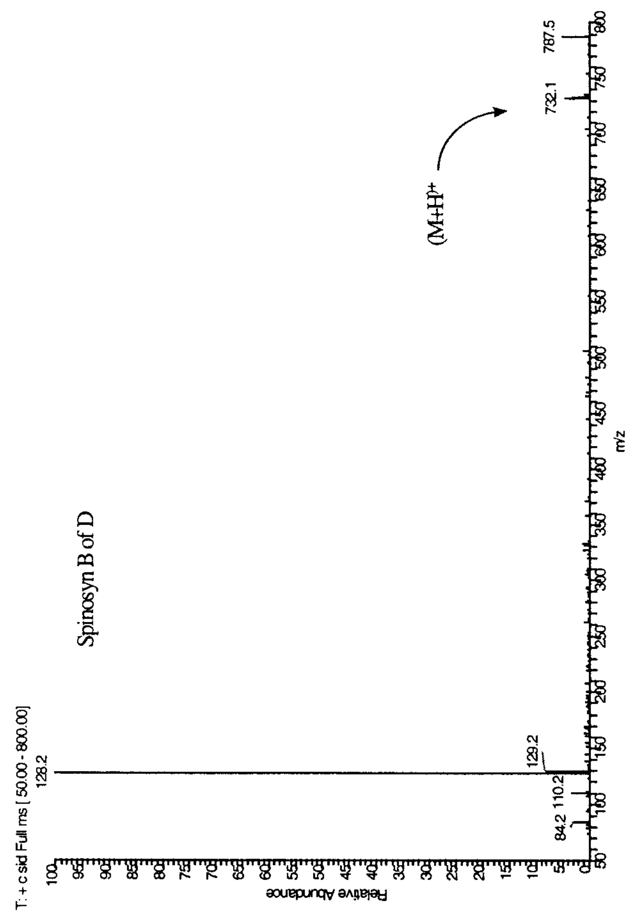
Spinosyn B
128.1
129.3
84.4 110.2
(M+H)+
718.4
773.4
Relative Abundance
m/z


T: + c sid Full ms [50.00 - 800.00]
Spinosyn B of D
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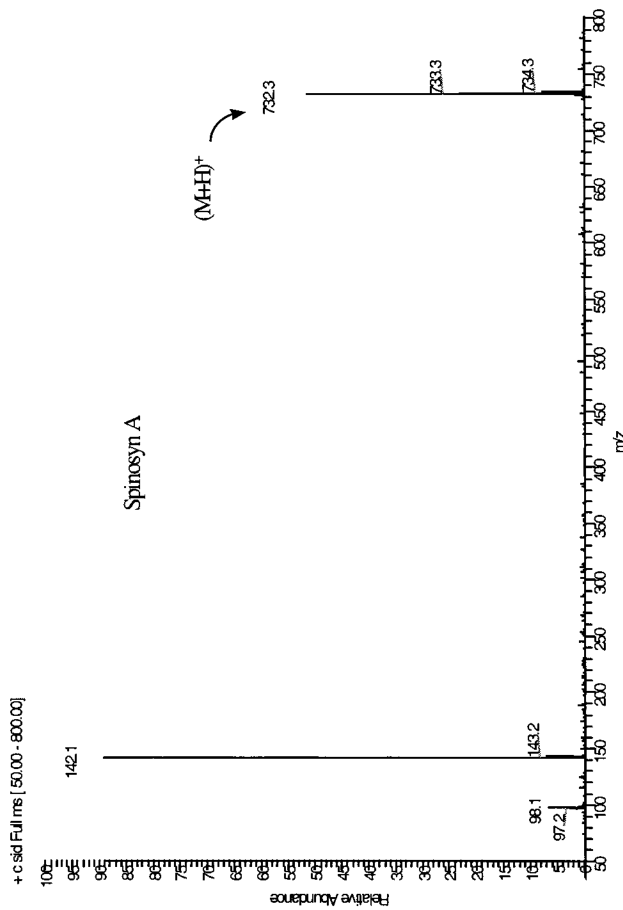
129.2
84.2 110.2
(M+H)+
732.1
787.5
Relative Abundance
m/z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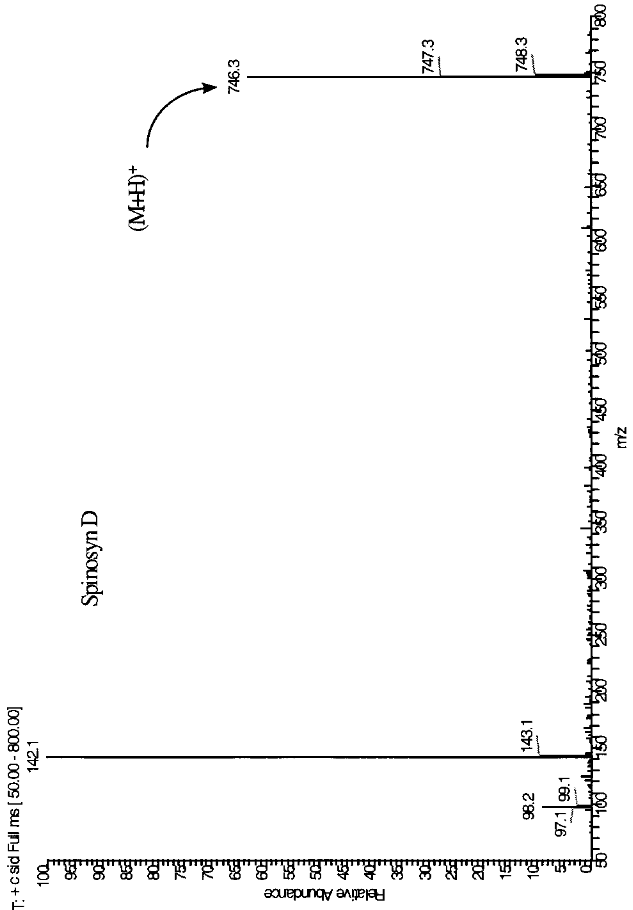
143.2
98.1
97.2
(M+H)+
732.3
733.3
734.3
Relative Abundance
m/z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Spinosyn D
142.1
143.1
98.2
99.1
97.1
(M+H)+
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747.3
748.3
Relative Abundance
m/z

