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Determination of Residues of Spinosad and its Metabolites in Agricultural Commodities by On-Line Solid Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry

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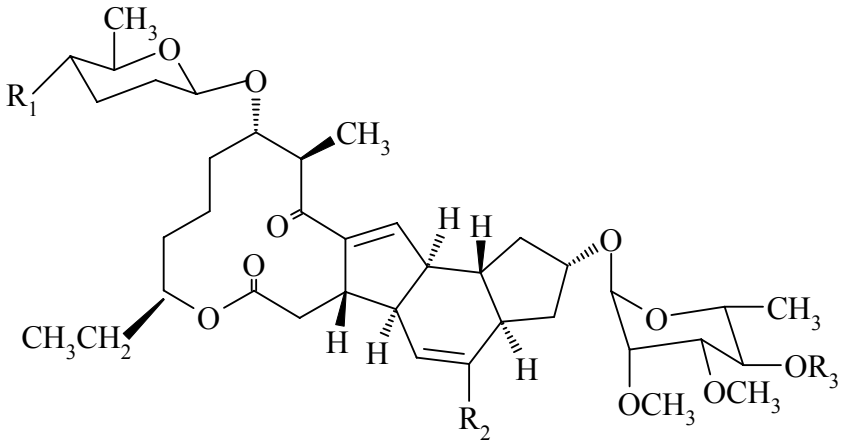
1. SCOPE

This method is applicable for the quantitative determination of spinosad (spinosyn A and spinosyn D), and their metabolites, (spinosyn B and *N*-demethyl spinosyn D), in agricultural commodities (apples, grass, lettuce, oranges, and tomatoes). The method was validated over the concentration range of 0.01-1.0 µg/g for all crops except lettuce, which was validated over the concentration range of 0.01-10.0 µg/g. The validated limit of quantitation for all crops was 0.01 µg/g.



Spinosyn A, $R_1 = N(CH_3)_2$, $R_2 = H$, $R_3 = CH_3$
Spinosyn D, $R_1 = N(CH_3)_2$, $R_2 = CH_3$, $R_3 = CH_3$

Common and chemical names along with other identifying information are given in Table 1.

2. PRINCIPLE

Residues of spinosad and its metabolites are extracted from the crop sample by homogenizing and shaking with an acetonitrile/water (80:20) solution. An aliquot is taken and a mixed XDE-175 and metabolites stable isotope internal standard solution is

added to each sample. The final solution is purified by on-line solid phase extraction using a cation exchange cartridge. The extract is loaded onto the solid phase extraction (SPE) cartridge with water. The SPE cartridge is washed with methanol and eluted onto the analytical column with an acetonitrile/methanol/water (4:4:2) solution containing 0.1 M ammonium acetate. Spinosad and its metabolites are analyzed by liquid chromatography with positive-ion atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (LC/MS/MS).

3. SAFETY PRECAUTIONS

3.1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non Dow AgroSciences LLC products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

3.2. Acetonitrile and methanol are flammable and volatile and should be used in well-ventilated areas away from ignition sources.

3.3. Formic acid is corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be used when handling all chemicals.

4. EQUIPMENT (Note 12.1.)

4.1. Laboratory Equipment

4.1.1. Balance, analytical, Model AE100, Mettler-Toledo, Inc., Columbus, OH 43240.

4.1.2. Balance, pan, Model PG2002, Mettler-Toledo, Inc.

4.1.3. Centrifuge, with rotor to accommodate 8-oz bottles, Model Centra-GP8, International Equipment Company, Needham Heights, MA 02494.

4.1.4. Dispenser, Bottle-Top, adjustable, Brinkmann, 20-100 mL, catalog number 13-688-136, Fisher Scientific, Pittsburgh, PA 15219.

4.1.5. Hammer mill, with 1/8 and 3/16-inch screen, Model 2001, AGVISE Laboratories, Inc., Northwood, ND 58267.

4.1.6. Homogenizer, Omni-mixer, Model ES, Omni International, Inc., Warrenton, VA 20187.

4.1.7. Homogenizer generator, 20-mm probe, catalog number 15020W, Omni International, Inc.

4.1.8. Pipettor, adjustable, Gilson Microman M50, 20-50 µL, catalog number F148503, Gilson Inc., Middleton, WI 53562.

4.1.9. Pipettor, adjustable, Gilson Microman M250, 50-250 µL, catalog number F148505, Gilson Inc.

4.1.10. Pipettor, adjustable, Gilson Microman M1000, 100-1000 µL, catalog number F148506, Gilson Inc.

4.1.11. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

4.1.12. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

4.2. Chromatographic System

4.2.1. Column, analytical, YMC ODS-AM, 50 x 4.6 mm, 5-µm, catalog number AM12S05-0546WT, Waters, Milford, MA 01757.

4.2.2. Column, confirmatory, Synergi Polar RP, 75 x 4.6 mm, 4-µm, catalog number 00C-4336-E0, Phenomenex, Torrance, CA 90501.

4.2.3. Mass spectrometer, Model API 4000, MDS/Sciex, Foster City, CA 94404.

4.2.4. Mass spectrometer data system, Analyst 1.4, MDS/Sciex.

4.2.5. On-line SPE/Liquid chromatograph, Symbiosis Pharma, Spark Holland Inc., Plainsboro, NJ 08536.

5. GLASSWARE AND MATERIALS (Note 12.1.)

5.1. Bottle, 250-mL, HDPE, catalog number 03-313-4D, Fisher Scientific.

5.2. Bottle, 1.0-L, media bottle, catalog number 06-423-3D, Fisher Scientific.

5.3. Bottle, 2.0-L, media bottle, catalog number 06-423-3E, Fisher Scientific.

5.4. Collection plate, 96-well, 2-mL, catalog number 121-5203, Biotage AB, Charlottesville, Virginia 22911.

5.5. Collection plate sealing cap, catalog number 121-5205, Biotage AB.

5.6. Column, SPE, BondElut SCX, 40-90 µm, catalog number 0722.141, Spark Holland Inc.

5.7. Cylinder, graduated, 100-mL, catalog number 08-557D, Fisher Scientific.

5.8. Cylinder, graduated, 500-mL, catalog number 08-557F, Fisher Scientific.

5.9. Cylinder, graduated, 1000-mL, catalog number 09-730-30, Fisher Scientific.

5.10. Cylinder, graduated, 2000-mL, catalog number 08-557H, Fisher Scientific.

5.11. Flask, volumetric, 100-mL, catalog number 5640-100, Corning, Inc., Acton, MA 01720.

5.12. Pipet, polyethylene disposable transfer, 3-mL, catalog number, 13-711-7, Fisher Scientific.

5.13. Pipet, volumetric, 0.5-mL, catalog number 13-650-3A, Fisher Scientific.

5.14. Pipet, volumetric, 1.0-mL, catalog number 13-650-3B, Fisher Scientific.

5.15. Pipet, volumetric, 2.0-mL, catalog number 13-650-3C, Fisher Scientific.

5.16. Pipet, volumetric, 3.0-mL, catalog number 13-650-3D, Fisher Scientific.

5.17. Pipet, volumetric, 5.0-mL, catalog number 13-650-3F Fisher Scientific.

5.18. Pipet, volumetric, 10.0-mL, catalog number 13-650-3L, Fisher Scientific.

5.19. Pipetter tips, Gilson Microman CP50, catalog number F148113, Gilson Inc.

5.20. Pipetter tips, Gilson Microman CP250, catalog number F148114, Gilson Inc.

5.21. Pipetter tips, Gilson Microman CP1000, catalog number F148560, Gilson Inc.

5.22. Vial, 40-mL, with PTFE-lined screw cap, catalog number 7587T, Qorpak, Bridgeville, PA 15017.

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS (Note 12.1.)

6.1. Reagents

6.1.1. Acetonitrile, ChromAR HPLC grade, catalog number 2856, Mallinckrodt-Baker, Inc., Paris, KY 40361.

6.1.2. Ammonium acetate, HPLC grade, catalog number A639-500, Fisher Scientific.

6.1.3. Formic acid, 96%, ACS grade, catalog number 251364, Sigma-Aldrich, Milwaukee, WI 53201.

6.1.4. Methanol, ChromAR HPLC grade, catalog number 3041, Mallinckrodt-Baker Inc.

6.1.5. Nitrogen, refrigerated liquid, BOC Group Inc., Murray Hill, NJ 07974.

6.1.6. Water, HPLC grade, catalog number WX0004-1, EMD Chemicals, Gibbstown, NJ 08027.

6.2. Standards

6.2.1. Analytical standard information for spinosyn A, spinosyn B, spinosyn D, and *N*-demethyl spinosyn D are listed in Table 1.

Compounds can be obtained from Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

6.2.2. Stable isotope labeled internal standards information for XDE-175-J, XDE-175-L, XDE-175-*N*-demethyl-J, XDE-175-*N*-demethyl-L are listed in Table 1.

Obtain from Specialty Synthesis Group, Dow AgroSciences LLC, 9330 Zionsville Road, Building 306, Indianapolis, IN 46268-1054. Dow AgroSciences will provide the internal standards free of charge.

6.3. Prepared Solutions

6.3.1. acetonitrile/water (1:1) containing 0.1% formic acid

Pipet 1.0 mL of formic acid into a 1-L bottle containing 500 mL of acetonitrile. Measure 500 mL of water using a 500-mL graduated cylinder and then transfer to the 1.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.2. acetonitrile/methanol/water (4:4:2) containing 0.1 M ammonium acetate

Weigh 7.7 g of ammonium acetate into a 40-mL vial and quantitatively transfer with 200 mL of HPLC water into a 1-L bottle. Measure 400 mL of methanol and 400 mL of acetonitrile using a 500-mL graduated cylinder and transfer to the 1.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.3. acetonitrile/methanol (1:1) containing 10 mM ammonium acetate

Weigh 0.77 g of ammonium acetate into a 40-mL vial and quantitatively transfer with 100 mL of methanol into a 1-L bottle. Add a further 400 mL of methanol to the bottle. Measure 500 mL of acetonitrile using a 500-mL graduated cylinder and then transfer to

the 1.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.4. acetonitrile/water (80:20)

Measure 1600 mL of acetonitrile using a 2-L graduated cylinder and then transfer into a 2.0-L bottle. Measure 400 mL of water using a 500-mL graduated cylinder and then transfer into the 2.0-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.5. water containing 10 mM ammonium acetate

Weigh 0.77 g of ammonium acetate into a 40-mL vial and quantitatively transfer with 100 mL of HPLC water into a 1-L bottle. Add a further 900 mL of HPLC water to the bottle. Cap the bottle and mix.

