Application of Empore Disk Extraction for Trace Analysis of Spinosad and Metabolites in Leafy Vegetables, Peppers, and Tomatoes by High-Performance Liquid Chromatography with Ultraviolet Detection

Li-Tain Yeh,* Debbie A. Schwedler, Gary E. Schelle, and Jesse L. Balcer

Global Environmental Chemistry Laboratory—Indianapolis Lab, DowElanco, 9330 Zionsville Road, Indianapolis, Indiana 46268

Residue methods were developed for the quantitation of spinosad and metabolites in leafy vegetables, peppers, and tomatoes. The compounds were extracted with a solution of acetonitrile/water. The extract was purified and concentrated by C_{18} Empore disk extraction, followed by silica and cyclohexyl solid-phase extraction. All five analytes in the purified extract were determined simultaneously by reversed-phase high-performance liquid chromatography with ultraviolet detection. For all analytes in six different commodities, the average recoveries ranged from 77 to 97% with standard deviations ranging from 2 to 7%. The limits of quantitation and detection were 0.01 and 0.003 μg/g, respectively. These results compare favorably with those obtained by replacing the Empore disk extraction with liquid–liquid partitioning in the method. Analysis of cabbages sprayed with [^{14}C]spinosyn A from a residue study indicated that the Empore disk extraction provided a cleaner final extract. Confirmation of analytes was performed by using liquid chromatography/mass spectrometry.

Keywords: *Empore disk extraction; spinosad; silica SPE; cyclohexyl SPE; vegetation; LC/UV; LC/MS*

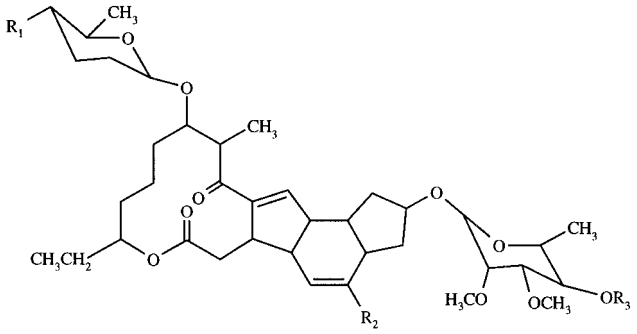
INTRODUCTION

Spinosad is a new product of DowElanco in the natural product class that can be applied for the management of many economically important insect pests in cotton, brassica, leafy vegetables, tomatoes, and other crops. It is produced naturally from living organisms in the fermentation process. For enforcement of proposed tolerances in various crops, new analytical methods have been developed for the quantitative determination of residues of spinosad and its metabolites in peppers, tomatoes, and leafy vegetables by high-performance liquid chromatography with ultraviolet detection (HPLC/UV).

Liquid–liquid partitioning using dichloromethane as an extraction solvent was initially used for the concentration of analytes in brassica, peppers, tomatoes, and apples. To avoid using large quantities of dichloromethane and to increase sample analysis efficiency, C_{18} Empore disk extraction was adapted to replace the liquid–liquid extraction. The use of disk extraction in water (Ho et al., 1995; Albanis and Hela, 1995; Barcelo et al., 1993) and body fluid (Ensing et al., 1992; Lensmeyer et al., 1991) matrices as an extraction and trace enrichment technique has been documented in several publications. The use of the disk extraction for residue analysis in vegetable and fruit matrices has so far been limited (Pavoni, 1992). This work demonstrates that C_{18} disk extraction can be applied to crop methods for trace level analysis.

MATERIALS AND METHODS

Apparatus. *The high-performance liquid chromatography system* consisted of a Varian 9012 solvent delivery system, a Varian 9050 UV detector, a Varian 9100 autosampler, and a Varian Star LC data station (Varian Associates Inc, Walnut Creek, CA). The analytes were separated on a 3 μm, 4.6 × 150 mm YMC ODS-AM column (YMC, Inc., Wilmington, NC) using isocratic elution. The mobile phase consisted of 42% acetonitrile, 42% methanol, and 16% of 2% ammonium acetate at a flow rate of 0.9 mL/min.



Spinosad and Metabolites

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

Figure 1. Molecular structures of spinosad and metabolites.

The liquid chromatography/mass spectrometry (LC/MS) system consisted of a Hewlett-Packard 1050 liquid chromatograph (Hewlett-Packard, Wilmington, DE) and a Finnigan TSQ-700 mass spectrometry (Finnigan MAT, San Jose, CA). The LC/MS used a electrospray interface with selected ion monitoring of ions 718 (spinosyns B and K), 732 (spinosyn A and *N*-demethyl spinosyn D), and 746 (spinosyn D). The analytes were separated on a 3 μm, 4.6 × 150 mm YMC ODS-AM column using isocratic elution. The mobile phase consisted of 40% acetonitrile, 40% methanol, and 20% of 2% ammonium acetate at a flow rate of 0.6 mL/min.

Empore Disk. C_{18} phase, 47 mm, part number 14-378E, Fisher Scientific, Pittsburgh, PA. Filter Aid 400, J. T. Baker, Phillipsburg, NJ.

