Determination of Spinosad and Its Metabolites in Food and Environmental Matrices. 1. High-Performance Liquid Chromatography with Ultraviolet Detection

Sheldon D. West,* Li-Tain Yeh, Larry G. Turner, Debbie A. Schwedler, Angelea D. Thomas, and Dennis O. Duebelbeis

Global Environmental Chemistry Laboratory—Indianapolis Laboratory, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054

Spinosad is an insect control agent that is derived from a naturally occurring soil bacterium and is effective on several classes of insects, especially Lepidoptera larvae. Spinosad is registered in many countries for use on a variety of crops, including cotton, corn, soybeans, fruits, and vegetables. Residue methods utilizing high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection have been described for determining spinosad and its metabolites in environmental and food matrices. These residue methods typically involve an extraction with organic solvents, followed by purification using liquid−liquid partitioning and/or solid phase extraction prior to measurement by HPLC-UV. The residue methods determine the active ingredients (spinosyns A and D) and up to three minor metabolites (spinosyn B, spinosyn K, and *N*-demethylspinosyn D). The methods have validated limits of quantitation ranging from 0.010 to 0.040 μg/g. This paper briefly reviews the residue methodology for spinosad and metabolites in food and environmental matrices and provides a summary of method validation results for 61 different sample types, including newly published results for 37 additional crop matrices and processed commodities.

Keywords: *Spinosad; spinosyn A; spinosyn D; spinosyn B; spinosyn K; N-demethylspinosyn D; food; crops; soil; water; beef; poultry; milk; eggs; HPLC-UV*

INTRODUCTION

Concern for human health and environmental safety has led to a search for safer pesticides, including those produced naturally (Nakanishi, 1978). Spinosad is a new insect control agent that is derived through the fermentation of a naturally occurring Actinomycetes bacterium, *Saccharopolyspora spinosa*. The organism was isolated from a soil sample taken at the site of a rum still in the Caribbean Islands. Spinosad is comprised of a mixture of spinosyns A and D and is the common name of the active ingredient that is present in Tracer Naturalyte, Success Naturalyte, SpinTor Naturalyte, and Conserve (all trademarks of Dow AgroSciences LLC) insect control products. These products are useful for the management of many insect pests, including caterpillars, thrips, flies, drywood termites, and some beetles.

Spinosad is currently registered in many countries for use on a variety of crops, including cotton, corn, soybeans, fruits, and vegetables (Table 1). Additional registrations and label expansions for other crops are pending in several countries. Efficacy, toxicity, and chemical and physical properties of the active ingredients have been presented (Sparks et al., 1995; Thompson et al., 1995). 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. It also has a favorable environmental profile in that it does not leach, bioaccumulate, volatilize, or persist in the environment.

* Author to whom correspondence should be addressed [fax (317) 337-3255; e-mail sdwest@dowagro.com].

One of the U.S. government's top environmental awards, the 1999 Green Chemistry Challenge Award, was presented to Dow AgroSciences LLC for the development of spinosad (Anastas et al., 1999). The award was presented by the U.S. Environmental Protection Agency on behalf of the White House to recognize technologies that incorporate the principals of green chemistry into chemical design, manufacture, and use. Green chemistry encompasses chemical processes that reduce negative impacts on human health and the environment.

Reliable analytical methods are an important aspect of monitoring pesticide residue levels to ensure human and environmental safety. Residue methods utilizing high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection have been previously reported for spinosad in cottonseed and cottonseed processed commodities (West, 1996), in soil, sediment, and water (West, 1997), in leafy vegetables, peppers, and tomatoes (Yeh et al., 1997), in meat, milk, cream, and eggs (West and Turner, 1998), and in citrus crops and citrus processed commodities (West and Turner, 2000). Complete details on the methods, including the specifics of how they differ, may be found in the referenced papers. In general, however, the methods involve extraction of the analytes using suitable organic solvents or aqueous−organic mixtures. Initial purification of the extracts is accomplished using aqueous−organic partitioning or 47-mm C_{18} solid phase extraction (SPE) disks (Empore, Fisher Scientific, Pittsburgh, PA). Further purification is achieved using cyclohexyl and/or silica SPE cartridges. The analytes are then separated and deter-

10.1021/jf000080q CCC: $19.00 © 2000 American Chemical Society
Published on Web 10/21/2000