7. PREPARATION OF STANDARD SOLUTIONS (Note 12.2.)

7.1. Preparation of Spinosad and Metabolite Spiking Solutions

7.1.1. Weigh 0.0100 g of each spinosad analytical standard (spinosyn A, spinosyn D, spinosyn B, and *N*-demethyl spinosyn D) corrected for purity if necessary and quantitatively transfer each standard to separate 100-mL volumetric flasks with acetonitrile. Dilute to volume with acetonitrile to obtain a 100-µg/mL stock solution of each analyte.

7.1.2. Pipet 10.0 mL of each 100-µg/mL solution (Section 7.1.1.) into a 100-mL volumetric flask. Dilute to volume with acetonitrile to obtain a 10.0-µg/mL mixed spinosad and metabolite spiking solution. Further dilute the 10.0-µg/mL mixed spinosad and metabolite spiking solution with acetonitrile according to the following suggested scheme:

Concentration of Initial Stock Solution	Aliquot of Stock Solution	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.[a]	Volume of Spiking Soln.
µg/mL	mL	mL	µg/mL	µg/g	µL
- -	- -	- -	100.0	10.0	500[b]
100.0	10.0	100	10.0	1.0	500
- -	- -	- -	10.0	0.1	50
10.0	10.0	100	1.0	0.01	50
1.0	10.0	100	0.1	0.003	150
0.1	10.0	100	0.01	- -	- -

[a] The equivalent sample concentration is based on fortifying a 5-g crop sample.
[b] 500 µL of each of the spinosad and metabolite 100-µg/mL spiking solutions (Section 7.1.1.).

7.2. Preparation of XDE-175 and Metabolite Stable Isotope Internal Standard Solutions

7.2.1. Weigh 0.0100 g of each XDE-175 stable isotope standard (XDE-175-J IS, XDE-175-L IS, XDE-175-*N*-demethyl-J IS and XDE-175-*N*-demethyl-L IS) and quantitatively transfer each standard to separate 100-mL volumetric flasks with acetonitrile. Dilute to volume with acetonitrile to obtain a 100-µg/mL stock solution of stable isotope standard.

7.2.2. Pipet 10.0 mL of each 100-µg/mL solution (Section 7.2.1.) into a 100-mL volumetric flask. Dilute to volume with acetonitrile to obtain a 10.0-µg/mL mixed XDE-175 and metabolite stable isotope internal standard solution.

7.2.3. Pipet 1.0 mL of the 10.0-µg/mL mixed XDE-175 stable isotope internal standard solution (Section 7.2.2.) into a 100-mL volumetric flask. Dilute to volume with acetonitrile to obtain a 0.1-µg/mL mixed XDE-175 and metabolite stable isotope internal standard solution.

7.2.4. Pipet 5.0 mL of the 0.1-µg/mL mixed XDE-175 stable isotope internal standard solution (Section 7.2.3.) into a 100-mL volumetric flask. Add 75 mL of acetonitrile to the flask. Measure 15 mL of water and transfer to the flask. Cap the flask and mix. Allow the solution to equilibrate to room temperature before diluting to volume with water to obtain an acetonitrile/water solution (80:20) containing 5 ng/mL of mixed XDE-175 and metabolite stable isotope internal standard solution.

7.3. Preparation of Mixed Spinosad and Metabolite Calibration Solutions

7.3.1. Prepare calibration standard solutions by pipeting 5.0 mL of the 0.1-µg/mL mixed XDE-175 and metabolites stable isotope solution, prepared in Section 7.2.3, into each 100-mL volumetric flask and diluting the 1.0, 0.1, and 0.01-µg/mL mixed spinosad spiking solutions (Section 7.1.2.) with acetonitrile/water (80:20) to give calibration standards over the range 0.1–50 ng/mL. Calibration standards may be prepared following the suggested scheme:

Concentration of Stock Solution	Aliquot of Spiking Solution	Aliquot of ISTD Solution	Final Soln. Volume	Calibration Soln. Final Conc.	Equivalent Sample Conc.[a]
µg/mL	mL	mL	mL	ng/mL	µg/g
1.0	5.0	5.0	100	50	1.0
1.0	3.5	5.0	100	35	0.7
1.0	2.0	5.0	100	20	0.4
1.0	1.0	5.0	100	10	0.2
0.1	5.0	5.0	100	5.0	0.1
0.1	1.0	5.0	100	1.0	0.02
0.01	5.0	5.0	100	0.5	0.01
0.01	1.0	5.0	100	0.1	0.002

[a] The equivalent sample concentration is based on extracting a 5-g crop sample.

8. ON-LINE SPE/LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

8.1. Typical Liquid Chromatography Operating Conditions (Note 12.3.)

Instrumentation:	Spark Holland Symbiosis Pharma MDS/Sciex API 4000 LC/MS/MS System MDS/Sciex Analyst 1.4 data system
Column:	YMC ODS-AM, 50 x 4.6 mm, 5-µm (Quantitation) Synergi Polar RP, 75 x 4.6 mm, 4-µm (Confirmation)
Column Temperature:	Ambient
Injection Volume:	30 µL
Autosampler Wash Program:	Autosampler loop and needle washed with: 1) 700 µL of acetonitrile/water (1:1) containing 0.1% formic acid 2) 700 µL of methanol 3) 700 µL of acetonitrile/water (1:1) containing 0.1% formic acid
Run Time:	Approx 6 mins 15 secs
Mobile Phase:	A –acetonitrile/methanol (1:1) containing 10 mM ammonium acetate B –water containing 10 mM ammonium acetate
Flow:	1.0 mL/min

Gradient:

Time, (min:secs)	Flow (mL/min)	A, %	B, %
00:01	1.0	70	30
00:05	0.8	70	30
01:00	0.8	70	30
01:05	1.0	70	30
03:05	1.0	100	0
05:00	1.0	100	0
05:15	1.0	70	30
06:15	1.0	70	30

Flow Diverter Program:
1) 0.0→3.0 min: flow to waste
2) 3.0→5.0 min: flow to source
3) 5.0→end of run: flow to waste

8.2. Typical On-Line Solid Phase Extraction Operating Conditions

SPE Cartridge:	BondElut SCX, 40-90 µm
SPE Solvation:	acetonitrile, 1 mL at 5 mL/min (SSM A)
SPE Equilibration:	water, 1 mL at 5 mL/min (SSM B)
Sample Extraction:	water, 2 mL at 2 mL/min (SSM B)
SPE Wash 1:	methanol, 2 mL at 2.5 mL/min (HPD1)
SPE Wash 2:	acetonitrile/methanol/water (4:4:2) containing 0.1 M ammonium acetate, 200 µL at 200 µL/min (HPD2)
SPE Elution:	focus mode, acetonitrile/methanol/water (4:4:2) containing 0.1 M ammonium acetate, 200 µL at 200 µL/min (HPD2)
Clamp Flush 1:	acetonitrile/methanol/water (4:4:2) containing 0.1 M ammonium acetate, 1 mL at 5.0 mL/min (HPD2)
Clamp Flush 2:	water, 2 mL at 5 mL/min (SSM B)

8.3. Typical Mass Spectrometry Operating Conditions (Note 12.2.)

Ionization Mode:	APCI
Polarity:	Positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR):	12 psi
Collision Gas (CAD):	4 psi
Temperature (TEM):	425 °C
Ion Source Gas 1 (GS1):	50 psi
Needle Current (NC)	5 µA
Declustering Potential (DP)	90 V

Period 1
Acquisition Time Delay: 3.0 mins
Period Duration: 2.0 mins

Compound:	Ion, *m/z*		Time, ms	Collision Energy, V
	Q1	Q3		
Spinosyn A	732.5	142.1	50	41
Spinosyn D	746.5	142.1	50	43
Spinosyn B	718.5	128.1	50	37
N-Demethyl Spinosyn D	732.5	128.1	50	37

XDE-175-J IS	757.9	146.2	50	31
XDE-175-L IS	769.9	146.2	50	37
XDE-175-*N*-Demethyl-J IS	739.9	128.2	50	33
XDE-175-*N*-Demethyl-L IS	751.7	128.2	50	33

8.4. Typical Mass Spectra

Typical mass spectra and product ion spectra of spinosad, its metabolites and the XDE-175 stable isotope internal standards are presented in Figures 1-8.

8.5. Typical Calibration Curves

Typical calibration curves for the determination of spinosad and its metabolites in wet crops (lettuce leaves) are shown in Figures 9-12.

8.6. Typical Chromatograms

Typical chromatograms of a 0.5-ng/mL calibration standard, a control lettuce sample, a control lettuce sample fortified at 0.01 µg/g (limit of quantitation), and a control lettuce sample fortified at 10 µg/g (1000 times the limit of quantitation) are presented in Figures 13-16. Typical chromatograms generated using the confirmatory HPLC column are presented in Figures 17-20.

9. DETERMINATION OF RECOVERY OF SPINOSAD AND ITS METABOLITES IN AGRICULTURAL COMMODITIES

9.1. Method Validation Prior to Field Sample Analysis

Unless otherwise specified, a sample set should contain, at the minimum, the following samples:

At least one reagent blank
At least one control
At least one control fortified at the limit of detection
At least two controls fortified at the limit of quantitation
At least two controls fortified at a higher concentration

9.2. Sample Preparation

Prepare samples for analysis by freezing the crop with dry ice or liquid nitrogen and then grinding or chopping with a hammer mill equipped with a 1/8 or 3/16-inch screen size.

9.3. Sample Analysis for Spinosad and Metabolites in Agricultural Commodities

9.3.1. Weigh 5 ± 0.05-g portions of sample into 250-mL HDPE bottles.

9.3.2. Add the required volume of the appropriate fortification solution to the recovery samples (Section 7.1.2.) using a positive displacement pipet.

9.3.3. Add 100 mL of acetonitrile/water (80:20).

9.3.4. Homogenize the samples with a 20-mm homogenizer generator for 1 minute. Cap the sample and shake for 30 minutes on a flat-bed shaker at approximately 180 excursions per minute.