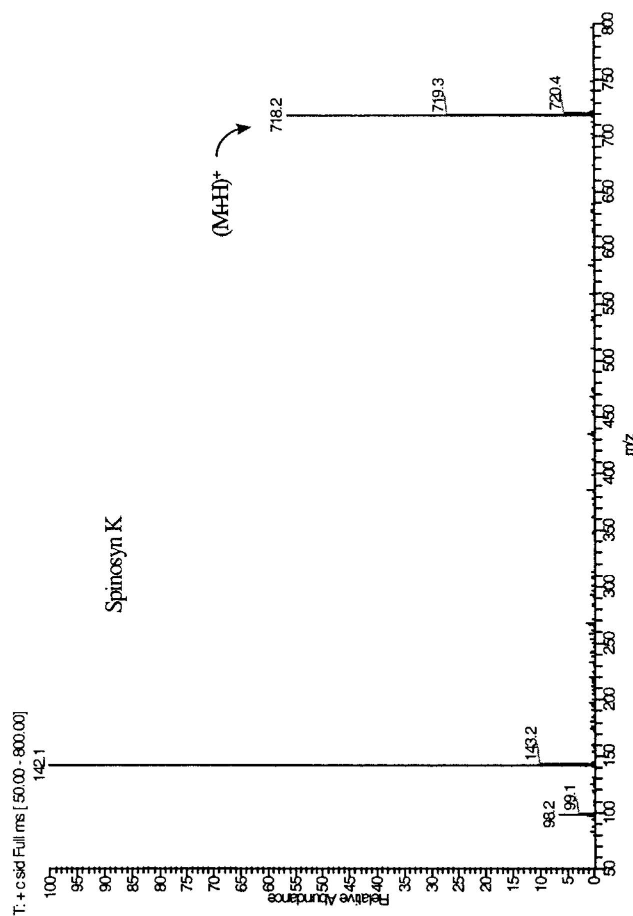

Figure 2. Mass spectra of spinosyns A, D, K, and B, and N-demethylspinosyn D using CID.

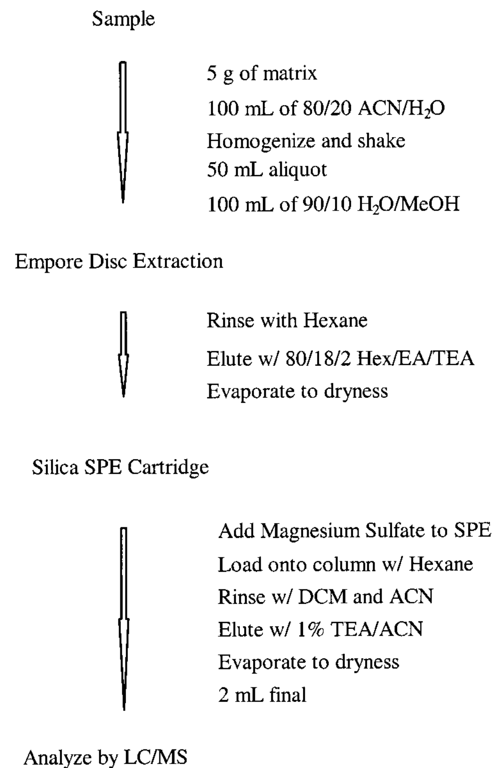

Figure 3. Flowchart for the determination of spinosad and metabolites in alfalfa hay, wheat hay, wheat straw, corn stover, and sorghum fodder by LC-MS (ACN, acetonitrile; MeOH, methanol; Hex, hexane; EA, ethyl acetate; TEA, triethylamine; SPE, solid phase extraction; DCM, dichloromethane).

coextracted organic matter that produced interfering peaks on the HPLC-UV chromatograms.

Liquid chromatography−mass spectrometry (LC-MS) can offer increased sensitivity and selectivity compared to HPLC-UV. The increased selectivity can be utilized to improve sample cleanup and/or to reduce the amount of sample cleanup that is required. The increased sensitivity afforded by LC-MS can result in a reduction of sample size requirements, which can also result in time and cost savings.