Table 1. Spinosad Registrations

country	crops
Argentina	cotton, soybean, tomato, cucumber
Australia	cotton, peppers, tomato, brassica, broccoli, Brussels sprouts, cabbage
Bolivia	cotton, soybean, corn, tomato
Brazil	cotton, tomato, corn, soybean, potato
Chile	alfalfa, nectarine, peach, tomato
China	cotton, cabbage
Colombia	cotton, beans, potato, flowers
Cyprus	brassica, citrus, grape, pepper, potato, spinach, strawberry, tomato, lettuce
Guatemala	cotton
Honduras	cotton
Indonesia	cabbage
Israel	potato, melon, apple, avocado, grape, nectarine, pepper, pear, plum, strawberry
Japan	brassica, turf, apple, cabbage, Chinese cabbage, Japanese radish, tea
Korea	Chinese cabbage, cucumber, roses
Lebanon	brassica, citrus, grape, lettuce, pepper, potato, spinach, stone fruit, tomato
Malaysia	cabbage
Mexico	cotton, broccoli, tomato, peppers, cucumber
New Zealand	Brassica, tomato
Nicaragua	cotton
Pakistan	cotton
Paraguay	corn, soybean, cotton
Peru	cotton, beans, cauliflower, citrus, corn, cucurbits, tomato
Philippines	cabbage
Taiwan	cabbage
Thailand	cabbage
Tunisia	vegetables
Turkey	potato
U.S.	almond, cotton, fruits, vegetables, beans, corn, potato, sorghum, turf
UAE	citrus, grape, strawberry
Uruguay	alfalfa, tomatoes, citrus, soybean, sugarbeet
Venezuela	corn

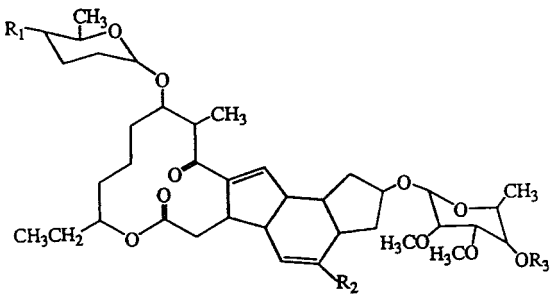
mined simultaneously using HPLC with UV detection at 250 nm.

Previous studies using radiolabeled (^{14}C) material demonstrated that the two active ingredients in spinosad (spinosyns A and D) were not metabolized in cotton plants. However, the parent compounds were metabolized and/or degraded to spinosyn B and *N*-demethylspinosyn D in soil, water, and animal tissues. Spinosyns B and K and *N*-demethylspinosyn D were also identified as minor metabolites in crops (D. P. Rainey, J. D. Magnussen, D. F. Berard, Dow AgroSciences LLC, personal communication, 1994). Consequently, the analytical methods for spinosad were developed to include the parent compounds and the appropriate metabolites for all of the sample matrices. The structures of spinosad and its metabolites are shown in Figure 1.

This paper briefly summarizes the method validation data for the various sample matrices and methods for determining spinosad and its metabolites by HPLC-UV. The corresponding method validation results are summarized for 61 sample matrices, including 37 additional crops and processed commodities that were analyzed using the previously published methods. This paper also describes modifications of the previous methods that were needed to obtain satisfactory results for some of the additional crop matrices.

EXPERIMENTAL PROCEDURES

Analytical Methodology and Method Validation. To determine if existing methods could be extended to additional sample types, >30 crop matrices and processed commodities



Spinosad and Metabolites

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.

were screened using two previously published methods. One of the methods (Yeh et al., 1997) utilized C_{18} Empore disk extraction for initial purification of the sample extracts, whereas the other method (West and Turner, 2000) utilized aqueous−organic partitioning. Additional purification was achieved in both methods by silica and cyclohexyl SPE prior to analysis by HPLC-UV. During method development, it was determined that the C_{18} disks worked well for nonoily and nonfatty crops but that oily and fatty sample types yielded low analyte recoveries. The oil and fat present in the sample extracts reduced recoveries by prematurely eluting the analytes from the disks during the purification process. In contrast, the aqueous−organic partitioning technique could be used with fatty/oily matrices as well as nonfatty/nonoily matrices. As a result, the aqueous−organic partitioning was successfully utilized instead of C_{18} SPE disks for oily and fatty matrices such as cottonseed and processed commodities (West, 1996), animal tissues (West and Turner, 1998), and orange oil (West and Turner, 2000). However, both techniques were utilized for the purpose of screening all of the new crop matrices to determine if the existing methods could be extended to additional crops.

To determine recovery of the analytes in the screening study, fortified recovery samples were prepared at 0.010−0.10 μg/g by adding 1.0 mL of the appropriate fortification standard solution to the appropriate control samples. Sample analysis and calculation of results were then conducted as described previously (West and Turner, 2000; Yeh et al., 1997). The additional crop matrices that were screened using the two approaches are listed in Tables 2−8 and included cucurbits, legumes, cereal grains, forage, stover, fodder, hay, straw, grain dust, potatoes, stone fruits, pears, grapes, and grape processed commodities.