9.3.5. Centrifuge the sample for 5 minutes at 2000 rpm.

9.3.6. Pipet 500 µL of the extraction solution into a 96-well plate.

9.3.7. Add 25 µL of the 0.1-µg/mL mixed XDE-175 and metabolite stable isotope standard (Section 7.2.3.) to each sample.

9.3.8. Add approximately 500 µL of each calibration standard (Section 7.3.1.) to empty wells of the 96-well plate, cap and vortex mix for approximately 30 seconds.

9.3.9. Chromatograph the samples and standard using the conditions given in Section 8, injecting the calibration standards evenly spaced throughout the run.

9.3.10. For sample extracts which contain spinosad and metabolite concentrations > 50 ng/mL (equivalent to >1 µg/g), dilute with acetonitrile:water (80:20) containing 5 ng/mL of mixed XDE-175 and metabolite stable isotope standard (Section 7.2.4.). Determine the suitability of the chromatographic system using the following criteria:

a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration.

b. Peak resolution: Determine visually that sufficient resolution has been achieved for the analyte relative to any background interferences.

c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 13-16 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the 0.5-ng/mL calibration standard (equivalent to 0.01 µg/g of spinosad and/or metabolites in the crop sample).

10. CALCULATIONS

10.1. Calculation of Standard Calibration Curve for Spinosad and its Metabolites

10.1.1. Inject a series of calibration standards (Section 7.3.) using the conditions described in Section 8 and determine the peak areas for spinosad, its metabolites and internal standards as indicated below:

Spinosyn A	*m/z* Q1/Q3 732.5/142.1
Spinosyn D	*m/z* Q1/Q3 746.5/142.1
Spinosyn B	*m/z* Q1/Q3 718.5/128.1
N-Demethyl Spinosyn D	*m/z* Q1/Q3 732.5/128.1
XDE-175-J IS	*m/z* Q1/Q3 757.9/146.2
XDE-175-L IS	*m/z* Q1/Q3 769.9/146.2
XDE-175-*N*-demethyl-J IS	*m/z* Q1/Q3 739.9/128.2
XDE-175-*N*-demethyl-L IS	*m/z* Q1/Q3 751.7/128.2

Quantitation of spinosyn A, spinosyn D, spinosyn B, and *N*-demethyl spinosyn D are performed using the XDE-175-J IS, XDE-175-L IS, XDE-175-*N*-demethyl-L IS, and XDE-175-*N*-demethyl-J IS as internal standards, respectively.

10.1.2. For each standard, calculate the spinosad quantitation ratio.

For example, using the data for spinosyn A from injection no. 15 in set 040107 S02, displayed in Figures 9 and 13:

$$\text{Quantitation Ratio} = \frac{\text{peak area of quantitation ion}}{\text{peak area of internal standard ion}}$$

$$\text{Quantitation Ratio} = \frac{\text{Spinosyn A peak area}}{\text{XDE - 175 - J IS stable isotope internal standard peak area}}$$

$$\text{Quantitation Ratio} = \frac{5600}{75420}$$

$$\text{Quantitation Ratio} = 0.0742$$

10.1.3. Prepare a standard curve by plotting the concentration of the analytes on the abscissa (x-axis) and the respective quantitation ratio on the ordinate (y-axis), as shown in Figures 9-12. Using linear regression analysis (13.1.) with a 1/x weighting (13.2.), determine the equation for the curve with respect to the abscissa (Note 12.4.).

For example, using the spinosyn A data from Figure 9:

$$X = \left(\frac{Y - \text{intercept}}{\text{slope}}\right)$$

$$\text{Spinosyn A (ng/mL)} = \left(\frac{\text{Spinosyn A quantitation ratio} - \text{intercept}}{\text{slope}}\right)$$

$$\text{Spinosyn A (ng/mL)} = \left(\frac{\text{Spinosyn A quantitation ratio} - (-0.0077)}{0.1627}\right)$$

10.2. Calculation of Percent Recovery for Spinosad and its Metabolites

10.2.1. Determine the gross concentration in each recovery sample by substituting the quantitation ratio obtained into the above equation and solving for the concentration.

For example, using the data for spinosyn A data from injection no. 14, Figure 13:

$$\text{Spinosyn A conc. (ng/mL)} = \left(\frac{\text{SpinosynA quantitation ratio} - (-0.0077)}{0.1627}\right)$$

$$\text{Spinosyn A conc. (grocss ng/mL)} = \left(\frac{0.0727 - (-0.0077)}{0.1627}\right)$$

$$\text{Spinosyn A conc. (gross)} = 0.4942 \text{ ng/mL}$$

Convert the concentration of ng/mL of spinosyn A found in the final sample extract prepared for analysis to µg/g of spinosyn A in the original crop sample as follows:

$$\text{Spinosyn A conc. (gross ng/g)} = 0.4942 \text{ ng/mL} \times \frac{\text{ext. vol. (mL)} \times \text{final vol. (mL)}}{\text{init. aliquot (mL)} \times \text{mass (g)}} \times \text{DF}$$

Where DF = Dilution Factor where applicable

$$\text{Spinosyn A conc. (gross ng/g)} = 0.4942 \text{ ng/mL} \times \frac{100 \text{ mL} \times 0.5 \text{ mL}}{0.5 \text{ mL} \times 5 \text{ g}} \times 1$$

$$\text{Spinosyn A conc. (gross)} = 9.88 \text{ ng/g or } 0.0099 \text{ µg/g}$$

10.2.2. Determine the net concentration in each recovery sample by subtracting the concentration found at the retention time of each analyte in the untreated control sample from that of the gross analyte concentration in the recovery sample.

For example, using the data for spinosyn A from Figure 13:

$$\text{Spinosyn A conc. (net µg/g)} = \text{Spinosyn A conc. (gross µg/g)} - \text{Spinosyn A conc. (control µg/g)}$$

$$\text{Spinosyn A conc. (net µg/g)} = 0.0099\ \mu g/g - 0.0011\ \mu g/g$$

$$\text{Spinosyn A conc. (net)} = 0.0088\ \mu g/g$$

10.2.3. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{conc. found}}{\text{conc. added}} \times 100\%$$

$$\text{Recovery} = \frac{0.0088\ \mu g/g}{0.010\ \mu g/g} \times 100\%$$

$$\text{Recovery} = 88\%$$

10.3. Determination of Spinosad and its Metabolites in Agricultural Commodities

10.3.1. Determine the gross concentration of spinosad and its metabolites in each sample by substituting the respective quantitation ratio into the equation for the calibration curve and calculating the uncorrected residue result as described in Section 10.2.1.

10.3.2. For those samples that require correction for the method procedural recovery, use the average recovery of all the recovery samples at or above the limit of quantitation, as described in Section 9.1, from a given sample set to correct for method efficiency. For example, continuing with the data from Figure 13 and the average recovery from Table 2 for the samples analyzed on 09-Nov-2004:

$$\text{Spinosyn A conc. (corrected µg/g)} = \text{Spinosyn A conc. (gross µg/g)} \times \left(\frac{100}{\text{Average \% Recovery}} \right)$$

$$\text{Spinosyn A conc. (corrected µg/g)} = 0.0088\ \mu g/g \times \frac{100}{98}$$

Spinosyn A conc. (corrected) = 0.0090 µg/g

11. RESULTS AND DISCUSSION

11.1. Method Validation

11.1.1. Recovery Levels and Precision

A method validation study was conducted to determine the recovery levels and the precision of the method for the determination of spinosad and its metabolites in agricultural commodities. Individual results are outlined in Tables 2-13 and are summarized in Tables 14-16.

For all of the analytes, the individual recoveries for all accepted samples in the validation study were between 74 and 119% with standard deviations less than or equal to 10.6%. For all analyses, the average recoveries for each analyte at each fortification level were between 70 and 110% except for the spinosyn B average recovery for dry crops (grass forage) which was 111%.

11.1.2. Standard Curve Linearity

For the linear regression analysis, the coefficients of determination (r^2) were greater or equal to 0.995 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.

11.1.3. Calculated Limits of Quantitation and Detection

Following established guidelines (13.3.), the limits of quantitation (LOQ) and detection (LOD) were calculated for spinosad and its metabolites using the standard deviation for the 0.01-µg/g (LOQ) recovery results. The LOQ was calculated as ten times the standard deviation (10*s*), and the LOD was calculated as three times the standard deviation (3*s*) of the LOQ results. The results are summarized in Tables 17-19.

The calculated method LOQ's support the validated LOQ of 0.01 µg/g for each analyte in each crop group. Since the lowest level of fortification for recovery samples was 0.01 µg/g, the method LOQ is considered to be 0.01 µg/g. The calculated method LOD's all support the validated LOD of 0.003 µg/g as well. The LOD is considered to be 0.003 µg/g. In actual residue samples, numerical results should be reported as less than the LOQ (<0.01 µg/g) for residues that are greater or equal to the LOD but less than the validated LOQ. For results less than the LOD, numerical results should be reported as not detected (ND).

11.2. Confirmation of Residue Identity

The presence of spinosad and its metabolites is confirmed by comparing the liquid chromatography retention times of the analyte in the calibration standards with those found in the samples while monitoring analyte specific MS/MS transitions. According to published guidelines (13.4.), when detection is performed by tandem mass spectrometry methods, confirmation of the presence of the analyte requires the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. Due to the lack of confirmatory MS/MS transitions, further confirmation of residue identity can be achieved, if necessary, by re-injecting the sample on the different selectivity column described in Section 8.

11.3. Assay Time and Stopping Points

A typical analytical run would consist of a minimum of eight standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of three fortified controls (two of which must be at the LOQ), and 15 samples. This typical analytical set can be prepared in approximately 3 hours followed by the chromatographic analysis.

There are four acceptable "stopping points" in the method, where sample preparation (Section 9) may be suspended, upon completion of a step, without deleterious effects on the sample analysis. These are indicated below:

Step 9.3.1. (store frozen)
Step 9.3.4. (store refrigerated)
Step 9.3.7. (store refrigerated)
Step 9.3.8. (store refrigerated)

12. NOTES

12.1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

12.2. Section 7 provides suggested concentrations for the preparation of fortification and calibration standards. Other dilution schemes may be followed.