Thus, LC-MS was investigated as a potential method for determining these analytes in the matrices that were difficult to analyze by HPLC-UV while also reducing analysis time and costs. The following LC-MS methods are presented for the determination of all five analytes in alfalfa hay, wheat hay, wheat straw, sorghum fodder, and corn stover.

EXPERIMENTAL PROCEDURES

Apparatus. LC-MS with Atmospheric Pressure Chemical Ionization (APCI) Interface. The LC-MS system consisted of an ion trap mass spectrometer (model LCQ, Finnigan MAT, San Jose, CA) and a high-performance liquid chromatograph (model 1050, Hewlett-Packard, Wilmington, DE). The APCI vaporizer temperature was set at 450 °C with a sheath gas (nitrogen) at 90 psi and an auxiliary gas (nitrogen) at 4 psi. The capillary temperature was set at 200 °C. The LC-MS was operated in the positive ion mode with selected ion monitoring (SIM) of ions *m/z* 718.4 (spinosyns B and K), *m/z* 732.4 (spinosyn A and *N*-demethylspinosyn D), and *m/z* 746.4 (spinosyn D). For confirmation, collision-induced dissociation (CID) was applied at 30% relative collision energy to produce a second ion for each of the spinosyns. The confirmation ion was *m/z* 128.0 for spinosyn B and *N*-demethylspinosyn D and was *m/z* 142.0 for spinosyns A, D, and K (Figure 2).

Table 1. Summary of Method Validation Data for Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (B of D) in Alfalfa Hay, Wheat Hay, Wheat Straw, Corn Stover, and Sorghum Fodder

matrix	μg/g fortified	*n*	analyte	recovery, %[a] range	$\bar{x}$	*s*	RSD
alfalfa hay	0.01−5.0	14	spinosyn A	79−104	92	9	10
			spinosyn D	74−109	88	10	11
			spinosyn K	84−115	96	9	9
			spinosyn B	58−105	87	15	17
			B of D	57−103	88	15	17
wheat hay	0.01−5.0	7	spinosyn A	87−102	90	4	4
			spinosyn D	88−98	86	8	9
			spinosyn K	78−96	87	10	11
			spinosyn B	78−94	71	8	11
			B of D	75−94	78	15	19
wheat straw	0.01−5.0	7	spinosyn A	86−104	93	6	6
			spinosyn D	81−103	93	7	8
			spinosyn K	79−94	87	5	6
			spinosyn B	59−88	72	12	17
			B of D	54−91	69	11	16
corn stover	0.01−5.0	7	spinosyn A	81−100	93	6	6
			spinosyn D	83−98	93	4	4
			spinosyn K	73−97	88	8	9
			spinosyn B	60−96	77	12	16
			B of D	63−102	82	15	18
sorghum fodder	0.01−5.0	7	spinosyn A	82−93	88	4	5
			spinosyn D	81−104	91	7	7
			spinosyn K	74−90	82	6	7
			spinosyn B	66−77	72	4	5
			B of D	61−82	72	7	10

[a] No residues were detected in untreated control samples.

The analytes were separated isocratically on an ODS-AM HPLC column [3-μm particle size, 150 × 4.6 mm i.d. (YMC, Inc., Wilmington, NC)]. The mobile phase consisted of acetonitrile/methanol/2% aqueous ammonium acetate (42:42:16). The flow rate was 0.9 mL/min, and the UV wavelength was 250 nm. The injection volume was 100 μL, and the total elution time was 30 min per injection.

Reagents. The following HPLC grade solvents and chemicals were obtained from Fisher Scientific: acetone, acetonitrile, ammonium acetate, dichloromethane, ethyl acetate, hexane, magnesium sulfate (anhydrous), methanol, triethylamine (TEA), and water. A new bottle of TEA was opened every two to three months to prevent the formation of impurities that produced interference peaks on the chromatogram. A citric acid and ascorbic acid antioxidant mixture from Sigma Chemical Co. (St. Louis, MO) was used to prevent the degradation of spinosyns A and D to their metabolites during the sample analysis. The purified active ingredients used for analytical standards were obtained from the Test Substance Coordinator, Dow AgroSciences LLC, Indianapolis, IN, and their purities ranged from 94 to 99%.

Standard Preparation. Analytical standards or pure active ingredients of spinosyns A, D, K, and B and *N*-demethylspinosyn D (corrected for purity if <95% pure) were separately weighed into 35-mL vials. Each standard (25.0 mg) was dissolved in 10 mL of acetonitrile/methanol (1:1), and all five of the standard solutions were then combined into one 250-mL volumetric flask. All of the vials were rinsed with 10 mL of acetonitrile/methanol/2% ammonium acetate (1:1:1), which was added to the volumetric flask. The solution was then diluted to volume with acetonitrile/methanol/2% ammonium acetate (1:1:1) to obtain a stock solution containing 100 μg/mL of each compound. Starting with the 100 μg/mL stock solution, additional dilutions with acetonitrile/methanol/2% ammonium acetate (1:1:1) were performed to obtain spiking solutions ranging from 0.015 to 25.0 μg/mL and LC-MS calibration solutions ranging from 0.005 to 1.0 μg/mL.