For the method screening study with the additional crop matrices, the method using aqueous−organic partitioning (West and Turner, 2000) was utilized as published. However, a few modifications were made for the method using the C_{18} disk (Yeh et al., 1997) to provide adequate cleanup and recoveries. The TurboVap LV evaporator (Zymark Corp., Hopkinton, MA) was replaced with a rotary vacuum evaporator and a 30 °C water bath. In addition, a few minor modifications in the C_{18} disk method were made to provide for adequate cleanup for some of the individual crop matrices. For alfalfa forage, sorghum forage, and wheat forage, the 80% acetonitrile/20% water extracting solution was replaced with 80% acetonitrile/20% water containing 0.25 g/L of a citric acid and ascorbic acid antioxidant mixture (Sigma Chemical Co., St. Louis, MO). For soybeans, the sample weight was reduced from 10 to 5 g, and the final volume was reduced from 2.0 to 1.0 mL. For sorghum grain dust, No. 5 filter paper (Whatman, Inc., Clifton, NJ) was used instead of No. 1 filter paper to improve filtration of the fine particles. For pears, grapes, grape

Table 2. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Cucurbits Fortified at 0.010−0.10 μg/g

		% recovery[a] (mean ± SD)				
sample type	method	A	D	B	NDSD	K
cantaloupe[b]	partitioning[c]	98 ± 11	98 ± 13	99 ± 6	103 ± 8	111 ± 5
	SPE[d]	100 ± 11	97 ± 14	88 ± 14	88 ± 13	97 ± 12
cucumber	partitioning	99 ± 4	99 ± 5	101 ± 2	103 ± 1	111 ± 2
	SPE	98 ± 3	94 ± 3	83 ± 18	81 ± 8	95 ± 2
squash	partitioning	99 ± 1	83 ± 8	93 ± 9	84 ± 11	103 ± 11
	SPE	85 ± 2	98 ± 5	87 ± 13	85 ± 8	102 ± 11

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Included both edible cantaloupe fruit and whole cantaloupe. [c] Aqueous−organic partitioning. [d] SPE with C_{18} disks.

Table 3. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Legumes Fortified at 0.010−0.10 μg/g

		% recovery[a] (mean ± SD)				
sample type	method	A	D	B	NDSD	K
snow peas	partitioning[b]	109 ± 5	105 ± 4	99 ± 3	96 ± 4	116 ± 6
	SPE[c]	101 ± 3	93 ± 10	81 ± 10	79 ± 11	92 ± 11
soybeans	partitioning	87 ± 4	75 ± 4	83 ± 3	79 ± 3	101 ± 3
	SPE	97 ± 6	94 ± 6	85 ± 7	84 ± 6	93 ± 4
snap beans	partitioning	73 ± 2	66 ± 3	78 ± 9	69 ± 5	96 ± 3
	SPE	100 ± 10	99 ± 8	86 ± 9	86 ± 7	100 ± 9

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Aqueous−organic partitioning. [c] SPE with C_{18} disks.

Table 4. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Cereal Grains Fortified at 0.010−0.10 μg/g

		% recovery[a] (mean ± SD)				
sample type	method	A	D	B	NDSD	K
field corn	partitioning[b]	83 ± 8	74 ± 7	99 ± 5	80 ± 8	103 ± 8
	SPE[c]	92 ± 7	82 ± 6	74 ± 5	69 ± 8	84 ± 3
sweet corn	partitioning	90 ± 9	83 ± 7	102 ± 14	86 ± 4	105 ± 3
	SPE	95 ± 4	86 ± 7	80 ± 3	77 ± 9	73 ± 5
sorghum	partitioning	82 ± 9	74 ± 4	87 ± 1	80 ± 2	95 ± 11
	SPE	96 ± 6	88 ± 5	79 ± 5	77 ± 6	80 ± 3
wheat	partitioning	84 ± 3	79 ± 6	88 ± 5	79 ± 7	106 ± 2
	SPE	95 ± 8	96 ± 6	87 ± 3	88 ± 6	79 ± 10

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Aqueous−organic partitioning. [c] SPE with C_{18} disks.

Table 5. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Forage Fortified at 0.010−0.10 μg/g

		% recovery[a] (mean ± SD)				
sample type	method	A	D	B	NDSD	K
sweet corn forage	partitioning[b]	101 ± 10	102 ± 10	92 ± 1	98 ± 4	103 ± 8
	SPE[c]	81 ± 5	79 ± 9	78 ± 2	75 ± 6	72 ± 4
sorghum forage	partitioning	interf[d]	interf	interf	interf	interf
	SPE	93 ± 2	92 ± 2	69 ± 3	70 ± 4	88 ± 3
wheat forage	partitioning	NA[e]	NA	NA	NA	NA
	SPE	85 ± 2	87 ± 4	81 ± 1	79 ± 2	81 ± 4
alfalfa forage	partitioning	65 ± 8	61 ± 8	81 ± 9	73 ± 7	67 ± 3
	SPE	94 ± 6	94 ± 7	74 ± 4	77 ± 6	73 ± 8

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Aqueous−organic partitioning. [c] SPE with C_{18} disks. [d] Interference due to insufficient cleanup. [e] Information not available due to an insufficient amount of control sample matrix.

juice, and wine, 25% dichloromethane/75% methanol was used instead of 2% triethylamine/98% acetonitrile to elute the analytes from the C_{18} extraction disk.