12.3. Operating conditions may be modified to obtain optimal chromatographic separation and performance, if necessary.

12.4. The type of regression model can be chosen to give the best fit for the data.

13. REFERENCES

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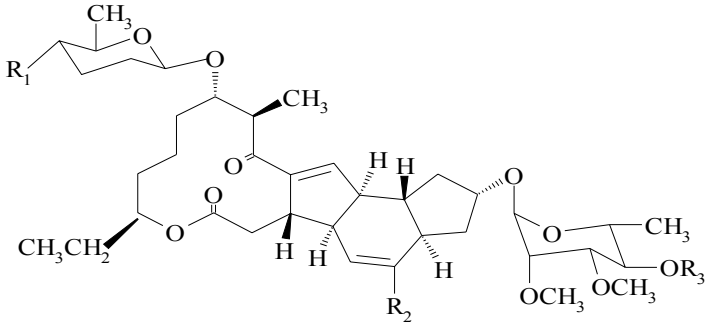
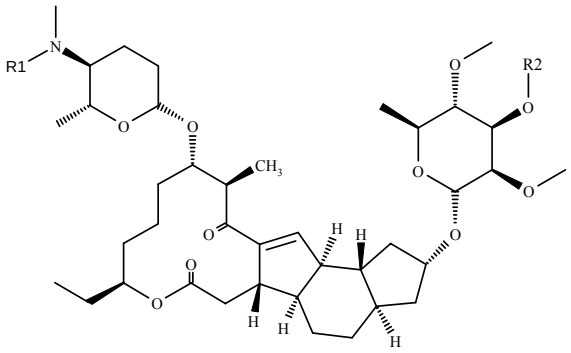
Table 1. Identity and Structure of Spinosad, its Metabolites, and XDE-175 Stable Isotope Internal Standards



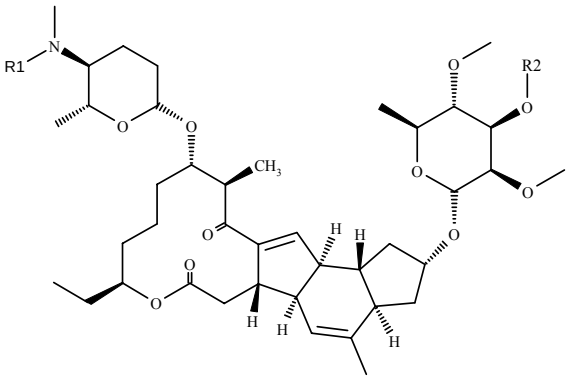
Spinosyn A, $R_1 = N(CH_3)_2$, $R_2 = H$, $R_3 = CH_3$
Spinosyn D, $R_1 = N(CH_3)_2$, $R_2 = CH_3$, $R_3 = CH_3$
Spinosyn B, $R_1 = N(CH_3)$, $R_2 = H$, $R_3 = CH_3$
N-demethyl Spinosyn D, $R_1 = N(CH_3)$, $R_2 = CH_3$, $R_3 = CH_3$

Common Name of Compound
Spinosyn A Molecular Formula: $C_{41}H_{65}NO_{10}$ Formula Weight: 731.9615 Nominal Mass: 731.5 CAS Registry Number: 131929-60-7 CAS Name: 2-[(6-deoxy-2,3,4-tri-O-methyl-(alpha)-L-mannopyranosyl)oxy]-13-[[(2R,5S,6R)-5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-, (2R,3aS,5aR,5bS,9S,13S,14R,16aS,16bR)-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione
Spinosyn D Molecular Formula: $C_{42}H_{67}NO_{10}$ Formula Weight: 745.988 Nominal Mass: 745.5 CAS Registry Number: 131929-63-0 CAS Name: 2-[(6-deoxy-2,3,4-tri-O-methyl-(alpha)-L-mannopyranosyl)oxy]-13-[[(2R,5S,6R)-5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-, (2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bS)-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione
Spinosyn B Molecular Formula: $C_{40}H_{63}NO_{10}$ Formula Weight: 717.9347 Nominal Mass: 717.4 CAS Registry Number: 131929-61-8 CAS Name: 2-[(6-deoxy-2,3,4-tri-O-methyl-alpha-L-mannopyranosyl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-13-[(tetrahydro-6-methyl-5-(methylamino)-2H-pyran-2-yl)oxy]-1H-as-indaceno[3,2-d]oxacyclododecin-7,15-dione
N-Demethyl Spinosyn D Molecular Formula: $C_{41}H_{65}NO_{10}$ Formula Weight: 731.9615 Nominal Mass: 731.5 CAS Registry Number: 149439-70-3 CAS Name: 2-[(6-deoxy-2,3,4-tri-O-methyl-alpha-L-mannopyranosyl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-13-[(tetrahydro-6-methyl-5-(methylamino)-2H-pyran-2-yl)-oxy]-1H-as-indaceno [3,2-d]oxacyclododecin-7,15-dione

Table 1. (Cont.) Identity and Structure of Spinosad, its Metabolites, and XDE-175 Stable Isotope Internal Standards



XDE-175-J, R1 = $^{13}CD_3$, R2 = C_2D_5
XDE-175-*N*-Demethyl-J, R1 = H, R2 = C_2D_5



XDE-175-L, R1 = $^{13}CD_3$, R2 = C_2D_5
XDE-175-*N*-Demethyl-L, R1 = H, R2 = C_2D_5

Common Name of Internal Standard	
XDE-175-J IS	
Molecular Formula:	$C_{41}{}^{13}CH_{61}D_8NO_{10}$
Formula Weight:	757.051
Nominal Mass:	756.5
CAS Registry Number:	N/A
XDE-175-L IS	
Molecular Formula:	$C_{42}{}^{13}CH_{61}D_8NO_{10}$
Formula Weight:	769.062
Nominal Mass:	768.5
CAS Registry Number:	N/A
XDE-175-*N*-Demethyl-J IS	
Molecular Formula:	$C_{41}H_{62}D_5NO_{10}$
Formula Weight:	739.014
Nominal Mass:	738.5
CAS Registry Number:	N/A
XDE-175-*N*-Demethyl-L IS	
Molecular Formula:	$C_{42}H_{62}D_5NO_{10}$
Formula Weight:	751.025
Nominal Mass:	750.5
CAS Registry Number:	N/A

Table 2. Recovery of Spinosyn A from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn A, µg/g		% Recovery[b]
			Added	Found	
15153	Orange Whole fruit	8-Nov-2004	NA[c]	0.0004	NA
17889	Tomato Fruit	8-Nov-2004	NA	0.0009	NA
20373	Apple Fruit	8-Nov-2004	NA	0.0004	NA
15153	Orange Whole fruit	9-Nov-2004	NA	ND[d]	NA
17889	Tomato Fruit	9-Nov-2004	NA	0.0017	NA
20373	Apple Fruit	9-Nov-2004	NA	0.0010	NA
17889	Tomato Fruit	30-Nov-2004	NA	0.0040	NA
20373	Apple Fruit	30-Nov-2004	NA	0.0014	NA
15153	Orange Whole fruit	10-Dec-2004	NA	0.0003	NA
17889	Tomato Fruit	10-Dec-2004	NA	0.0009	NA
20373	Apple Fruit	10-Dec-2004	NA	0.0007	NA
15153	Orange Whole fruit	17-Dec-2004	NA	0.0007	NA
17889	Tomato Fruit	17-Dec-2004	NA	0.0008	NA
20373	Apple Fruit	17-Dec-2004	NA	0.0007	NA
15153	Orange Whole fruit	21-Dec-2004	NA	0.0007	NA
17889	Tomato Fruit	21-Dec-2004	NA	0.0013	NA
20373	Apple Fruit	21-Dec-2004	NA	0.0003	NA
20373	Apple Fruit	8-Nov-2004	0.003	0.0035[e]	NA
20373	Apple Fruit	9-Nov-2004	0.003	0.0025	NA
20373	Apple Fruit	30-Nov-2004	0.003	0.0023	NA
20373	Apple Fruit	10-Dec-2004	0.003	0.0028	NA
20373	Apple Fruit	17-Dec-2004	0.003	0.0021	NA
20373	Apple Fruit	21-Dec-2004	0.003	0.0030	NA
15153	Orange Whole fruit	8-Nov-2004	0.01	0.0100	100
20373	Apple Fruit	8-Nov-2004	0.01	0.0095	95
15153	Orange Whole fruit	9-Nov-2004	0.01	0.0106	106
17889	Tomato Fruit	9-Nov-2004	0.01	0.0090	90
20373	Apple Fruit	9-Nov-2004	0.01	0.0097	97
17889	Tomato Fruit	30-Nov-2004	0.01	0.0074	74
20373	Apple Fruit	30-Nov-2004	0.01	0.0093	93
15153	Orange Whole fruit	10-Dec-2004	0.01	0.0117	117
17889	Tomato Fruit	10-Dec-2004	0.01	0.0088	88
20373	Apple Fruit	10-Dec-2004	0.01	0.0111	111
15153	Orange Whole fruit	17-Dec-2004	0.01	0.0097	97
17889	Tomato Fruit	17-Dec-2004	0.01	0.0110	110
20373	Apple Fruit	17-Dec-2004	0.01	0.0109	109
15153	Orange Whole fruit	21-Dec-2004	0.01	0.0096	96
17889	Tomato Fruit	21-Dec-2004	0.01	0.0090	90
20373	Apple Fruit	21-Dec-2004	0.01	0.0101	101

Table 2. (Cont.) Recovery of Spinosyn A from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis	Spinosyn A, µg/g		% Recovery
			Added	Found	
20373	Apple Fruit	8-Nov-2004	0.1	0.1096	110
20373	Apple Fruit	9-Nov-2004	0.1	0.1007	101
20373	Apple Fruit	30-Nov-2004	0.1	0.1048	105
20373	Apple Fruit	10-Dec-2004	0.1	0.1054	105
20373	Apple Fruit	17-Dec-2004	0.1	0.1039	104
20373	Apple Fruit	21-Dec-2004	0.1	0.1017	102
15153	Orange Whole fruit	8-Nov-2004	1.0	1.0531	105
17889	Tomato Fruit	8-Nov-2004	1.0	1.0335	103
20373	Apple Fruit	8-Nov-2004	1.0	1.1053	111
15153	Orange Whole fruit	9-Nov-2004	1.0	0.9981	100
17889	Tomato Fruit	9-Nov-2004	1.0	0.9507	95
20373	Apple Fruit	9-Nov-2004	1.0	0.9472	95
17889	Tomato Fruit	30-Nov-2004	1.0	1.0069	101
20373	Apple Fruit	30-Nov-2004	1.0	1.0607	106
15153	Orange Whole fruit	10-Dec-2004	1.0	1.0503	105
17889	Tomato Fruit	10-Dec-2004	1.0	1.0331	103
20373	Apple Fruit	10-Dec-2004	1.0	1.0100	101
15153	Orange Whole fruit	17-Dec-2004	1.0	1.0259	103
17889	Tomato Fruit	17-Dec-2004	1.0	1.0498	105
20373	Apple Fruit	17-Dec-2004	1.0	1.0413	104
15153	Orange Whole fruit	21-Dec-2004	1.0	1.0255	103
17889	Tomato Fruit	21-Dec-2004	1.0	1.0246	102
20373	Apple Fruit	21-Dec-2004	1.0	1.0205	102

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

[d] Not Detected.