Precautionary Protection from Light. During the sample extraction and purification steps, the extracts were protected from light to prevent photolysis of the analytes. Protective measures included working under reduced lighting conditions (e.g., turning off the lights in fume hoods during SPE cleanup steps) and placing the samples in the dark for any interruptions during sample processing. Long interruptions were generally avoided during sample analysis, except that the analysis could be delayed overnight prior to the silica SPE procedure by storing the sample extracts in a refrigerator at ∼4 °C.

Initial Sample Preparation. Samples were frozen with liquid nitrogen and then ground through a hammermill with a $^{3}/_{16}$-in. screen (model 2001, Agvise Laboratories, Inc., Northwood, ND). The samples were stored in a freezer at −20 °C until removed for analysis. The primary steps in the residue method for determining spinosad and its metabolites in the five sample matrices are summarized in the flowchart in Figure 3.

Sample Weighing and Fortification of Recovery Samples. Untreated control samples (5.0 ± 0.1 g) were weighed into a series of 8-oz (237-mL) glass bottles. Fortified recovery samples were prepared by adding 1.0 mL of the appropriate fortification solutions to result in samples fortified with all five analytes at concentrations of 0.003, 0.010, 1.0, and 5.0 μg/g. A reagent blank (containing no sample or analytes) and an untreated control sample (containing no analytes) were also analyzed with each sample set.

Sample Homogenization and Extraction. Samples were homogenized by adding 100 mL of acetonitrile/water (80:20) containing 0.25 g/L of a citric acid and ascorbic acid antioxidant (Sigma Chemical Co.) and blending for 1 min with a homogenizer (model 15105, Omni International, Waterbury, CT). The probe was cleaned between samples by rinsing with acetonitrile, which was discarded.

The bottles were capped with PTFE-lined closure caps and shaken on a reciprocating shaker (model 6010, Eberbach Corp., Ann Arbor, MI) at 180 excursions/min for 10 min. The samples were centrifuged for 10 min at ∼2200 rpm. Aliquots (50 mL) of the sample extracts were decanted into graduated cylinders and were stored in the dark until preparation of the SPE disks was completed as described below.

Purification by C_{18} SPE Disk. The 50-mL aliquots were purified by SPE using 47-mm, C_{18} disks (Empore, Fisher Scientific) and an SPE disk manifold (Kontes, Vineland, NJ). Approximately 20 mL (∼54 g) of Filter Aid 400 glass beads (Fisher Scientific) was added to each reservoir on the manifold apparatus. The glass beads and the SPE disks were conditioned by adding 15 mL of methanol. A vacuum of ∼10 in. (∼250 mm) was applied to draw a small amount of methanol through the disks, but the disks were not allowed to dry. The vacuum was turned off to soak the disks with the remaining methanol. After 2 min, the vacuum was turned on to draw the methanol through the disks until only a thin layer remained on top of the beads. Water (20 mL) was added to each reservoir, and the vacuum was applied until only a thin layer of liquid remained on top of the beads.

A 50-mL aliquot of water/methanol (90:10) was added to the reservoir, and the 50-mL extract from Sample Homogenization and Extraction was transferred to the reservoir. The graduated cylinder was rinsed with 50 mL of water/methanol solution (90:10), which was also added to the reservoir. Full vacuum (∼25 in. or 635 mm of Hg) was applied to pull the sample through the SPE disk at a flow rate of 35−70 mL/min. After the solution had eluted completely, the disk was dried for 10 min under full vacuum. The Empore disk was rinsed with 15 mL of hexane under full vacuum, and the disk was dried under full vacuum for at least 10 min after the hexane had eluted.

The vacuum was turned off, and a 40-mL amber glass vial (Fisher Scientific) was placed inside the vacuum manifold. A solution of hexane/ethyl acetate/TEA (80:18:2) was prepared fresh daily to ensure proper elution of the analytes from the disk. The fume hood lights were turned off to prevent photodegradation of the analytes in the presence of TEA, and 25 mL of hexane/ethyl acetate/TEA (80:18:2) was added to the reservoir. A small amount of the solution was pulled through the disk using a vacuum of ∼10 in. (∼250 mm) of Hg, and the