S0021-8561(96)00579-1 CCC: $14.00 © 1997 American Chemical Society

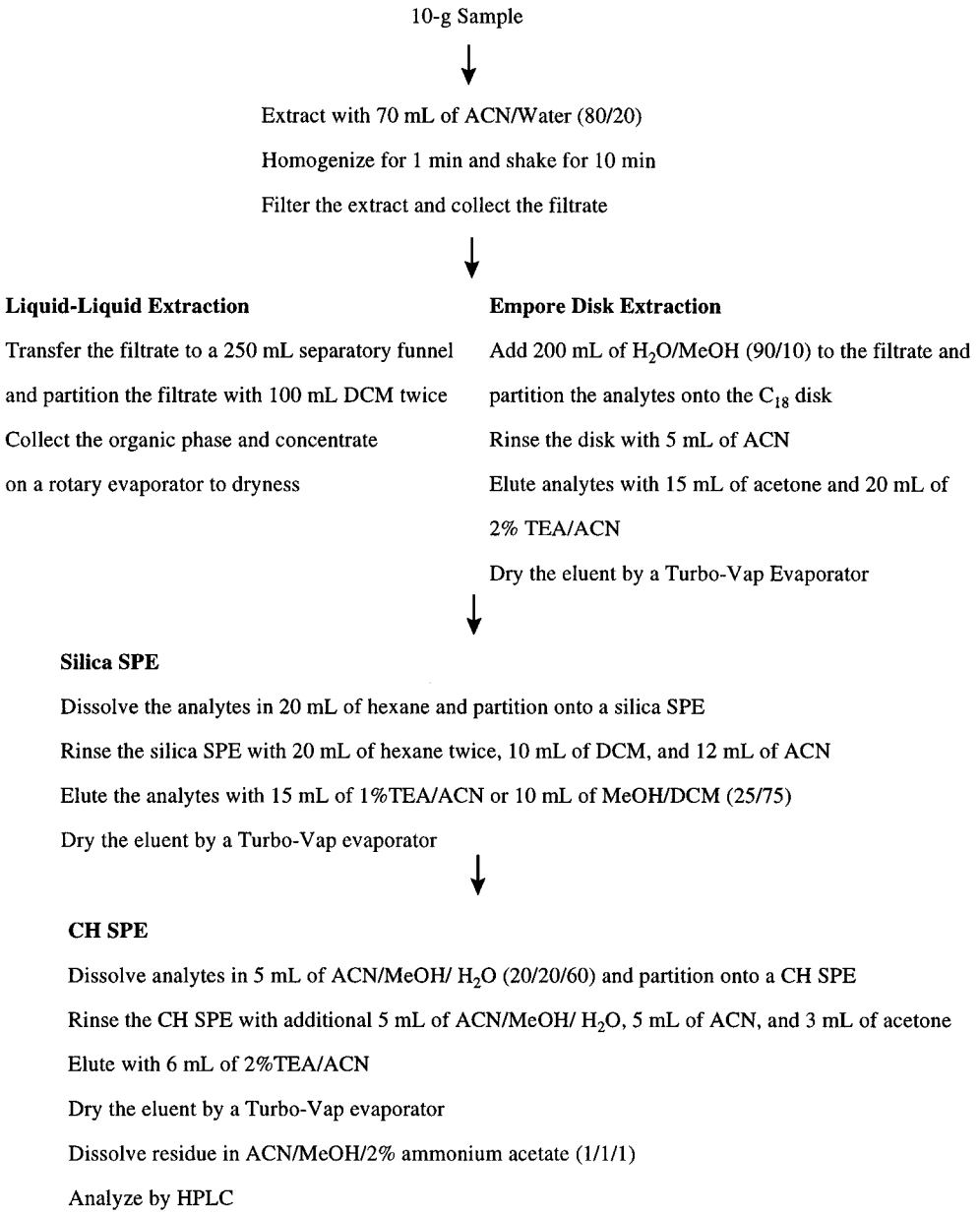


Figure 2. Flow chart of methods for the determination of spinosad and metabolites in cabbage, leafy vegetables, peppers, and tomatoes.

Solid-Phase Extraction (SPE) Cartridges. Silica Sep-Pak Plus, 690 mg, part number WAT20520, Waters, Milford, MA. Cyclohexyl (CH), Bond Elute LRC, 500 mg, part number 1211-3032, Varian Sample Preparation Products.

Evaporator. Zymark TurboVap LV, Zymark Corporation, Hopkinton, MA.

Homogenizer. Model 17105 equipped with 20 mm diameter × 145 mm generator probe, Omni International, Waterbury, CT.

Reagents. HPLC-grade solvents and chemicals (acetone, acetonitrile, ammonium acetate, dichloromethane, hexane, methanol, triethylamine, and water) were obtained from Fisher Scientific (Pittsburgh, PA) and were used without further purification.

Safety Precautions. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in the method before commencing laboratory work. Volatile and flammable organic solvents such as acetone, acetonitrile, dichloromethane, hexane, methanol, and triethylamine must be used in well-ventilated areas away from ignition sources.