[e] Samples were fortified at the method's proposed limit of detection (0.003 µg/g). Values are reported with a lower degree of confidence than values above the limit of quantitation.

Table 3. Recovery of Spinosyn D from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn D, µg/g		% Recovery[b]
			Added	Found	
15153	Orange Whole fruit	8-Nov-2004	NA[c]	ND[d]	NA
17889	Tomato Fruit	8-Nov-2004	NA	ND	NA
20373	Apple Fruit	8-Nov-2004	NA	ND	NA
15153	Orange Whole fruit	9-Nov-2004	NA	ND	NA
17889	Tomato Fruit	9-Nov-2004	NA	ND	NA
20373	Apple Fruit	9-Nov-2004	NA	ND	NA
17889	Tomato Fruit	30-Nov-2004	NA	0.0015	NA
20373	Apple Fruit	30-Nov-2004	NA	0.0010	NA
15153	Orange Whole fruit	10-Dec-2004	NA	ND	NA
17889	Tomato Fruit	10-Dec-2004	NA	ND	NA
20373	Apple Fruit	10-Dec-2004	NA	0.0001	NA
15153	Orange Whole fruit	17-Dec-2004	NA	0.0008	NA
17889	Tomato Fruit	17-Dec-2004	NA	0.0009	NA
20373	Apple Fruit	17-Dec-2004	NA	0.0007	NA
15153	Orange Whole fruit	21-Dec-2004	NA	ND	NA
17889	Tomato Fruit	21-Dec-2004	NA	ND	NA
20373	Apple Fruit	21-Dec-2004	NA	ND	NA
20373	Apple Fruit	8-Nov-2004	0.003	0.0039[e]	NA
20373	Apple Fruit	9-Nov-2004	0.003	0.0041	NA
20373	Apple Fruit	30-Nov-2004	0.003	0.0028	NA
20373	Apple Fruit	10-Dec-2004	0.003	0.0028	NA
20373	Apple Fruit	17-Dec-2004	0.003	0.0029	NA
20373	Apple Fruit	21-Dec-2004	0.003	0.0036	NA
15153	Orange Whole fruit	8-Nov-2004	0.01	0.0107	107
20373	Apple Fruit	8-Nov-2004	0.01	0.0110	110
15153	Orange Whole fruit	9-Nov-2004	0.01	0.0107	107
17889	Tomato Fruit	9-Nov-2004	0.01	0.0107	107
20373	Apple Fruit	9-Nov-2004	0.01	0.0102	102
17889	Tomato Fruit	30-Nov-2004	0.01	0.0097	97
20373	Apple Fruit	30-Nov-2004	0.01	0.0093	93
15153	Orange Whole fruit	10-Dec-2004	0.01	0.0106	106
17889	Tomato Fruit	10-Dec-2004	0.01	0.0094	94
20373	Apple Fruit	10-Dec-2004	0.01	0.0113	113
15153	Orange Whole fruit	17-Dec-2004	0.01	0.0100	100
17889	Tomato Fruit	17-Dec-2004	0.01	0.0098	98
20373	Apple Fruit	17-Dec-2004	0.01	0.0104	104
15153	Orange Whole fruit	21-Dec-2004	0.01	0.0101	101
17889	Tomato Fruit	21-Dec-2004	0.01	0.0107	107
20373	Apple Fruit	21-Dec-2004	0.01	0.0100	100

Table 3. (Cont.) Recovery of Spinosyn D from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis	Spinosyn D, µg/g Added	Spinosyn D, µg/g Found	% Recovery
20373	Apple Fruit	8-Nov-2004	0.1	0.1024	102
20373	Apple Fruit	9-Nov-2004	0.1	0.1009	101
20373	Apple Fruit	30-Nov-2004	0.1	0.1052	105
20373	Apple Fruit	10-Dec-2004	0.1	0.0981	98
20373	Apple Fruit	17-Dec-2004	0.1	0.1056	106
20373	Apple Fruit	21-Dec-2004	0.1	0.1005	101
15153	Orange Whole fruit	8-Nov-2004	1.0	1.0513	105
17889	Tomato Fruit	8-Nov-2004	1.0	1.0188	102
20373	Apple Fruit	8-Nov-2004	1.0	1.0512	105
15153	Orange Whole fruit	9-Nov-2004	1.0	1.0611	106
17889	Tomato Fruit	9-Nov-2004	1.0	0.9942	99
20373	Apple Fruit	9-Nov-2004	1.0	1.0015	100
17889	Tomato Fruit	30-Nov-2004	1.0	1.0610	106
20373	Apple Fruit	30-Nov-2004	1.0	1.0237	102
15153	Orange Whole fruit	10-Dec-2004	1.0	1.1458	115
17889	Tomato Fruit	10-Dec-2004	1.0	1.0949	109
20373	Apple Fruit	10-Dec-2004	1.0	0.9905	99
15153	Orange Whole fruit	17-Dec-2004	1.0	1.0615	106
17889	Tomato Fruit	17-Dec-2004	1.0	1.0639	106
20373	Apple Fruit	17-Dec-2004	1.0	1.0290	103
15153	Orange Whole fruit	21-Dec-2004	1.0	1.0407	104
17889	Tomato Fruit	21-Dec-2004	1.0	1.0470	105
20373	Apple Fruit	21-Dec-2004	1.0	1.0109	101

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

[d] Not Detected.

[e] Samples were fortified at the method's proposed limit of detection (0.003 µg/g). Values are reported with a lower degree of confidence than values above the limit of quantitation.

Table 4. Recovery of Spinosyn B from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn B, µg/g Added	Spinosyn B, µg/g Found	% Recovery[b]
15153	Orange Whole fruit	8-Nov-2004	NA[c]	0.0009	NA
17889	Tomato Fruit	8-Nov-2004	NA	0.0011	NA
20373	Apple Fruit	8-Nov-2004	NA	0.0011	NA
15153	Orange Whole fruit	9-Nov-2004	NA	0.0011	NA
17889	Tomato Fruit	9-Nov-2004	NA	0.0010	NA
20373	Apple Fruit	9-Nov-2004	NA	0.0008	NA
17889	Tomato Fruit	30-Nov-2004	NA	0.0016	NA
20373	Apple Fruit	30-Nov-2004	NA	0.0016	NA
15153	Orange Whole fruit	10-Dec-2004	NA	0.0011	NA
17889	Tomato Fruit	10-Dec-2004	NA	0.0011	NA
20373	Apple Fruit	10-Dec-2004	NA	0.0011	NA
15153	Orange Whole fruit	17-Dec-2004	NA	0.0014	NA
17889	Tomato Fruit	17-Dec-2004	NA	0.0013	NA
20373	Apple Fruit	17-Dec-2004	NA	0.0013	NA
15153	Orange Whole fruit	21-Dec-2004	NA	0.0013	NA
17889	Tomato Fruit	21-Dec-2004	NA	0.0013	NA
20373	Apple Fruit	21-Dec-2004	NA	0.0012	NA
20373	Apple Fruit	8-Nov-2004	0.003	0.0026[d]	NA
20373	Apple Fruit	9-Nov-2004	0.003	0.0025	NA
20373	Apple Fruit	30-Nov-2004	0.003	0.0021	NA
20373	Apple Fruit	10-Dec-2004	0.003	0.0027	NA
20373	Apple Fruit	17-Dec-2004	0.003	0.0026	NA
20373	Apple Fruit	21-Dec-2004	0.003	0.0026	NA
15153	Orange Whole fruit	8-Nov-2004	0.01	0.0099	99
20373	Apple Fruit	8-Nov-2004	0.01	0.0086	86
15153	Orange Whole fruit	9-Nov-2004	0.01	0.0098	98
17889	Tomato Fruit	9-Nov-2004	0.01	0.0096	96
20373	Apple Fruit	9-Nov-2004	0.01	0.0094	94
17889	Tomato Fruit	30-Nov-2004	0.01	0.0082	82
20373	Apple Fruit	30-Nov-2004	0.01	0.0095	95
15153	Orange Whole fruit	10-Dec-2004	0.01	0.0112	112
17889	Tomato Fruit	10-Dec-2004	0.01	0.0091	91
20373	Apple Fruit	10-Dec-2004	0.01	0.0105	105
15153	Orange Whole fruit	17-Dec-2004	0.01	0.0097	97
17889	Tomato Fruit	17-Dec-2004	0.01	0.0091	91
20373	Apple Fruit	17-Dec-2004	0.01	0.0104	104
15153	Orange Whole fruit	21-Dec-2004	0.01	0.0096	96
17889	Tomato Fruit	21-Dec-2004	0.01	0.0094	94
20373	Apple Fruit	21-Dec-2004	0.01	0.0092	92
20373	Apple Fruit	8-Nov-2004	0.1	0.0933	93
20373	Apple Fruit	9-Nov-2004	0.1	0.1030	103
20373	Apple Fruit	30-Nov-2004	0.1	0.1076	108

Table 4. (Cont.) Recovery of Spinosyn B from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis	Spinosyn B, µg/g Added	Found	% Recovery
20373	Apple Fruit	10-Dec-2004	0.1	0.1041	104
20373	Apple Fruit	17-Dec-2004	0.1	0.1009	101
20373	Apple Fruit	21-Dec-2004	0.1	0.0986	99
15153	Orange Whole fruit	8-Nov-2004	1.0	1.0119	101
17889	Tomato Fruit	8-Nov-2004	1.0	0.9861	99
20373	Apple Fruit	8-Nov-2004	1.0	1.0136	101
15153	Orange Whole fruit	9-Nov-2004	1.0	1.0781	108
17889	Tomato Fruit	9-Nov-2004	1.0	1.0654	107
20373	Apple Fruit	9-Nov-2004	1.0	0.9787	98
17889	Tomato Fruit	30-Nov-2004	1.0	1.0767	108
20373	Apple Fruit	30-Nov-2004	1.0	1.1047	110
15153	Orange Whole fruit	10-Dec-2004	1.0	1.1610	116
17889	Tomato Fruit	10-Dec-2004	1.0	1.1027	110
20373	Apple Fruit	10-Dec-2004	1.0	1.0585	106
15153	Orange Whole fruit	17-Dec-2004	1.0	1.0512	105
17889	Tomato Fruit	17-Dec-2004	1.0	1.0789	108
20373	Apple Fruit	17-Dec-2004	1.0	1.0835	108
15153	Orange Whole fruit	21-Dec-2004	1.0	1.0926	109
17889	Tomato Fruit	21-Dec-2004	1.0	1.0779	108
20373	Apple Fruit	21-Dec-2004	1.0	1.0075	101

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

[d] Samples were fortified at the method's proposed limit of detection (0.003 µg/g). Values are reported with a lower degree of confidence than values above the limit of quantitation.