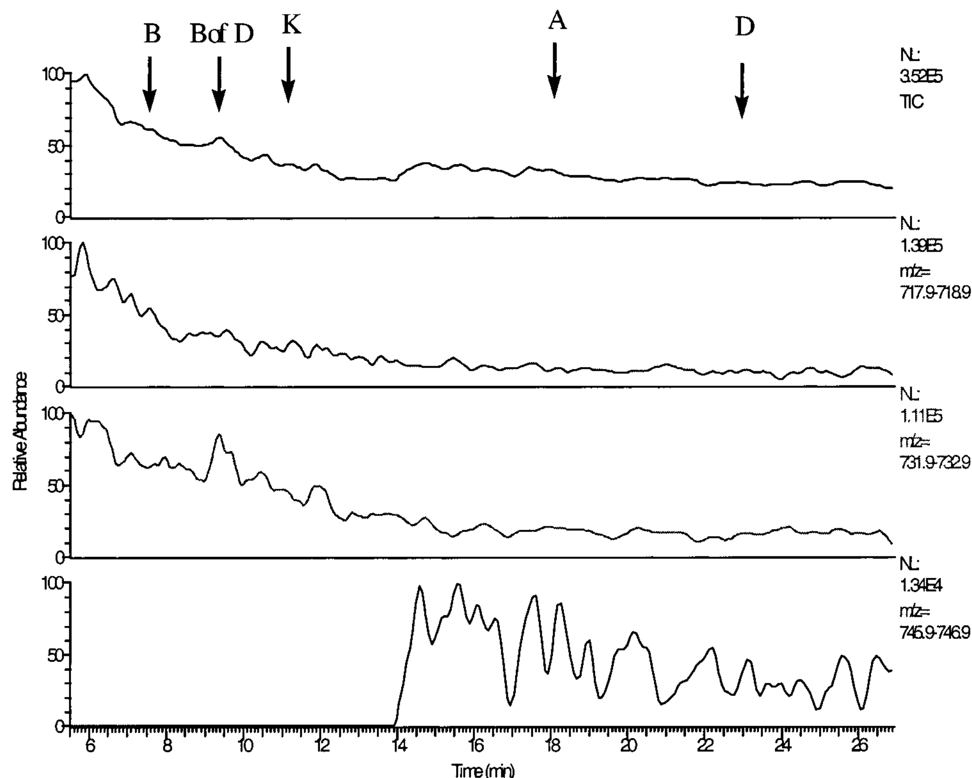

Figure 4. Typical total ion chromatogram (TIC) and ion chromatograms (*m/z* 718, 732, and 746) for a control alfalfa hay sample containing no detectable residue of spinosad.

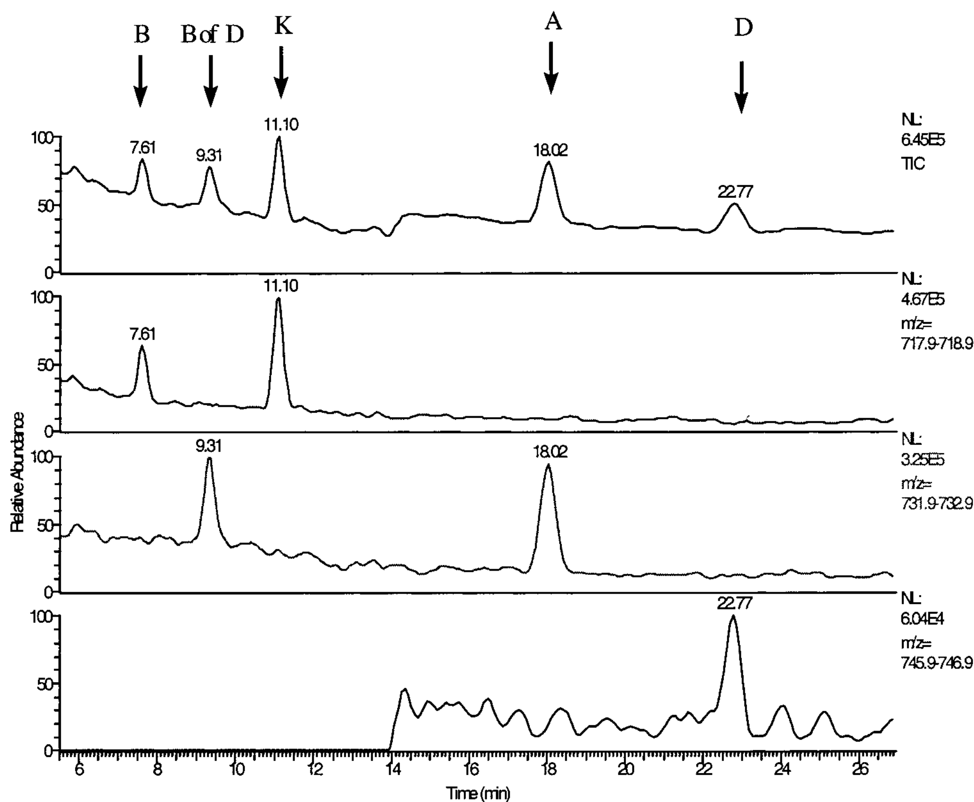

Figure 5. Typical TIC and ion chromatograms (*m/z* 718, 732, and 746) for a control alfalfa hay sample fortified with 0.010 μg/g of each analyte, equivalent to recoveries of 95% for spinosyn A, 95% for spinosyn D, 93% for spinosyn B, 97% for spinosyn K, and 90% for *N*-demethylspinosyn D.

eluate was collected in the 40-mL vial. The vacuum was turned off to allow the remaining solvent to soak on the disk for 1 min, followed by the application of full vacuum to pull the remaining solvent into the 40-mL vial.