Preparation of Standard Solutions. Analytical standard or pure active ingredients of spinosyns A, D, K, B and *N*-demethylspinosyn D were weighed into separate 40-mL vials. Each compound was dissolved in 10 mL of acetonitrile/methanol (50/50). The resulting solutions were transferred into a 200-mL volumetric flask. The 40-mL vials were then rinsed with 10 mL of acetonitrile/methanol/2% ammonium acetate (1/1/1), and the rinsates were transferred to the 200-mL volumetric flask and filled to the mark with acetonitrile/methanol/2% ammonium acetate (1/1/1) to attain a stock solution containing 50 μg/mL of each compound. The stock solution was serially diluted with acetonitrile/methanol/2% ammonium acetate (1/1/1) to attain solutions containing 10.0, 5.0, 1.0, 0.50, 0.10, 0.050, and 0.030 μg/mL of each compound. Solutions with concentrations ranging from 0.030 to 1.0 μg/mL were used as calibration standards, and solutions with concentrations ranging from 0.10 to 10.0 μg/mL were used as spiking solutions. The solution at 0.030 μg/mL was used to confirm the claimed limit of detection (LOD). The structures of these compounds are presented in Figure 1.

Sample Preparations. A method flow chart is presented in Figure 2. The samples were processed by freezing with liquid nitrogen and grinding through an AGVISE Model 2001 Hammermill with a 3/16-in. screen. The processed samples were weighed (10 g) into a 240-mL bottle. For each recovery sample, 1.0 mL of the appropriate fortification solution was added into the samples. A reagent blank and a control were

Table 1. Recovery of Spinosyns A, D, K, B, and *N*-Demethylspinosyn D (B of D) in Celery, Head Lettuce, Leaf Lettuce, and Spinach

			average recovery[a] (%)				
			spinosyn				
matrix	fortification (μg/g)	*n*	A	D	K	B	B of D
celery	0.01	8	93	93	88	85	84
	0.05	3	93	93	88	86	85
	0.5	3	94	94	85	86	86
	2.0	3	93	93	78	85	85
	5.0	3	91	92	75	87	88
	overall recovery	20	93	93	84	86	85
	overall SD[b]		2	2	6	2	2
head lettuce	0.01	8	89	90	88	78	76
	0.05	3	87	86	83	73	72
	0.5	3	86	86	84	76	75
	2.0	3	89	89	85	80	79
	5.0	3	86	85	82	84	84
	overall recovery	20	88	88	85	78	77
	overall SD[b]		3	3	4	4	4
leaf lettuce	0.01	8	92	90	87	81	77
	0.05	3	94	94	89	79	78
	0.5	3	93	93	89	83	84
	2.0	3	91	91	86	85	85
	5.0	3	92	91	88	87	87
	overall recovery	20	93	91	88	83	81
	overall SD[b]		3	2	2	4	5
spinach	0.01	8	89	89	87	80	79
	0.05	3	96	94	92	90	89
	0.5	3	94	94	88	87	87
	2.0	3	90	91	85	86	85
	5.0	3	90	90	82	86	87
	overall recovery	20	91	91	87	84	84
	overall SD[b]		4	3	4	4	5

[a] Data obtained by the Empore disk extraction method. [b] Standard deviation.

Table 2. Recovery of Spinosyns A, D, K, B, and *N*-Demethylspinosyn D (B of D) in Peppers and Tomatoes

			average recovery (%)				
			spinosyn				
matrix	fortification (μg/g)	*n*	A	D	K	B	B of D
peppers[a]	0.01	6	96	94	92	85	85
	0.05	4	96	95	84	77	77
	0.1	7	100	99	86	87	86
	overall recovery	13	97	96	88	85	84
	overall SD[b]		3	3	7	5	5
tomatoes[a]	0.01	8	87	83	82	83	81
	0.1	5	90	89	83	83	81
	overall recovery	13	88	85	82	83	81
	overall SD[b]		3	4	3	4	6
peppers[c]	0.01	8	96	92	95	83	82
	0.05	3	97	95	95	89	88
	0.5	3	94	93	94	89	87
	2.0	3	93	92	91	81	79
	5.0	3	95	94	94	85	84
	overall recovery	20	95	93	94	85	84
	overall SD[b]		2	2	2	4	3
tomatoes[c]	0.01	8	99	94	91	86	86
	0.05	3	95	93	93	81	80
	0.5	3	94	93	93	84	83
	2.0	3	93	93	90	86	85
	5.0	3	91	91	89	84	84
	overall recovery	20	96	93	91	85	84
	overall SD[b]		4	3	3	3	3

[a] Data obtained by the Empore disk extraction method. [b] Standard deviation. [c] Data obtained by the liquid−liquid partition method.

carried through the method with each sample set. The reagent blank contained 1 mL of acetonitrile/methanol/2% ammonium acetate (1/1/1), and the control contained 10 g of matrix and 1 mL of acetonitrile/methanol/2% ammonium acetate (1/1/1). A solution of acetonitrile/water (80/20, 70 mL) was added to each sample, and each resulting mixture was homogenized for 1 min. The generator probe was rinsed with acetonitrile (~10 mL), and the rinsate was added to the sample bottle. Each bottle was sealed with a TFE-lined closure and shaken for 10 min. The extract was then filtered through a Whatman No. 1 filter paper using a Büchner funnel into a 500-mL side arm flask under vacuum. Acetonitrile (~15 mL) was used to rinse the bottle and the plant tissue during the filtration process and collected in the side arm flask. The filtrate was stored in the dark until the preparation of the C_{18} extraction disk was completed.