Table 5. Recovery of *N*-Demethyl Spinosyn D from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis[a]	*N*-Demethyl Spinosyn D, µg/g Added	Found	% Recovery[b]
15153	Orange Whole fruit	8-Nov-2004	NA[c]	0.0005	NA
17889	Tomato Fruit	8-Nov-2004	NA	0.0007	NA
20373	Apple Fruit	8-Nov-2004	NA	0.0005	NA
15153	Orange Whole fruit	9-Nov-2004	NA	0.0006	NA
17889	Tomato Fruit	9-Nov-2004	NA	0.0006	NA
20373	Apple Fruit	9-Nov-2004	NA	0.0006	NA
17889	Tomato Fruit	30-Nov-2004	NA	0.0012	NA
20373	Apple Fruit	30-Nov-2004	NA	0.0012	NA
15153	Orange Whole fruit	10-Dec-2004	NA	ND[d]	NA
17889	Tomato Fruit	10-Dec-2004	NA	ND	NA
20373	Apple Fruit	10-Dec-2004	NA	0.0012	NA
15153	Orange Whole fruit	17-Dec-2004	NA	0.0015	NA
17889	Tomato Fruit	17-Dec-2004	NA	0.0015	NA
20373	Apple Fruit	17-Dec-2004	NA	0.0014	NA
15153	Orange Whole fruit	21-Dec-2004	NA	0.0011	NA
17889	Tomato Fruit	21-Dec-2004	NA	0.0011	NA
20373	Apple Fruit	21-Dec-2004	NA	0.0011	NA
20373	Apple Fruit	8-Nov-2004	0.003	0.0026[e]	NA
20373	Apple Fruit	9-Nov-2004	0.003	0.0027	NA
20373	Apple Fruit	30-Nov-2004	0.003	0.0025	NA
20373	Apple Fruit	10-Dec-2004	0.003	0.0026	NA
20373	Apple Fruit	17-Dec-2004	0.003	0.0027	NA
20373	Apple Fruit	21-Dec-2004	0.003	0.0027	NA
15153	Orange Whole fruit	8-Nov-2004	0.01	0.0090	90
20373	Apple Fruit	8-Nov-2004	0.01	0.0091	91
15153	Orange Whole fruit	9-Nov-2004	0.01	0.0101	101
17889	Tomato Fruit	9-Nov-2004	0.01	0.0097	97
20373	Apple Fruit	9-Nov-2004	0.01	0.0089	89
17889	Tomato Fruit	30-Nov-2004	0.01	0.0092	92
20373	Apple Fruit	30-Nov-2004	0.01	0.0097	97
15153	Orange Whole fruit	10-Dec-2004	0.01	0.0113	113
17889	Tomato Fruit	10-Dec-2004	0.01	0.0100	100
20373	Apple Fruit	10-Dec-2004	0.01	0.0093	93
15153	Orange Whole fruit	17-Dec-2004	0.01	0.0092	92
17889	Tomato Fruit	17-Dec-2004	0.01	0.0088	88
20373	Apple Fruit	17-Dec-2004	0.01	0.0100	100
15153	Orange Whole fruit	21-Dec-2004	0.01	0.0095	95
17889	Tomato Fruit	21-Dec-2004	0.01	0.0094	94
20373	Apple Fruit	21-Dec-2004	0.01	0.0095	95
20373	Apple Fruit	8-Nov-2004	0.1	0.1025	103
20373	Apple Fruit	9-Nov-2004	0.1	0.1012	101

Table 5. (Cont.) Recovery of *N*-Demethyl Spinosyn D from Acidic Crops (Apples, Oranges, and Tomatoes)

Sample Number	Matrix	Date of Analysis	*N*-Demethyl Spinosyn D, µg/g Added	Found	% Recovery
20373	Apple Fruit	30-Nov-2004	0.1	0.1066	107
20373	Apple Fruit	10-Dec-2004	0.1	0.0980	98
20373	Apple Fruit	17-Dec-2004	0.1	0.1040	104
20373	Apple Fruit	21-Dec-2004	0.1	0.0978	98
15153	Orange Whole fruit	8-Nov-2004	1.0	0.9735	97
17889	Tomato Fruit	8-Nov-2004	1.0	0.9364	94
20373	Apple Fruit	8-Nov-2004	1.0	1.0492	105
15153	Orange Whole fruit	9-Nov-2004	1.0	1.0470	105
17889	Tomato Fruit	9-Nov-2004	1.0	0.9851	99
20373	Apple Fruit	9-Nov-2004	1.0	0.9789	98
17889	Tomato Fruit	30-Nov-2004	1.0	1.0432	104
20373	Apple Fruit	30-Nov-2004	1.0	1.0197	102
15153	Orange Whole fruit	10-Dec-2004	1.0	1.0558	106
17889	Tomato Fruit	10-Dec-2004	1.0	1.0932	109
20373	Apple Fruit	10-Dec-2004	1.0	1.0213	102
15153	Orange Whole fruit	17-Dec-2004	1.0	1.0814	108
17889	Tomato Fruit	17-Dec-2004	1.0	1.0538	105
20373	Apple Fruit	17-Dec-2004	1.0	1.0506	105
15153	Orange Whole fruit	21-Dec-2004	1.0	1.0740	107
17889	Tomato Fruit	21-Dec-2004	1.0	1.0454	105
20373	Apple Fruit	21-Dec-2004	1.0	1.0122	101

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

[d] Not Detected.

[e] Samples were fortified at the method's proposed limit of detection (0.003 µg/g). Values are reported with a lower degree of confidence than values above the limit of quantitation.

Table 6. Recovery of Spinosyn A from Wet Crops (Lettuce Leaves)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn A, µg/g		% Recovery[b]
			Added	Found	
15155	Lettuce Leaves	8-Nov-2004	NA[c]	0.0006	NA
15155	Lettuce Leaves	9-Nov-2004	NA	0.0011	NA
15155	Lettuce Leaves	30-Nov-2004	NA	0.0013	NA
15155	Lettuce Leaves	10-Dec-2004	NA	0.0005	NA
15155	Lettuce Leaves	17-Dec-2004	NA	0.0008	NA
15155	Lettuce Leaves	21-Dec-2004	NA	0.0006	NA
15155	Lettuce Leaves	9-Nov-2004	0.01	0.0088	88
15155	Lettuce Leaves	30-Nov-2004	0.01	0.0096	96
15155	Lettuce Leaves	10-Dec-2004	0.01	0.0095	95
15155	Lettuce Leaves	17-Dec-2004	0.01	0.0104	104
15155	Lettuce Leaves	21-Dec-2004	0.01	0.0096	96
15155	Lettuce Leaves	8-Nov-2004	10	9.6882	97
15155	Lettuce Leaves	9-Nov-2004	10	9.5897	96
15155	Lettuce Leaves	30-Nov-2004	10	10.2341	102
15155	Lettuce Leaves	10-Dec-2004	10	10.0248	100
15155	Lettuce Leaves	17-Dec-2004	10	9.5468	95

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

Table 7. Recovery of Spinosyn D from Wet Crops (Lettuce Leaves)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn D, µg/g		% Recovery[b]
			Added	Found	
15155	Lettuce Leaves	8-Nov-2004	NA[c]	ND[d]	NA
15155	Lettuce Leaves	9-Nov-2004	NA	ND	NA
15155	Lettuce Leaves	30-Nov-2004	NA	0.0012	NA
15155	Lettuce Leaves	10-Dec-2004	NA	ND	NA
15155	Lettuce Leaves	17-Dec-2004	NA	0.0007	NA
15155	Lettuce Leaves	21-Dec-2004	NA	ND	NA
15155	Lettuce Leaves	9-Nov-2004	0.01	0.0106	106
15155	Lettuce Leaves	30-Nov-2004	0.01	0.0094	94
15155	Lettuce Leaves	10-Dec-2004	0.01	0.0108	108
15155	Lettuce Leaves	17-Dec-2004	0.01	0.0111	111
15155	Lettuce Leaves	21-Dec-2004	0.01	0.0104	104
15155	Lettuce Leaves	8-Nov-2004	10	9.4897	95
15155	Lettuce Leaves	9-Nov-2004	10	9.7671	98
15155	Lettuce Leaves	30-Nov-2004	10	9.7551	98
15155	Lettuce Leaves	10-Dec-2004	10	9.5441	95
15155	Lettuce Leaves	17-Dec-2004	10	9.8640	99

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

[d] Not Detected.