In addition to the study in which the additional crop matrices were screened using the existing methods, methods for several other crops were also developed by modifying the previously published methods. To provide adequate cleanup and recovery for the determination of spinosad in some of these crops, it was necessary to make a few modifications in the cleanup procedures. For cotton gin trash, it was necessary to utilize the extraction and aqueous−organic partitioning techniques described for cottonseed (West, 1996) in combination with the silica and cyclohexyl SPE cleanup procedures that were utilized for citrus crops (West and Turner, 2000).

Table 6. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Potatoes Fortified at 0.010−0.10 μg/g

		% recovery[a] (mean ± SD)				
sample type	method	A	D	B	NDSD	K
Irish potatoes	partitioning[b]	104 ± 12	102 ± 10	102 ± 8	95 ± 15	111 ± 11
	SPE[c]	93 ± 10	86 ± 2	74 ± 2	92 ± 17	88 ± 10
red potatoes	partitioning	92 ± 9	85 ± 9	82 ± 15	73 ± 13	103 ± 11
	SPE	NA[d]	NA	NA	NA	NA

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Aqueous−organic partitioning. [c] SPE with C_{18} disks. [d] Information not available.

Table 7. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Stone Fruits and Pears Fortified at 0.010−0.10 μg/g

		% recovery[a] (mean ± SD)				
sample type	method	A	D	B	NDSD	K
cherries[b]	partitioning[c]	108 ± 4	106 ± 4	99 ± 6	97 ± 6	104 ± 6
	SPE[d]	100 ± 6	101 ± 6	86 ± 8	87 ± 6	98 ± 7
peaches	partitioning	118 ± 3	118 ± 2	110 ± 4	106 ± 5	112 ± 3
	SPE	98 ± 8	97 ± 8	86 ± 9	88 ± 6	94 ± 7
plums	partitioning	87 ± 3	86 ± 3	83 ± 8	78 ± 2	84 ± 5
	SPE	99 ± 7	106 ± 18	81 ± 3	76 ± 6	85 ± 5
prunes	partitioning	103 ± 1	92 ± 3	85 ± 3	85 ± 3	83 ± 4
	SPE	104 ± 18	86 ± 13	83 ± 3	71 ± 12	67 ± 3
pears	partitioning	111 ± 8	120 ± 11	92 ± 11	106 ± 11	106 ± 11
	SPE	94 ± 4	93 ± 3	89 ± 4	97 ± 6	83 ± 5

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Included both sweet and sour cherries. [c] Aqueous−organic partitioning. [d] SPE with C_{18} disks.

Table 8. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Grapes, Juice, and Wine Fortified at 0.010−0.10 μg/g

		% recovery (mean ± SD)[a]				
sample type	method	A	D	B	NDSD	K
grapes	partitioning[b]	110 ± 2	122 ± 7	95 ± 7	104 ± 7	104 ± 7
	SPE[c]	99 ± 3	73 ± 5	87 ± 2	87 ± 2	73 ± 7
grape juice	partitioning	90 ± 9	101 ± 9	86 ± 6	91 ± 7	97 ± 7
	SPE	94 ± 5	73 ± 5	87 ± 2	87 ± 2	84 ± 21
grape wine	partitioning	98 ± 3	106 ± 7	92 ± 1	98 ± 1	103 ± 3
	SPE	84 ± 7	71 ± 9	72 ± 8	70 ± 4	71 ± 10

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Aqueous−organic partitioning. [c] Solid phase extraction with C_{18} disks.

For several crop matrices, the aqueous−organic partitioning described in the cottonseed method was modified by replacing hexane with another organic partitioning solvent. The organic partitioning solvent was 1-chlorobutane for pecans and almonds and was dichloromethane for apples, processed apple commodities, cabbage, mustard greens, and broccoli. In addition, it was necessary to utilize both the silica and the cyclohexyl SPE columns for these matrices.

The method for green and cured tobacco utilized the C_{18} extraction disk approach. However, it was necessary to add the previously mentioned antioxidant mixture to the extraction solution, and 25% dichloromethane/75% methanol instead of 2% triethylamine/98% acetonitrile was used to elute the analytes from the disk.