C_{18} Disk Extraction. For each sample, the extract was purified and concentrated with a C_{18} disk by using the following procedure: A C_{18} disk was placed on the support base of the vacuum manifold, followed by a 500-mL funnel reservoir. The reservoir was tightly clamped to the support base, and approximately 50 g of Filter Aid 400 glass beads were added to the reservoir. The glass beads and the C_{18} disk were conditioned with 15 mL of methanol and then 20 mL of water. A solution of water/methanol solution (90/10, 200 mL) was added to each filtrate in the 500-mL side arm flask. The resulting solution was immediately transferred to the reservoir and eluted under full vacuum (~64 mmHg, ~35−70 mL/min). The disk was dried for 5 min after the solution had eluted. The side arm flask was rinsed with 5 mL of acetonitrile, and the rinsate was added to the reservoir and eluted under full vacuum. The disk was dried for at least 2 min after the acetonitrile had eluted. The vacuum was stopped, and a 40-mL amber vial was placed inside the vacuum manifold. The side arm flask was rinsed with 15 mL of acetone, and the rinsate was added to the reservoir. A small vacuum (~25 mmHg) was applied to draw a small amount of acetone through the disk, and the eluent was collected in the 40-mL vial. The vacuum was then stopped to allow the remaining solvent to wet the disk for 1 min before the vacuum (~65 mmHg) was applied again to pull the remaining solvent through disk into the vial. Once acetone was completely eluted, a solution of 2% triethylamine/acetonitrile was used to rinse the side arm flask, and the rinsate was added to the reservoir. A small vacuum (~25 mmHg) was applied to draw a small amount of solvent through the disk, and the eluent was collected in the 40-mL vial. The vacuum was then stopped to allow the remaining solvent to wet the disk for 1 min before the vacuum (~65 mmHg) was applied to pull the remaining solvent through disk into the vial. The eluent was immediately evaporated to dryness using a TurboVap evaporator (60 °C, ~8 psi N_2). The residue was dissolved in 20 mL of hexane, and the solution was sonicated for 10−20 s.

Liquid−Liquid Partitioning. For comparison, liquid−liquid partitioning was used to replace the C_{18} disk extraction procedure. The sample filtrate was concentrated as follows: The filtrate from the sample preparation procedure was transferred to a 250-mL separatory funnel. The side arm flask was rinsed with 50 mL of dichloromethane twice, and the rinsates were transferred to the separatory funnel. The analytes were partitioned into organic solvents by vigorously shaking the solution for 20−30 s. Sufficient time was allowed for the layers to separate, and the organic (lower) layer was drained into a 500-mL evaporating flask. The hood light was turned off during the liquid−liquid partitioning step to avoid photolysis of the analytes. Methanol (4 mL) was added to the aqueous phase, and sodium hydroxide (1 N) was also added to the aqueous phase in 0.1-mL increments until the pH was greater than 9; an additional 0.1 mL of 1 N sodium hydroxide was then added to the aqueous phase. The aqueous phase was partitioned with an additional 100 mL of dichloromethane, and the organic (lower) layer was collected in the same 500-mL evaporating flask. Any emulsion present in the liquid interface was not collected in the process. The extract was evaporated to dryness using a rotary vacuum evaporator with the water bath heated between 45 and 55 °C. After the solvent

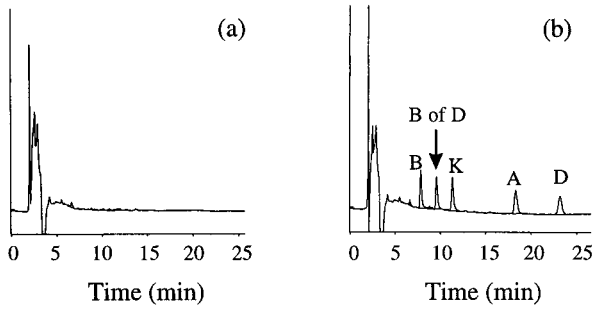


Figure 3. Typical chromatograms of the final extract obtained from (a) control celery (no detectable interference peaks) and (b) celery fortified at 0.01 μg/g using Empore disk extraction. HPLC conditions: YMC ODS-AM column (15 cm × 4.6 mm i.d. 3 μm); isocratic elution, acetonitrile/methanol/2% ammonium acetate (42/42/16) at 0.9 mL/min. Injection volume: 250 μL. Approximate retention time: spinosyn B (B, 7.8 min), *N*-demethylspinosyn B (B of D, 9.6 min), spinosyn K (K, 10.7 min), spinosyn A (A, 17.1 min), and spinosyn D (D, 21.6 min).