Table 8. Recovery of Spinosyn B from Wet Crops (Lettuce Leaves)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn B, µg/g		% Recovery[b]
			Added	Found	
15155	Lettuce Leaves	8-Nov-2004	NA[c]	0.0011	NA
15155	Lettuce Leaves	9-Nov-2004	NA	0.0010	NA
15155	Lettuce Leaves	30-Nov-2004	NA	0.0015	NA
15155	Lettuce Leaves	10-Dec-2004	NA	0.0011	NA
15155	Lettuce Leaves	17-Dec-2004	NA	0.0014	NA
15155	Lettuce Leaves	21-Dec-2004	NA	0.0013	NA
15155	Lettuce Leaves	9-Nov-2004	0.01	0.0100	100
15155	Lettuce Leaves	30-Nov-2004	0.01	0.0093	93
15155	Lettuce Leaves	10-Dec-2004	0.01	0.0092	92
15155	Lettuce Leaves	17-Dec-2004	0.01	0.0100	100
15155	Lettuce Leaves	21-Dec-2004	0.01	0.0099	99
15155	Lettuce Leaves	8-Nov-2004	10	9.7086	97
15155	Lettuce Leaves	9-Nov-2004	10	10.3450	103
15155	Lettuce Leaves	30-Nov-2004	10	10.3397	103
15155	Lettuce Leaves	10-Dec-2004	10	10.1060	101
15155	Lettuce Leaves	17-Dec-2004	10	9.8659	99

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

Table 9. Recovery of *N*-Demethyl Spinosyn D from Wet Crops (Lettuce Leaves)

Sample Number	Matrix	Date of Analysis[a]	*N*-Demethyl Spinosyn D, µg/g Added	Found	% Recovery[b]
15155	Lettuce Leaves	8-Nov-2004	NA[c]	0.0005	NA
15155	Lettuce Leaves	9-Nov-2004	NA	0.0006	NA
15155	Lettuce Leaves	30-Nov-2004	NA	0.0011	NA
15155	Lettuce Leaves	10-Dec-2004	NA	0.0013	NA
15155	Lettuce Leaves	17-Dec-2004	NA	0.0014	NA
15155	Lettuce Leaves	21-Dec-2004	NA	0.0013	NA
15155	Lettuce Leaves	9-Nov-2004	0.01	0.0093	93
15155	Lettuce Leaves	30-Nov-2004	0.01	0.0098	98
15155	Lettuce Leaves	10-Dec-2004	0.01	0.0095	95
15155	Lettuce Leaves	17-Dec-2004	0.01	0.0095	95
15155	Lettuce Leaves	21-Dec-2004	0.01	0.0090	90
15155	Lettuce Leaves	8-Nov-2004	10	9.3970	94
15155	Lettuce Leaves	9-Nov-2004	10	10.0332	100
15155	Lettuce Leaves	30-Nov-2004	10	10.4881	105
15155	Lettuce Leaves	10-Dec-2004	10	10.5044	105
15155	Lettuce Leaves	17-Dec-2004	10	10.1697	102

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

Table 10. Recovery of Spinosyn A from Dry Crops (Grass Forage)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn A, µg/g		% Recovery[b]
			Added	Found	
14251	Grass Forage	9-Nov-2004	NA[c]	0.0011	NA
14251	Grass Forage	30-Nov-2004	NA	0.0059	NA
14243	Grass Forage	10-Dec-2004	NA	0.0006	NA
14243	Grass Forage	17-Dec-2004	NA	0.0011	NA
14243	Grass Forage	21-Dec-2004	NA	0.0007	NA
14251	Grass Forage	9-Nov-2004	0.01	0.0094	94
14243	Grass Forage	10-Dec-2004	0.01	0.0105	105
14243	Grass Forage	17-Dec-2004	0.01	0.0109	109
14243	Grass Forage	21-Dec-2004	0.01	0.0099	99
14251	Grass Forage	9-Nov-2004	1.0	0.9836	98
14251	Grass Forage	30-Nov-2004	1.0	1.1099	111
14243	Grass Forage	10-Dec-2004	1.0	1.0957	110
14243	Grass Forage	17-Dec-2004	1.0	1.1221	112
14243	Grass Forage	21-Dec-2004	1.0	1.0889	109

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

Table 11. Recovery of Spinosyn D from Dry Crops (Grass Forage)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn D, µg/g		% Recovery[b]
			Added	Found	
14251	Grass Forage	9-Nov-2004	NA[c]	ND[d]	NA
14251	Grass Forage	30-Nov-2004	NA	0.0019	NA
14243	Grass Forage	10-Dec-2004	NA	ND	NA
14243	Grass Forage	17-Dec-2004	NA	0.0009	NA
14243	Grass Forage	21-Dec-2004	NA	ND	NA
14251	Grass Forage	9-Nov-2004	0.01	0.0108	108
14243	Grass Forage	10-Dec-2004	0.01	0.0112	112
14243	Grass Forage	17-Dec-2004	0.01	0.0107	107
14243	Grass Forage	21-Dec-2004	0.01	0.0110	110
14251	Grass Forage	9-Nov-2004	1.0	1.0705	107
14251	Grass Forage	30-Nov-2004	1.0	1.1286	113
14243	Grass Forage	10-Dec-2004	1.0	1.0844	108
14243	Grass Forage	17-Dec-2004	1.0	1.1293	113
14243	Grass Forage	21-Dec-2004	1.0	1.0555	106

[a] The date of analysis represents the date the samples were extracted.
[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.
[c] Not Applicable.
[d] Not Detected.

Table 12. Recovery of Spinosyn B from Dry Crops (Grass Forage)

Sample Number	Matrix	Date of Analysis[a]	Spinosyn B, µg/g		% Recovery[b]
			Added	Found	
14251	Grass Forage	9-Nov-2004	NA[c]	0.0010	NA
14251	Grass Forage	30-Nov-2004	NA	0.0017	NA
14243	Grass Forage	10-Dec-2004	NA	0.0010	NA
14243	Grass Forage	17-Dec-2004	NA	0.0016	NA
14243	Grass Forage	21-Dec-2004	NA	0.0012	NA
14251	Grass Forage	9-Nov-2004	0.01	0.0103	103
14243	Grass Forage	10-Dec-2004	0.01	0.0100	100
14243	Grass Forage	17-Dec-2004	0.01	0.0104	104
14243	Grass Forage	21-Dec-2004	0.01	0.0108	108
14251	Grass Forage	9-Nov-2004	1.0	1.1148	111
14251	Grass Forage	30-Nov-2004	1.0	1.1471	115
14243	Grass Forage	10-Dec-2004	1.0	1.1846	118
14243	Grass Forage	17-Dec-2004	1.0	1.1935	119
14243	Grass Forage	21-Dec-2004	1.0	1.1761	118

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

Table 13. Recovery of *N*-Demethyl Spinosyn D from Dry Crops (Grass Forage)

Sample Number	Matrix	Date of Analysis[a]	*N*-Demethyl Spinosyn D, µg/g		
			Added	Found	% Recovery[b]
14251	Grass Forage	9-Nov-2004	NA[c]	0.0006	NA
14251	Grass Forage	30-Nov-2004	NA	0.0012	NA
14243	Grass Forage	10-Dec-2004	NA	0.0013	NA
14243	Grass Forage	17-Dec-2004	NA	0.0014	NA
14243	Grass Forage	21-Dec-2004	NA	0.0011	NA
14251	Grass Forage	9-Nov-2004	0.01	0.0093	93
14243	Grass Forage	10-Dec-2004	0.01	0.0092	92
14243	Grass Forage	17-Dec-2004	0.01	0.0091	91
14243	Grass Forage	21-Dec-2004	0.01	0.0089	89
14251	Grass Forage	9-Nov-2004	1.0	1.0481	105
14251	Grass Forage	30-Nov-2004	1.0	1.0599	106
14243	Grass Forage	10-Dec-2004	1.0	1.0325	103
14243	Grass Forage	17-Dec-2004	1.0	1.0623	106
14243	Grass Forage	21-Dec-2004	1.0	1.0337	103

[a] The date of analysis represents the date the samples were extracted.

[b] All calculations were performed using Microsoft Excel 2002 with full precision. Recovery values corrected for control value where appropriate.

[c] Not Applicable.

Table 14. Recovery Summary of Spinosad and its Metabolites from Acidic Crops (Apples, Oranges, and Tomatoes)

Compound	Fortification Level (µg/g)	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)	n
Spinosyn A	0.01	98	74 - 117	10.6	10.8	16
	0.10	104	101 - 110	3.2	3.0	6
	1.00	103	95 - 111	3.8	3.7	17
	0.01 – 1.00	101	74 - 117	7.6	7.5	39
Spinosyn D	0.01	103	93 - 113	5.7	5.6	16
	0.10	102	98 - 106	2.9	2.9	6
	1.00	104	99 - 115	3.9	3.7	17
	0.01 – 1.00	103	93 - 115	4.6	4.5	39
Spinosyn B	0.01	96	82 - 112	7.3	7.7	16
	0.10	101	93 - 108	4.9	4.9	6
	1.00	106	98 - 116	4.8	4.5	17
	0.01 – 1.00	101	82 - 116	7.5	7.5	39
N-Demethyl Spinosyn D	0.01	95	88 - 113	6.1	6.4	16
	0.10	102	98 - 107	3.4	3.4	6
	1.00	103	94 - 109	4.2	4.1	17
	0.01 – 1.00	100	88 - 113	6.1	6.1	39

Table 15. Recovery Summary of Spinosad and its Metabolites from Wet Crops (Lettuce Leaves)

Compound	Fortification Level (µg/g)	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)	n
Spinosyn A	0.01	96	88 - 104	5.8	6.0	5
	10	98	95 - 102	3.0	3.0	5
	0.01 - 10	97	88 - 104	4.5	4.6	10
Spinosyn D	0.01	105	94 - 111	6.4	6.1	5
	10	97	95 - 99	1.6	1.6	5
	0.01 - 10	101	94 - 111	6.1	6.0	10
Spinosyn B	0.01	96	92 - 100	3.9	4.1	5
	10	101	97 - 103	2.8	2.8	5
	0.01 - 10	99	92 - 103	3.9	4.0	10
N-Demethyl Spinosyn D	0.01	94	90 - 98	2.9	3.1	5
	10	101	94 - 105	4.5	4.5	5
	0.01 - 10	98	90 - 105	5.1	5.2	10

Table 16. Recovery Summary of Spinosad and its Metabolites from Dry Crops (Grass Forage)

Compound	Fortification Level (µg/g)	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)	n
Spinosyn A	0.01	102	94 - 109	6.8	6.6	4
	1.0	108	98 - 112	5.5	5.1	5
	0.01 - 1	105	94 - 112	6.6	6.3	9
Spinosyn D	0.01	109	107 - 112	2.2	2.0	4
	1.0	109	106 - 113	3.4	3.1	5
	0.01 - 1	109	106 - 113	2.7	2.5	9
Spinosyn B	0.01	104	100 - 108	3.1	3.0	4
	1.0	116	111 - 119	3.2	2.8	5
	0.01 - 1	111	100 - 119	7.3	6.6	9
N-Demethyl Spinosyn D	0.01	91	89 - 93	1.7	1.8	4
	1.0	105	103 - 106	1.4	1.3	5
	0.01 - 1	99	89 - 106	7.2	7.2	9