Apparatus. HPLC with a UV Detector. Although the exact chromatographic conditions varied somewhat over time, typical HPLC conditions were as follows: 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-AM [3-μm particle size, 150 × 4.6 mm i.d. (YMC, Inc., Wilmington, NC)], maintained at 30 °C. (ODS-AM is a high-carbon load C_{18} packing that has been subjected to an endcapping step that improves peak shape with some analytes.) The mobile phase consisted of 44% reservoir A/44% reservoir B/12% reservoir C (isocratic), with reservoir A containing methanol, reservoir B containing acetonitrile, and reservoir C containing 2% aqueous ammonium acetate/acetonitrile (67:33). The flow rate was 0.4 mL/min. The injection volume was 175 μL, the integrator attenuation was 2^3, and the chart speed was 0.2 cm/min. The UV detector was operated at 250 nm.

Table 9. Overall Summary of Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosin D (NDSD) from the Crop Matrices in Tables 2−8

		% recovery (mean ± SD)				
method	no. of recoveries	A	D	B	NDSD	K
partitioning[a]	72	97 ± 12	95 ± 16	93 ± 10	90 ± 16	103 ± 10
SPE[b]	72	96 ± 8	91 ± 10	81 ± 8	81 ± 10	86 ± 11

[a] Aqueous−organic partitioning. [b] SPE with C_{18} disks.

RESULTS AND DISCUSSION

The results of the method screening study with the additional sample types are summarized for the individual matrices in Tables 2−8, and an overall summary for all of the matrices using both methods is contained in Table 9. For all 26 commodities summarized in Table 9, the average recoveries ranged from 96 to 97% for

Table 10. Recovery of Spinosyns A and D from Cotton Gin Trash Fortified at 0.020−1.0 μg/g

method	no. of recoveries	% recovery[a] (mean ± SD) A	D	B	NDSD	K
partitioning[b]	14	82 ± 6	79 ± 7	NA[c]	NA	NA

[a] No residues were detected in untreated control samples at a detection limit of 0.006 μg/g. [b] Aqueous−organic partitioning. [c] Not applicable. (These analytes are not metabolites of spinosad in cotton.)

Table 11. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Tree Nuts (Almond and Pecan Nutmeat and Almond Hulls) Fortified at 0.010−0.10 μg/g

sample type	no. of recoveries	% recovery[a] (mean ± SD) A	D	B	NDSD	K
nutmeat[b]	20	86 ± 4	83 ± 4	85 ± 6	89 ± 9	82 ± 4
almond hulls	20	87 ± 4	82 ± 5	81 ± 3	83 ± 3	79 ± 4

[a] No residues were detected in untreated control samples at a detection limit of 0.003 μg/g. [b] Included almond and pecan nutmeat.

Table 12. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Green and Cured Tobacco Fortified at 0.040−0.10 μg/g

sample type	no. of recoveries	% recovery[a] (mean ± SD) A	D	B	NDSD	K
green tobacco	20	91 ± 3	90 ± 3	78 ± 4	78 ± 4	90 ± 2
cured tobacco	20	85 ± 3	85 ± 2	73 ± 4	83 ± 3	82 ± 3

[a] No residues were detected in untreated control samples at a detection limit of 0.012 μg/g.

spinosyn A, from 91 to 95% for spinosyn D, from 81 to 93% for spinosyn B, from 81 to 90% for *N*-demethylspinosyn D, and from 86 to 103% for spinosyn K. The corresponding standard deviations ranged from 8 to 12% for spinosyn A, from 10 to 16% for spinosyn D, from 8 to 10% for spinosyn B, from 10 to 16% for *N*-demethylspinosyn D, and from 10 to 11% for spinosyn K. For all five analytes, average recoveries were higher with the aqueous−organic partitioning than with the SPE disk. However, the SPE disk yielded lower standard deviations for all of the analytes except for spinosyn K.

For a large majority of the matrices analyzed, the recoveries of all of the analytes were >70%. However, for a few of the matrices, the average recovery of one or more of the analytes was <70%. For the method utilizing aqueous−organic partitioning, average recoveries were <70% for two analytes in snap beans (Table 3) and for three analytes in alfalfa forage (Table 5). For the method utilizing SPE, average recoveries were <70% for one analyte in field corn (Table 4), for one analyte in sorghum forage (Table 5), and for one analyte in prunes (Table 7).

In addition to the method screening study, several new crop matrices were validated using slight modifications of the previously published methods. The modifications that were used for these sample types are noted under Experimental Procedures. The average recoveries for all of the analytes ranged from 79 to 82% for cotton gin trash (Table 10), from 79 to 89% for almonds and pecans (Table 11), and from 73 to 91% for tobacco (Table 12).

One of the objectives of the study was to determine if the existing methods could be extended to a wide variety of additional sample types. The method validation data indicate that 32 new crop matrices could be analyzed with the published methods or with minor modifications to the published methods. The method validation recovery data for the new matrices (Tables 2−12) compare favorably with previously published validation data for 24 diverse sample types (Tables 13−16).