To minimize the potential for photodegradation of the analytes, the sample was immediately transferred to a 100-mL evaporating flask. The sample vial was rinsed three times with 5 mL of methanol/dichloromethane (25:75), which was added to the flask. Prior to evaporating the sample, the rotary vacuum evaporator was rinsed with methanol to prevent cross-

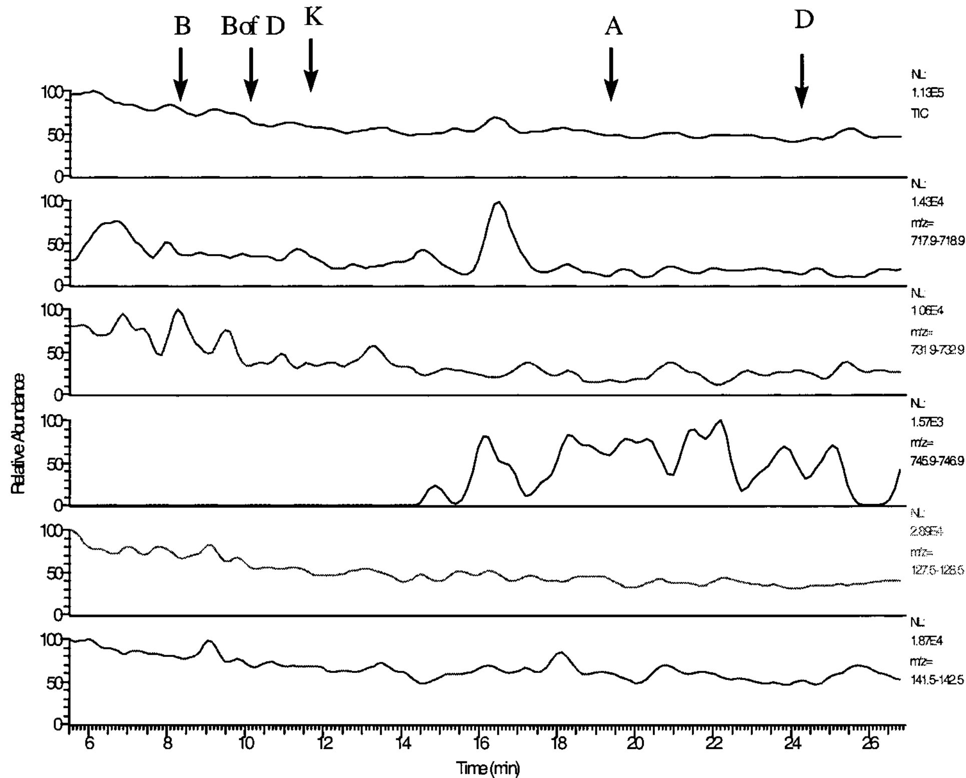

Figure 6. Typical TIC and ion chromatograms (*m/z* 718, 732, 746, 128, and 142) for the confirmation of a control corn stover sample containing no detectable residue of spinosad.

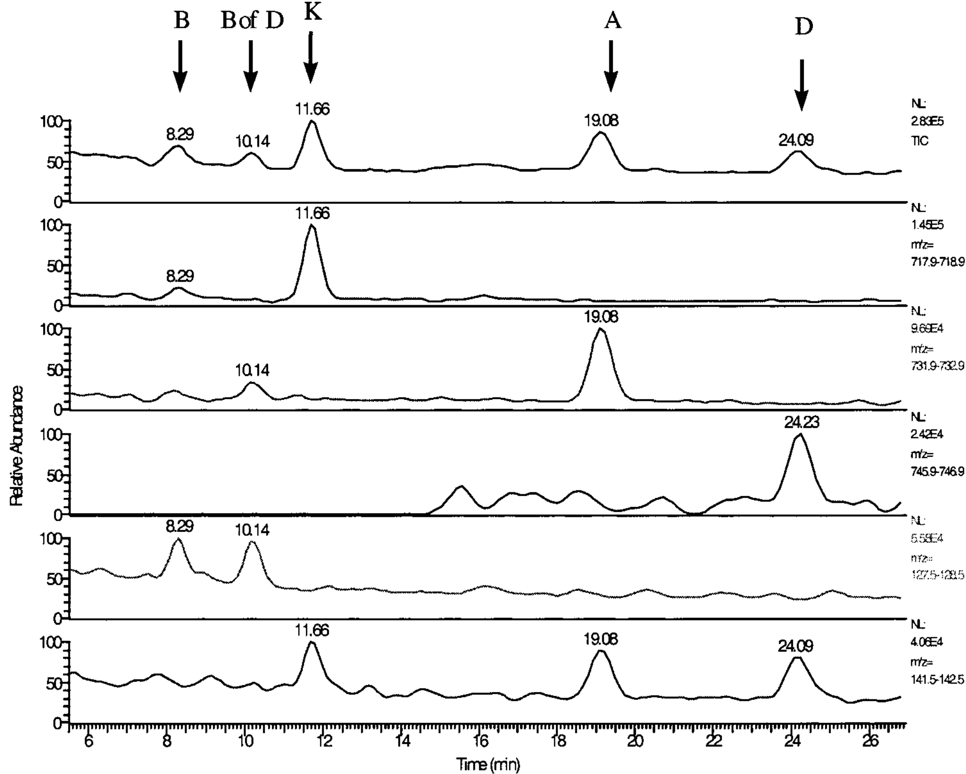

Figure 7. Typical TIC and ion chromatograms (*m/z* 718, 732, 746, 128, and 142) for the confirmation of a control corn stover sample fortified with 0.010 μg/g of each analyte.

contamination. The solvent was evaporated with the aid of a water bath heated to ~30 °C, after which 10 mL of methanol was added to the flask and evaporated to remove traces of water. The residue was dissolved in 20 mL of hexane with the aid of an ultrasonic bath (model FS1414H, Fisher Scientific) for 10−20 s, and the solution was mixed for 5 s with a vortex mixer (model G-560, Scientific Industries, Inc., Bohemia, NY).