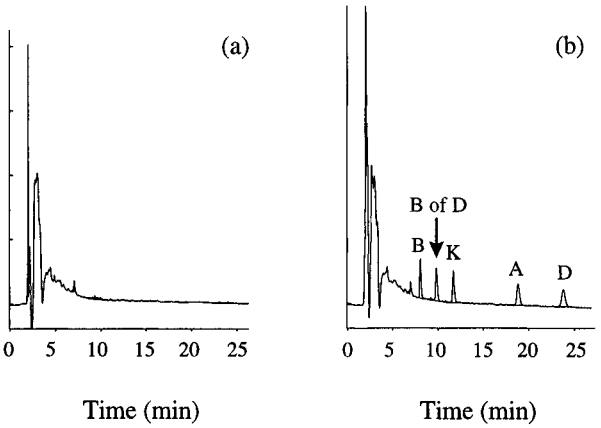


Figure 4. Typical chromatograms of the final extract obtained from (a) control spinach (no detectable interference peaks) and (b) spinach fortified at 0.01 μg/g using Empore disk extraction. HPLC conditions are the same as in Figure 3.

was removed, approximately 20 mL of methanol was added to the flask and the solution was evaporated to dryness again. The residue was dissolved in 20 mL of hexane, and the solution was sonicated for 10−20 s.

Silica SPE. The sample extract in hexane was purified on a silica SPE cartridge utilizing the following procedures: A 30-mL SPE cartridge reservoir was attached to a 1.0-μm glass fiber filter and then a silica SPE cartridge, followed by a stopcock, to an extraction vacuum manifold. The cartridge was conditioned with 20 mL of hexane under vacuum. The sample solution in hexane was added to the silica SPE cartridge and eluted at approximately 6 mL/min. After the hexane was eluted, the 40-mL sample vial (or 500-mL evaporating flask if liquid−liquid partition was used) was rinsed with two separate 20-mL aliquots of hexane, and each rinsate was added to the cartridge separately. After the hexane was eluted, the sample vial was rinsed with 10 mL of dichloromethane. The rinsate was added to the cartridge and eluted at approximately 5 mL/min. The column was dried under vacuum for at least 2 min after the dichloromethane had eluted. The sample vial was rinsed with 12 mL of acetonitrile, and the rinsate was added to the cartridge. Acetonitrile was allowed to pass through the column at approximately 6 mL/min. After acetonitrile had passed through the column completely, a 40-mL vial was placed in the vacuum manifold. The sample vial was rinsed with 15 mL of 1% triethylamine/acetonitrile or 10 mL of methanol/dichloromethane (25/75), and the rinsate was added to the cartridge. The solution was eluted at approximately 3 mL/min and collected in the 40-mL vial. The eluent was immediately evaporated to dryness using a TurboVap evaporator (60 °C, 8 psi N_2). The residue was dissolved in 5 mL of

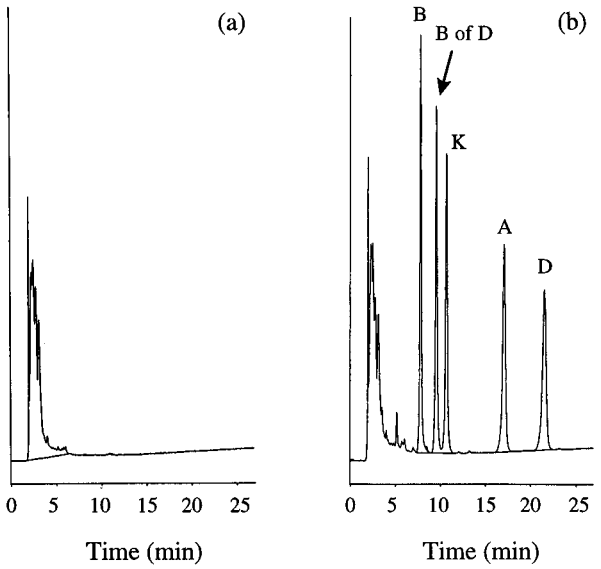


Figure 5. Typical chromatograms of the final extract obtained from (a) control tomatoes (no detectable interference peaks) and (b) tomatoes fortified at 0.1 μg/g using Empore disk extraction. HPLC conditions are the same as in Figure 3.

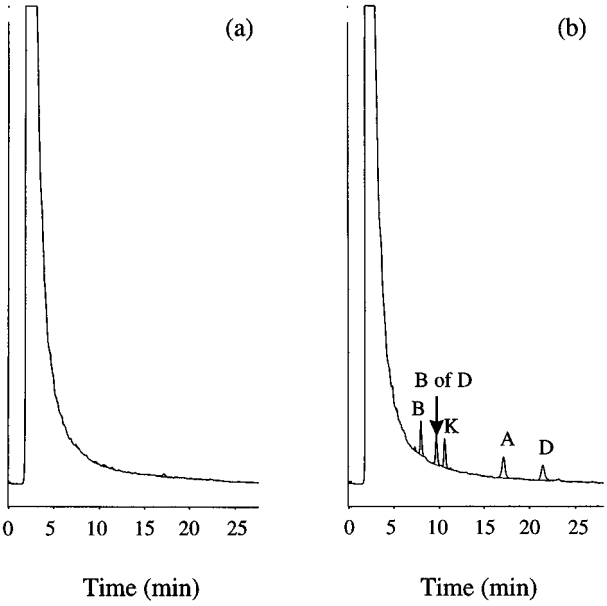


Figure 6. Typical chromatograms of the final extract obtained from (a) control tomatoes (no detectable interference peaks) and (b) tomatoes fortified at 0.01 μg/g using liquid−liquid extraction. HPLC conditions are the same as in Figure 3.

acetonitrile/methanol/water (20/20/60), and the resulting solution was sonicated and vortexed to completely dissolve the residue.