Table 13. Recovery of Spinosyns A and D from Cottonseed and Cottonseed Processed Commodities (Meal, Hulls, Oil, and Soapstock) Fortified at 0.010−0.10 μg/g (West, 1996)

sample type	no. of recoveries	% recovery (mean ± SD) A	D	B	NDSD	K
cottonseed	18	99 ± 14	95 ± 11	NA[a]	NA	NA
cottonseed meal	10	90 ± 6	85 ± 8	NA	NA	NA
cottonseed hulls	10	100 ± 10	100 ± 10	NA	NA	NA
crude oil	18	96 ± 7	93 ± 5	NA	NA	NA
refined oil	10	92 ± 10	86 ± 11	NA	NA	NA
soapstock	18	99 ± 4	102 ± 4	NA	NA	NA

[a] Not applicable. (These analytes are not metabolites of spinosad in cotton.)

Table 14. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Soil and Sediment Fortified at 0.010−0.10 μg/g and from Water Fortified at 0.001−0.100 μg/mL (West, 1997)

sample type	no. of recoveries	% recovery (mean ± SD) A	D	B	NDSD	K
soil and sediment[a]	35	82 ± 5	83 ± 6	78 ± 6	76 ± 6	NA[c]
water[b]	35	93 ± 8	90 ± 8	87 ± 6	90 ± 8	NA[c]

[a] Included pond sediment, sandy loam soil, and clay loam soil. [b] Included pond water, well water, and tap water. [c] Not applicable. (Spinosyn K is not a degradation product of spinosad in soil, sediment, or water.)

Table 15. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Leafy Vegetables, Peppers, and Tomatoes Fortified at 0.010−5.0 μg/g (Yet et al., 1997)

sample type	no. of recoveries	% recovery (mean ± SD) A	D	B	NDSD	K
celery	20	93 ± 2	93 ± 2	86 ± 2	95 ± 11	84 ± 6
head lettuce	20	88 ± 3	88 ± 3	78 ± 4	95 ± 11	85 ± 4
leaf lettuce	20	93 ± 3	91 ± 2	83 ± 4	95 ± 11	88 ± 2
spinach	20	91 ± 4	91 ± 3	84 ± 4	95 ± 11	87 ± 4
peppers	13	97 ± 3	96 ± 3	95 ± 11	85 ± 5	88 ± 7
tomatoes	13	88 ± 3	85 ± 4	95 ± 11	83 ± 4	82 ± 3

Table 16. Recovery of Spinosyns A, D, K, and B and *N*-Demethylspinosyn D (NDSD) from Citrus Crops and Orange Processed Commodities Fortified at 0.010−10 μg/g (West and Turner, 2000)

sample type	no. of recoveries	% recovery (mean ± SD) A	D	B	NDSD	K
whole fruit[a]	20	104 ± 4	101 ± 4	98 ± 7	95 ± 8	99 ± 5
edible oranges	20	101 ± 4	98 ± 5	92 ± 7	90 ± 7	98 ± 6
orange peel	20	97 ± 9	95 ± 7	90 ± 8	90 ± 7	98 ± 5
dried orange pulp	20	113 ± 5	110 ± 4	99 ± 8	96 ± 8	110 ± 4
orange juice	20	102 ± 5	94 ± 5	94 ± 6	90 ± 6	104 ± 4
orange oil	20	88 ± 7	87 ± 6	76 ± 7	77 ± 5	88 ± 5

[a] Included oranges, grapefruit, lemons, and mandarins.

Although HPLC-UV methods were successfully developed for 56 sample types, 5 crop matrices could not be analyzed using either of the method approaches (West and Turner, 2000; Yeh et al., 1997.) Alfalfa hay, wheat hay and straw, corn stover, and sorghum fodder yielded low recoveries and/or insufficient cleanup with HPLC-UV, and it was necessary to develop a method utilizing liquid chromatography−mass spectrometry (LC-MS) for these analytes (Schwedler et al., 2000).