Silica SPE. An SPE cartridge reservoir (70-mL with 20-μm polyethylene frits, Jones Chromatography, Lakewood, CO) was attached to a 25-mm i.d., 1-μm glass fiber filter (Fisher Scientific), followed by a silica SPE cartridge (Sep-Pak Plus, Waters, Milford, MA) and a stopcock. The SPE assembly was attached to an SPE vacuum manifold (Supelco, Inc., Bellefonte, PA). Anhydrous magnesium sulfate (~1 g) was added to the

Table 2. Calculated Limits of Detection and Quantitation for Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (B of D)

			μg/g			
matrix	*n*	analyte	$(\bar{x})^a$	$(s)^b$	LOD[c] (3*s*)	LOQ[d] (10*s*)
alfalfa hay	8	spinosyn A	0.0098	0.0007	0.002	0.007
		spinosyn D	0.0094	0.0008	0.003	0.008
		spinosyn K	0.0100	0.0009	0.003	0.009
		spinosyn B	0.0099	0.0005	0.002	0.005
		B of D	0.0099	0.0004	0.001	0.004
others[e]	12	spinosyn A	0.0092	0.0005	0.002	0.005
		spinosyn D	0.0094	0.0009	0.003	0.009
		spinosyn K	0.0087	0.0008	0.002	0.008
		spinosyn B	0.0081	0.0007	0.002	0.007
		B of D	0.0087	0.0011	0.003	0.011

[a] Mean values of the μg/g results for samples fortified at the validated method LOQ (0.010 μg/g). [b] Standard deviation of the μg/g results for samples fortified at the validated method LOQ (0.010 μg/g). [c] Limit of detection, calculated as 3*s*. [d] Limit of quantitation, calculated as 10*s*. [e] Due to a limited supply of control samples, results for wheat hay, wheat straw, corn stover, and sorghum fodder were combined by commodity grouping to provide a sufficient number of data points for statistical analysis.

reservoir. The cartridge was conditioned by rinsing with 20 mL of hexane, and the column was not allowed to go dry.

The sample solution that was purified by the C_{18} SPE disk was added to the silica SPE cartridge. The vacuum was increased to ~4 in. (~10 cm) of Hg to pull the solvent through the cartridge at a flow rate of ~5 mL/min. After the hexane had eluted, the sample vial was rinsed with two separate 20-mL aliquots of hexane, and both rinsates were added separately to the cartridge. After the hexane had eluted, the vacuum was increased to ~20 in. (~50 cm) of Hg for ~10 s to briefly dry the cartridge. The vacuum was then decreased to ~2 in. (~5 cm) of Hg. The sample vial was rinsed with 15 mL of dichloromethane, and the rinsate was added to the cartridge. The vacuum was increased to ~5 in. (~13 cm) of Hg to pull the solvent through the cartridge at a flow rate of ~5 mL/min. After the dichloromethane had eluted, the vacuum was increased to 20 in. of Hg for ~10 s to briefly dry the cartridge. The vacuum was decreased to ~2 in. of Hg. The sample vial was rinsed with 15 mL of acetonitrile, which was added to the cartridge. The vacuum was increased to ~2.5 in. (~6 cm) of Hg to pull the solvent through the cartridge at a flow rate of ~5 mL/min. After the acetonitrile had completely eluted from the cartridge, the vacuum was increased to ~20 in. of Hg for ~10 s to briefly dry the cartridge.

The vacuum was turned off, and a 40-mL amber glass vial was placed in the vacuum manifold. A solution of 1% TEA/99% acetonitrile was prepared fresh daily to ensure proper elution of the analytes from the SPE cartridge. The fume hood lights were turned off to prevent photodegradation of the analytes in the presence of TEA, and the analytes were eluted with 15 mL of 1% TEA/acetonitrile to the SPE. The eluate was collected in the 40-mL amber vial by increasing the vacuum to ~2.5 in. of Hg to obtain a flow rate of ~3 mL/min.

To minimize the potential for photodegradation of the analytes, the sample was immediately evaporated to dryness using a TurboVap evaporator (model LV, Zymark Corp., Hopkinton, MA) at 60 °C with a nitrogen pressure of ~8 psi. The evaporation step required ~15 min. The residue was dissolved in 2.0 mL of acetonitrile/methanol/2% ammonium acetate (1:1:1) with the aid of an ultrasonic bath for 10−20 s. The solution was mixed with a vortex mixer for ~5 s to dissolve any residue remaining on the glass vial. If the fortification levels were ≥1.0 μg/g, further dilution with acetonitrile/methanol/2% ammonium acetate (1:1:1) was made to result in a concentration within the linear range of the calibration curve. The solution was transferred to an HPLC sample vial.

LC-MS. Solutions were analyzed by LC-MS using the conditions described previously under Apparatus. After the instrument had been calibrated with the manufacturer's recommended solution, two spinosyn factors (spinosyn D and *N*-demethylspinosyn D) were used to tune the instrument, and the results were saved as two separate tune files. The experimental method was then established with two analysis segments using the tune files that were generated. The first segment (~14 min) utilized the tune file for *N*-demethylspinosyn D and included the analysis of spinosyns B, K, and *N*-demethylspinosyn D. The second segment (~13 min) utilized the tune file for spinosyn D and included the analysis of spinosyns A and D.