Cyclohexyl (CH) SPE. The extract obtained from silica SPE cleanup was purified on a CH SPE cartridge utilizing the following procedures: A CH SPE cartridge was attached to a stopcock and then to a SPE vacuum manifold. The CH cartridge was conditioned under vacuum using 9 mL of methanol and then 18 mL of water. The solvent flow was stopped by controlling the stopcock to maintain approximately 1 mm of solvent on top of the CH packing. The sample in the acetonitrile/methanol/water solution was added to the CH cartridge and eluted at approximately 2.5 mL/min. Acetonitrile/methanol/water (20/20/60, 5 mL) was used to rinse the sample vial, and the rinsate was added to the column. After the solution eluted, the column was dried under vacuum for approximately 2 min. The evaporating vial was rinsed with 5 mL of acetonitrile, and the rinsate was added to the cartridge. The acetonitrile was eluted at approximately 3 mL/

Table 3. Comparision of Liquid−Liquid Partition and Empore Disk Extraction Using Incurred Cabbages Sprayed with Radiolabeled Spionosyn A

	radioactivity detection			
	Empore disk extraction[a]		liquid−liquid partition	
phase	ppm	recovery (%)	ppm	recovery (%)
cabbage extract	4.88	--	4.16	--
after liq−liq or Empore extraction	1.22	25	3.20	77

	UV detection (ppm)					
	spinosyn[b]			spinosyn		
	A	B	K	A	B	K
final extract	0.56	0.05	0.27	0.52	0.07	0.30

[a] The sample was stored frozen for over 1.5 y after the analysis with the liquid−liquid partition method. The higher radioactivity extracted was due to the loss of moisture during storage. [b] These results have been corrected by a factor of 0.85 (4.16/4.88) to compensate for the moisture loss in storage.

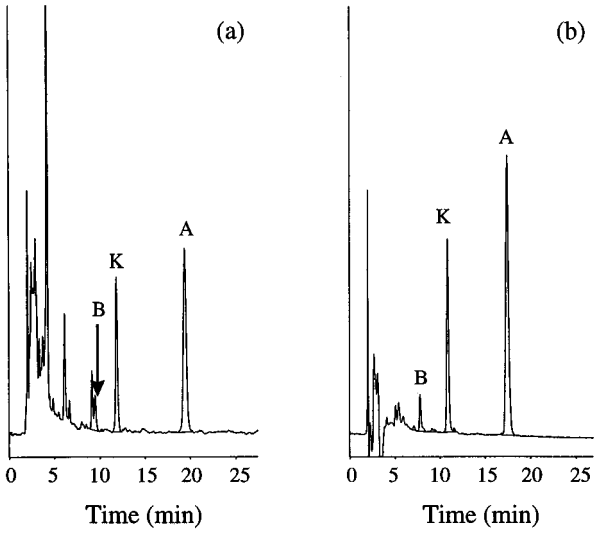


Figure 7. Chromatograms of cabbages sprayed with [^{14}C]-spinosyn A carried through residue methods using (a) liquid−liquid extraction and (b) Empore disk extraction. HPLC conditions are the same as in Figure 3.

min, and the cartridge was dried under vacuum for approximately 5 min. The vial was rinsed with 3 mL of acetone, and the rinsate was added to the cartridge under vacuum (~5 mmHg). A full vacuum was applied when the acetone level was below the frit. After the acetone had completely eluted, the vacuum was turned off and a 40-mL vial was placed in the vacuum manifold. The sample vial was rinsed with 6 mL of 2% triethylamine/acetonitrile solution, and the rinsate was added to the cartridge. The eluent was collected in the 40-mL amber vial, and the solution was immediately evaporated to dryness using a TurboVap evaporator (60 °C and 8 psi N_2). The residue was dissolved in 2.0 mL of acetonitrile/methanol/2% ammonium acetate (1/1/1). For fortification levels at 0.5 μg/g or higher, further dilution was performed to bring the final concentration within the calibration range. The solution was transferred to an HPLC sample vial and analyzed with standard solutions by HPLC.

RESULTS AND DISCUSSION

Recovery Levels and Precision. A method validation study was conducted to determine the percent recovery and the precision of the residue method. The results are given in Tables 1 and 2. For the six commodities that used the C_{18} extraction disk as part of the method, the average recoveries for spinosyn A ranged from 88 to 97% with standard deviations ranging from 2 to 4%. For spinosyn D, the average recoveries ranged from 85 to 96% with the standard deviations ranging from 2 to 4%. For spinosyn K, the average recoveries ranged from 82 to 88% with the standard deviations ranging from 2 to 7%. For spinosyn B, the average recoveries ranged from 78 to 86% with the standard deviations ranging from 2 to 5%. For *N*-demethylspinosyn D, the average recoveries ranged from 77 to 85% with the standard deviations ranging from 2 to 6%. Typical chromatograms of samples fortified at the validated limit of quantitation (LOQ) are illustrated in Figures 3−5.