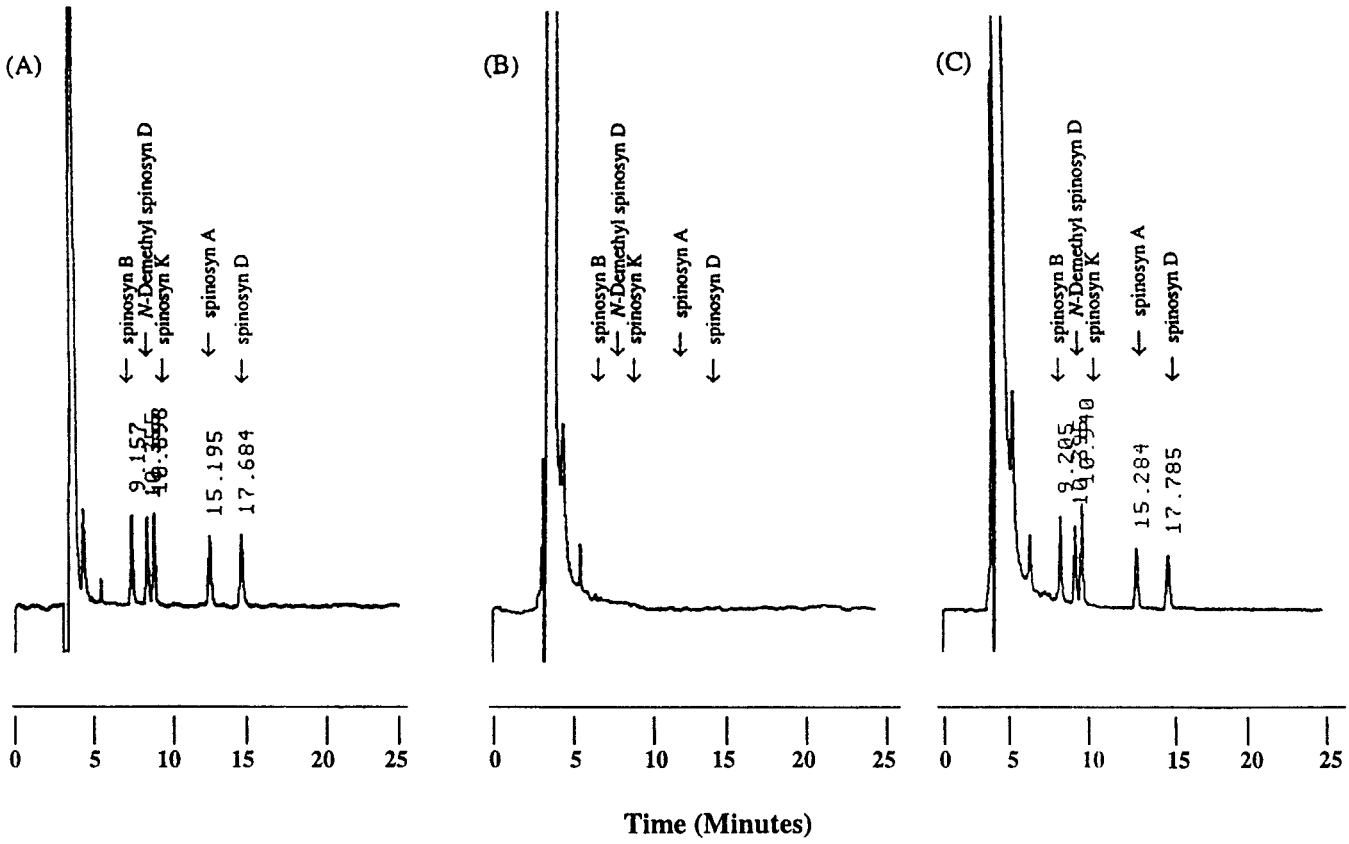


Figure 2. Typical chromatograms from the determination of spinosyns A, D, K, and B and *N*-demethylspinosyn D in field corn grain using the aqueous−organic partitioning technique (West and Turner, 2000): (A) standard, 17.5 ng of each analyte; (B) control grain containing no detectable residue; (C) control grain fortified with 0.01 μg/g (limit of quantitation), equivalent to recoveries of 88% (spinosyn B), 87% (*N*-demethylspinosyn D), 112% (spinosyn K), 91% (spinosyn A), and 80% (spinosyn D).