The suitability of the chromatographic system was determined using the following performance criteria: (a) It was determined that the correlation coefficient (r^2) exceeded 0.995 for the least-squares equation that described the detector response as a function of the concentration of the calibration standards for linear regression calculations. (b) It was visually determined that a signal-to-noise ratio of approximately 5:1 was achievable for the 0.015 μg/mL calibration standard.

Calculation of Results. Using simple linear regression, separate standard calibration curves were prepared for each of the five analytes. The concentration (*C*) of the analytes in the final solution was calculated from the measured peak area responses (PR) and the least-squares coefficients for the slope (*m*) and *y*-axis intercept (*b*) as follows:

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

The residue concentration (μg/g) of the analytes in the fortified recovery sample was calculated from the concentration in the final solution (*C*), the final volume (*V*), the weight of the sample that was extracted (*W*), and the aliquot factor (AF) using the following equation:

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

The aliquot factor was calculated from the appropriate extraction and aliquot volumes:

$$\mathrm{AF} = (\text{total extraction volume/aliquot volume}) \qquad (3)$$

The percent recovery (*R*) was determined by dividing the net concentration (μg/g) found in fortified recovery samples by the theoretical concentration added:

$$R = [(\mu\text{g/g found})/(\text{added } \mu\text{g/g})] \times 100\% \qquad (4)$$

Confirmation of Results. Confirmation of the identity of spinosyns A, D, K, and B and *N*-demethylspinosyn D was achieved by reanalyzing representative final solutions using LC-MS with CID as described under Apparatus. To confirm the residues, the resulting retention times of the analytes in the sample were matched with those of the standards in the total ion chromatogram. In addition to retention time matches, the confirmation ions (*m*/*z* 128 and 142) were also required to be present along with the parent ions (*m*/*z* 718, 732, and 746).

If desired, additional confirmation may be obtained by injecting the same final solutions onto a different HPLC column, a C_{18}/Cation Mixed Mode, 5 μm, 150 mm × 4.6 mm i.d. (Alltech Associates, Inc., Deerfield, IL) (West and Turner, 2000).

RESULTS AND DISCUSSION

Advantages of LC-MS. The increased sensitivity and selectivity afforded by LC-MS permitted the determination of spinosad and its metabolites in five sample matrices that could not be purified sufficiently for analysis by HPLC-UV. By using LC-MS, it was also possible to reduce sample size and cleanup requirements, thereby reducing sample analysis time and costs. A further advantage of LC-MS was the ability to provide both quantitation and confirmation of residues.

Method Validation. A method validation study was conducted to determine the recovery levels and the precision of the residue method. The results are presented in Table 1. For spinosyn A, average recoveries ranged from 88 to 93% with the standard deviation ranging from 4 to 9%. For spinosyn D, average recoveries ranged from 86 to 93% with the standard deviation ranging from 4 to 10%. For spinosyn K, average recoveries ranged from 82 to 96% with the standard deviation ranging from 5 to 10%. For spinosyn B, average recoveries ranged from 70 to 87% with the standard deviation ranging from 4 to 15%. For *N*-demethylspinosyn D, average recoveries ranged from 69 to 88% with the standard deviation ranging from 7 to 15%.

Chromatograms. Typical chromatograms for the determination of all five analytes obtained under the primary and confirmatory LC-MS conditions for alfalfa hay and corn stover are included in Figures 4–7. Chromatograms for the other sample matrices were similar.

Linearity. The correlation coefficient (r^2) for the least-squares equations describing the detector response as a function of concentration was >0.995 for each validation set for all five analytes. Linearity at concentrations exceeding the range of the calibration curve (0.0–1.0 μg/mL) was not investigated.

Limits of Detection and Quantitation. Following a published technique (Keith et al., 1983), the limits of detection (LOD) and quantitation (LOQ) for the residue method were calculated from the standard deviation (s). The calculated LOD and LOQ were determined using the standard deviation from samples fortified at 0.010 μg/g.

The calculated values for the LOD (3s) and LOQ (10s) are presented in Table 2. For all five analytes in all five matrices, the calculated LOD ranged from 0.001 to 0.003 μg/g. These calculated values support a method LOD of 0.003 μg/g. To confirm the ability to detect the analytes at an LOD of 0.003 μg/g, one control sample from each crop type was fortified at 0.003 μg/g. Peaks that were distinguishable from background were detected in all of the 0.003 μg/g recovery samples.

Likewise, for all five analytes in all five matrices, the calculated LOQ ranged from 0.004 to 0.011 μg/g (Table 2). These calculated values support a method LOQ of 0.01 μg/g. The determination and confirmation of the analytes at the method LOQ are illustrated in Figures 5 and 7, respectively.

Conclusions. Reliable residue methodology exists for spinosad in food, feed, and environmental matrices using immunoassay, HPLC-UV, and/or LC-MS. An LC-MS method has been developed and validated for the determination and/or confirmation of spinosad and its three major metabolites in five crop matrices that could not be purified sufficiently for analysis by HPLC-UV. The greater sensitivity and selectivity of LC-MS also reduced cost and analysis time by reducing the requirements for sample size and purification.

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