Table 4. Calculated Limits of Detection and Quantitation for Spinosyns A, D, K, B and *N*-Demethylspinosyn D (B of D) in Celery, Spinach, Leaf Lettuce, Head Lettuce, Peppers, and Tomatoes

		(μg/g)			
matrix	analyte	$(\bar{x})^a$	$(s)^b$	LOD[c] (3s)	LOQ[d] (10s)
celery	spinosyn A	0.0093	0.0002	0.0006	0.002
	spinosyn D	0.0093	0.0003	0.0009	0.003
	spinosyn K	0.0088	0.0003	0.0009	0.003
	spinosyn B	0.0085	0.0003	0.0009	0.003
	B of D	0.0084	0.0002	0.0006	0.002
spinach	spinosyn A	0.0089	0.0002	0.0006	0.002
	spinosyn D	0.0089	0.0001	0.0003	0.001
	spinosyn K	0.0087	0.0002	0.0006	0.002
	spinosyn B	0.0080	0.0002	0.0006	0.002
	B of D	0.0079	0.0002	0.0006	0.002
leaf lettuce	spinosyn A	0.0092	0.0004	0.0012	0.004
	spinosyn D	0.0089	0.0003	0.0009	0.003
	spinosyn K	0.0087	0.0003	0.0009	0.003
	spinosyn B	0.0083	0.0003	0.0009	0.003
	B of D	0.0078	0.0004	0.0012	0.004
head lettuce	spinosyn A	0.0089	0.0004	0.0012	0.004
	spinosyn D	0.0090	0.0004	0.0012	0.004
	spinosyn K	0.0088	0.0003	0.0009	0.003
	spinosyn B	0.0078	0.0003	0.0009	0.003
	B of D	0.0076	0.0003	0.0009	0.003
peppers	spinosyn A	0.0092	0.0001	0.0003	0.001
	spinosyn D	0.0089	0.0002	0.0006	0.002
	spinosyn K	0.0087	0.0001	0.0003	0.001
	spinosyn B	0.0083	0.0002	0.0006	0.002
	B of D	0.0078	0.0002	0.0006	0.002
tomatoes	spinosyn A	0.0087	0.0003	0.0009	0.003
	spinosyn D	0.0083	0.0004	0.0012	0.004
	spinosyn K	0.0082	0.0003	0.0009	0.003
	spinosyn B	0.0083	0.0003	0.0009	0.003
	B of D	0.0081	0.0004	0.0012	0.004

[a] Mean values for μg/g recovery results at 0.01 μg/g (LOQ). [b] Standard deviation of the μg/g recovery results for the validated LOQ. [c] Limit of detection, calculated as 3s. [d] Limit of quantation, calculated as 10s.

C_{18} Disk Extraction versus Liquid−Liquid Partitioning. A comparison study was conducted to determine the differences in recovery and precision, if any, between the C_{18} disk extraction and liquid−liquid partitioning. The results are summarized in Table 2. Except for a slightly lower average recovery of spinosyn K in tomatoes, the average recoveries and standard deviations are similar from these two different processes. The chromatograms of the final extracts from tomatoes are presented in Figures 5 and 6. These chromatograms indicate that the C_{18} disk extraction reduced the amount of polar material in the final extract as demonstrated by a reduced solvent front in the chromatogram.

Extraction Efficiency. Cabbage plants sprayed with [^{14}C]spinosyn A generated as a portion of a nature

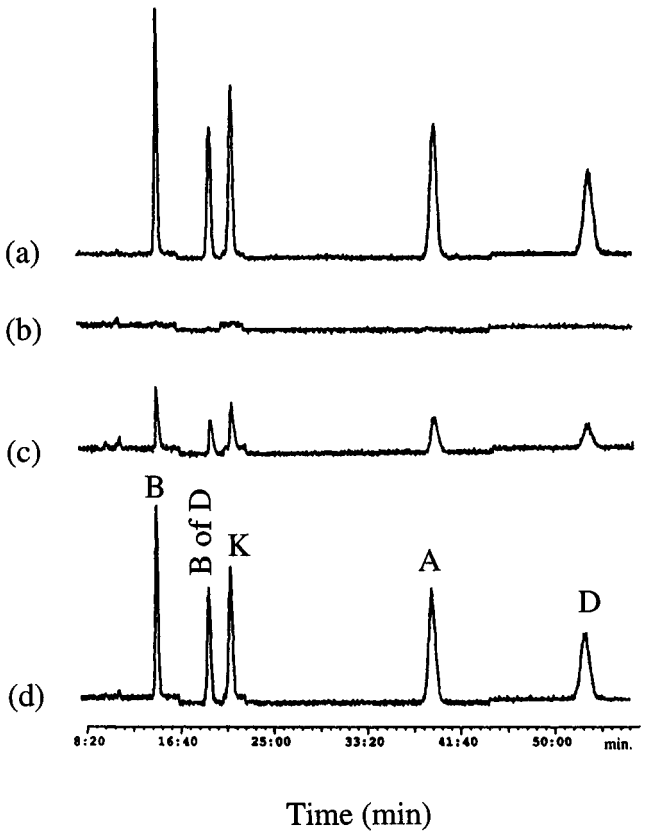


Figure 8. Total ion chromatograms for LC/MS confirmation analysis (a) a 0.05 μg/mL standard; (b) a control spinach sample; (c) a 0.003 μg/g fortified spinach sample; and (d) a 0.01 μg/g fortified spinach sample. HPLC conditions: YMC ODS-AM column (15 cm × 4.6 mm i.d. 3 μm); isocratic elution, acetonitrile/methanol/2% ammonium acetate (40/40/20) at 0.6 mL/min. Injection volume: 100 μL. Approximate retention time: spinosyn B (B, 10.5 min), *N*-demethylspinosyn B (B of D, 13.7 min), spinosyn K (K, 16.7 min), spinosyn A (A, 28.0 min), and spinosyn D (D, 33.5 min).