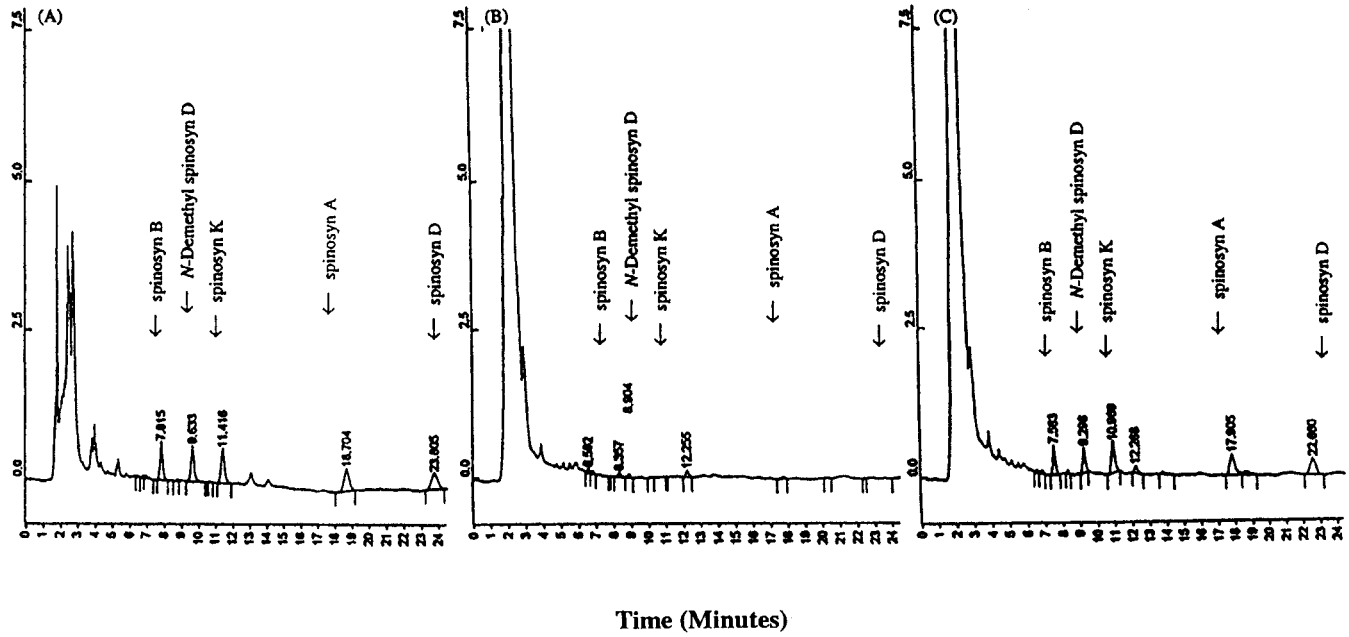


Figure 3. Typical chromatograms from the determination of spinosyns A, D, K, and B and *N*-demethylspinosyn D in field corn grain using the C_{18} SPE disk technique (Yeh et al., 1997): (A) standard, 12.5 ng of each analyte; (B) control grain containing no detectable residue; (C) control grain fortified with 0.01 μg/g (limit of quantitation), equivalent to recoveries of 82% (spinosyn B), 84% (*N*-demethylspinosyn D), 92% (spinosyn K), 96% (spinosyn A), and 96% (spinosyn D).

Chromatograms. Typical chromatograms resulting from the analysis of field corn grain by HPLC-UV are illustrated for both the aqueous−organic partitioning approach (Figure 2) and the C_{18} SPE disk approach (Figure 3). Both techniques yielded adequate cleanup and analyte recoveries.

Conclusions. Residue methodology utilizing HPLC-UV has been validated for the determination of the

active ingredients of spinosad (spinosyns A and D) and up to three minor metabolites (spinosyns B and K and *N*-demethylspinosyn D) in a very wide variety of crops, processed commodities, and environmental matrices. The accuracy and precision of the methodologies are suitable for residue or environmental monitoring or for tolerance enforcement. This study expands the list of sample matrices in which spinosad residues may be successfully determined.

LITERATURE CITED

Anastas, P.; Kirchchoff, M.; Williamson, T. Spinosad—a new natural product for insect control. *Green Chem*. **1999**, *1*, G88.

Nakanishi, K. Main Topic III. Natural products with biological activity. In *Advances in Pesticide Science*; Geissbuhler, H., Brooks, G. T., Kearney, P. C., Eds.; Pergamon Press: Oxford, U.K., 1978; Part 2, p 283.

Schwedler, D. A.; Thomas, A. D.; Yeh, L. T. Determination of spinosad and its metabolites in food and environmental matrices. 2. Liquid chromatography—mass spectrometry. *J. Agric. Food Chem*. **2000**, *48*, 5138−5145.

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.

West, S. D. Determination of the naturally derived insect control agent spinosad in cottonseed and processed commodities by high-performance liquid chromatography with ultraviolet detection. *J. Agric. Food Chem*. **1996**, *44*, 3170−3177.

West, S. D. Determination of the naturally derived insect control agent spinosad and its metabolites in soil, sediment, and water by high-performance liquid chromatography with ultraviolet detection. *J. Agric. Food Chem*. **1997**, *45*, 3107−3113.

West, S. D.; Turner, L. G. Determination of the naturally derived insect control agent spinosad and its metabolites in meat, milk, cream, and eggs by high-performance liquid chromatography with ultraviolet detection. *J. Agric. Food Chem*. **1998**, *46*, 4620−4627.

West, S. D.; Turner, L. G. Determination of spinosad and its metabolites in citrus crops and orange processed commodities by HPLC with UV detection. *J. Agric. Food Chem*. **2000**, *48*, 366−372.

Yeh, L. T.; Schwedler, D. A.; Schelle, G. B.; Balcer, J. L. 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. *J. Agric. Food Chem*. **1997**, *45*, 1746−1751.

Received for review January 18, 2000. Revised manuscript received July 31, 2000. Accepted August 3, 2000.

JF000080Q