of residue study were also examined by both methods. In this study, the radioactivity in the extract was monitored in the process. The results are presented in Table 3. The final HPLC/UV analysis shows a similar amount of spinosyns A, K, and B residue in the ^{14}C-treated cabbage. The radioactivity shows that most of the polar degradation products were not retained in the Empore disk extraction and hence provide cleaner final extract for analysis. The chromatograms of the final extracts from cabbages are presented in Figure 7. These chromatograms again indicate that the C_{18} disk extraction provided a cleaner extract for analysis.

Calculated Limits of Detection and Quantitation. The calculated LOD and LOQ were determined using the standard deviation from the validated LOQ recovery results. Following a published technique (Keith et al., 1983), the LOD was calculated as 3× the standard deviation, and the LOQ was calculated as 10× the standard deviation. For all five analytes, the calculated LOD ranged from 0.0003 to 0.0015 μg/g for all matrices (Table 4). These calculated results support a LOD of 0.003 μg/g.

For all five analytes, the calculated LOQ ranged from 0.001 to 0.005 μg/g for all matrices (Table 4). These calculated results support the validated LOQ of 0.010 μg/g. However, results should not be quantified at levels below the validated LOQ unless recovery samples have been analyzed to support a lower LOQ.

Standard Curve Linearity. The average correlation coefficient (r^2) of the least squares equations describing the detector response as a function of concentration was greater than 0.9999 for each validation set for spinosyns A, D, K, B, and *N*-demethylspinosyn D.

Confirmation of Residue Identity. Confirmation of the identity of spinosyns A, D, K, B, and *N*-demethylspinosyn D can be achieved by re-analyzing representative final solutions using LC/MS. To confirm the residues, the resulting retention time of the analytes in the sample are compared with those of the standards in selected ion monitoring at *m/z* 718, 732, and 746. Typical chromatograms demonstrating the confirmation of spinosad and metabolites at the LOD and LOQ are illustrated in Figure 8.

Conclusion. The results presented here demonstrate that the C_{18} disk extraction can be used in the residue analysis of spinosad and its metabolites in crop matrices. The recoveries and standard deviations obtained by using the C_{18} disk extraction are similar to those obtained by using liquid−liquid partitioning. The use of C_{18} disk extraction to replace liquid−liquid extraction produces a streamlined procedure that allows for faster through put of samples and elimination of chlorinated solvent. Typically, the final extract was cleaner when C_{18} disk extraction was used in the method.

LITERATURE CITED

Albanis, T. A.; Hela, D. G. Multi-Residue Pesticide Analysis in Environmental Water Samples Using Solid-Phase Extraction Discs and Gas Chromatography with Flame Thermionic and Mass-Selective Detection. *J. Chromatogr. A* **1995**, *707* (2), 283−292.

Barcelo, D.; Durand, G.; Bouvot, V.; Nielen, M. Use of Extraction Disks for Trace Enrichment of Various Pesticides from River Water and Simulated Seawater Samples Followed by Liquid Chromatography-Rapid-Scanning UV-Visible and Thermospray-Mass Spectrometry Detection. *Environ. Sci. Technol.* **1993**, *27*, 271−277.

Ensing, K.; Franke, J. P.; Temmink, A.; Chen, X.-.H; De Zeeuw, R. A. Application of Empore C8 Disks for Screening Urine in Systematic Toxicological Analysis. *J. Forensic Sci.* **1992**, *37* (2), 460−466.

Ho, J. S.; Tang, P. H.; Eichelberger, J. W.; Budde, W. L. Liquid-Solid Disk Extraction by SFE and GC-Ion-Trap MS for the Determination of Trace Organic Pollutants in Water. *J. Chromatogr. Sci.* **1995**, *33*, 1−8.

Lensmeyer, G. L.; Wibbe, D. A.; Darcy, B. A. Application of a Novel Form of Solid-Phase Sorbent Empore Membrane to the Isolation of Tricyclic Antidepressant Drugs from Blood. *J. Chromatogr. Sci.* **1991**, *29* (10), 444−449.

Keith, L. H.; Crummett, W.; Deegan, J.; Libby, R. A.; Taylor, J. K.; Wentler, G. Principle of Environmental Analysis. *Anal. Chem.* **1983**, *55*, 2210−2218.

Pavoni, G. Determination of Fungicides in Fruits, Vegetables, and Water using Empore Disk SPE Membrane. *Boll. Chim. Ig., Parte Sci.* **1992**, *43* (S1), 45−51.

Received for review August 5, 1996. Revised manuscript received February 18, 1997. Accepted February 20, 1997.[⊗]

JF960579D

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1997.