Table 17. Calculated Limits of Detection and Quantitation for Spinosad and its Metabolites in Acidic Crops (Apples, Oranges, and Tomatoes)

Compound	Average Recovery (µg/g)	Standard Deviation (*s*)	Calculated Limit of Detection (3*s*)	Calculated Limit of Quantitation (10*s*)	Number of Samples (n)
Spinosyn A	0.00985	0.00106	0.00318	0.01061	16
Spinosyn D	0.01028	0.00057	0.00172	0.00575	16
Spinosyn B	0.00958	0.00073	0.00220	0.00734	16
N-Demethyl Spinosyn D	0.00954	0.00061	0.00184	0.00615	16

Table 18. Calculated Limits of Detection and Quantitation for Spinosad and its Metabolites in Wet Crops (Lettuce Leaves)

Compound	Average Recovery (µg/g)	Standard Deviation (*s*)	Calculated Limit of Detection (3*s*)	Calculated Limit of Quantitation (10*s*)	Number of Samples (n)
Spinosyn A	0.00958	0.00058	0.00173	0.00575	5
Spinosyn D	0.01047	0.00064	0.00193	0.00643	5
Spinosyn B	0.00965	0.00039	0.00118	0.00393	5
N-Demethyl Spinosyn D	0.00944	0.00029	0.00088	0.00294	5

Table 19. Calculated Limits of Detection and Quantitation for Spinosad and its Metabolites in Dry Crops (Grass Forage)

Compound	Average Recovery (µg/g)	Standard Deviation (*s*)	Calculated Limit of Detection (3*s*)	Calculated Limit of Quantitation (10*s*)	Number of Samples (n)
Spinosyn A	0.01017	0.00068	0.00203	0.00676	4
Spinosyn D	0.01090	0.00022	0.00065	0.00217	4
Spinosyn B	0.01037	0.00031	0.00094	0.00315	4
N-Demethyl Spinosyn D	0.00914	0.00017	0.00050	0.00167	4

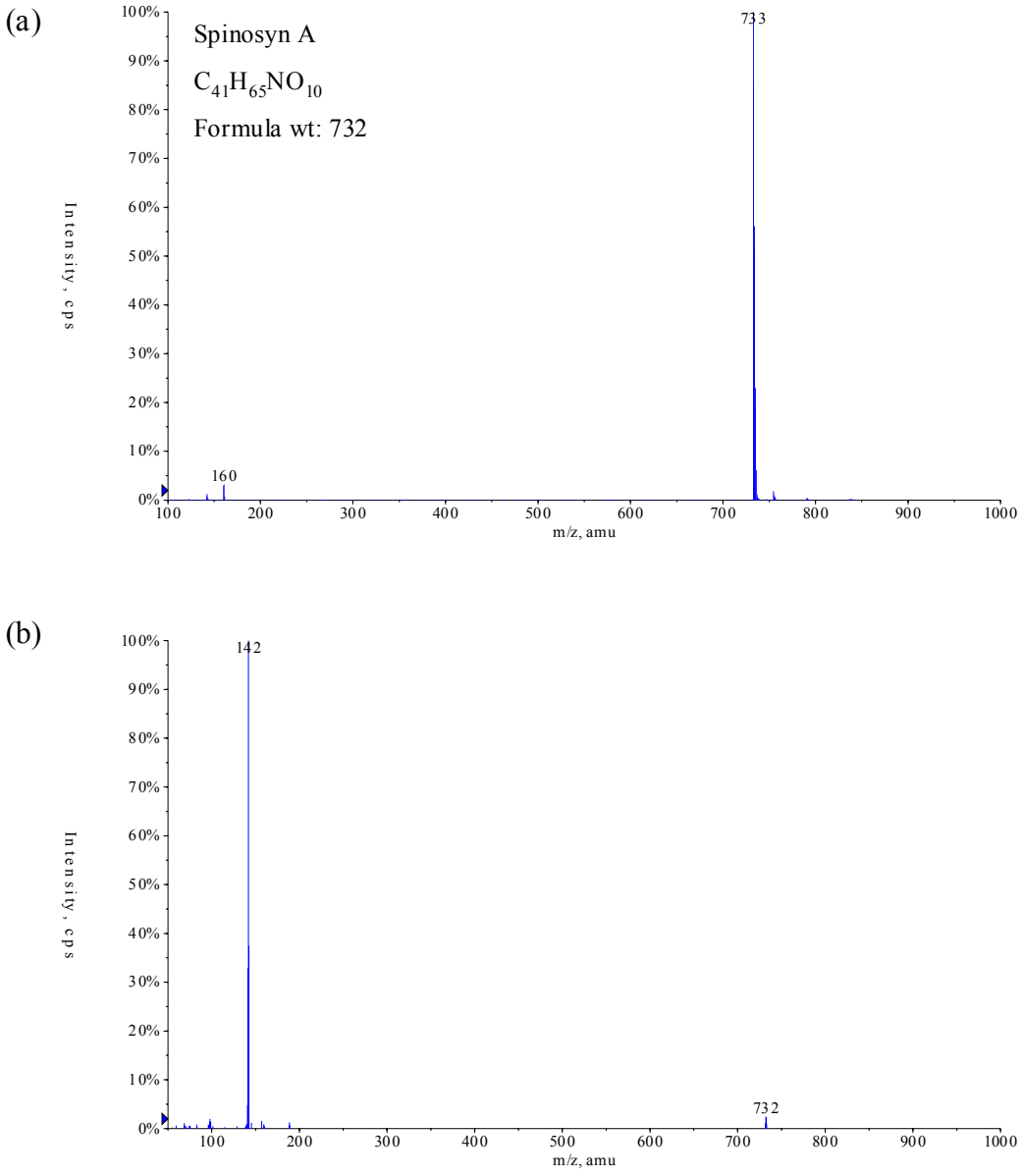


Figure 1. Mass Spectra for Spinosyn A: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 733 (b) Product-Ion Mass Spectrum of Spinosyn A Showing Fragment Ion at *m/z* 142

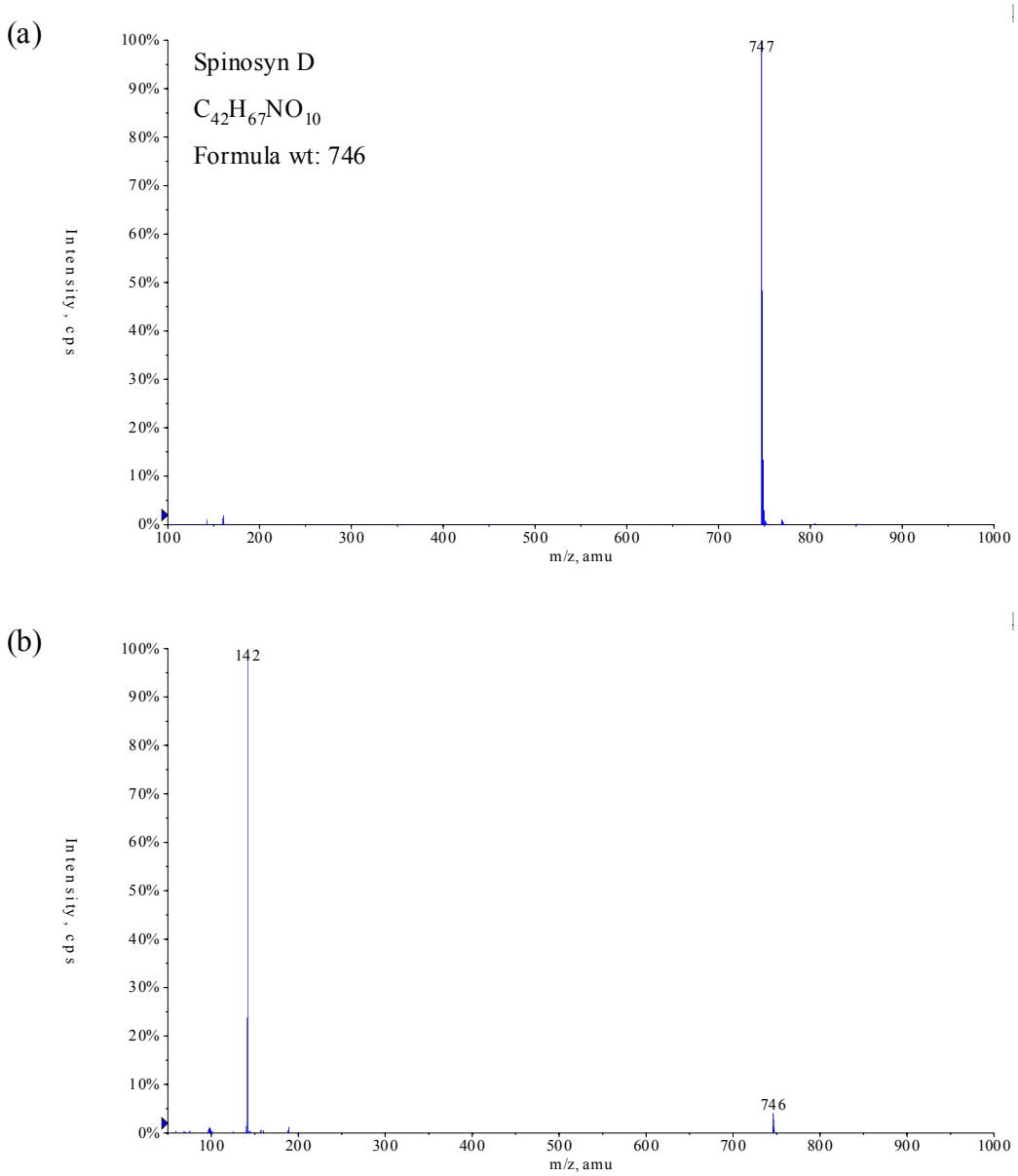


Figure 2. Mass Spectra for Spinosyn D: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 747 (b) Product-Ion Mass Spectrum of Spinosyn D Showing Fragment Ion at *m/z* 142

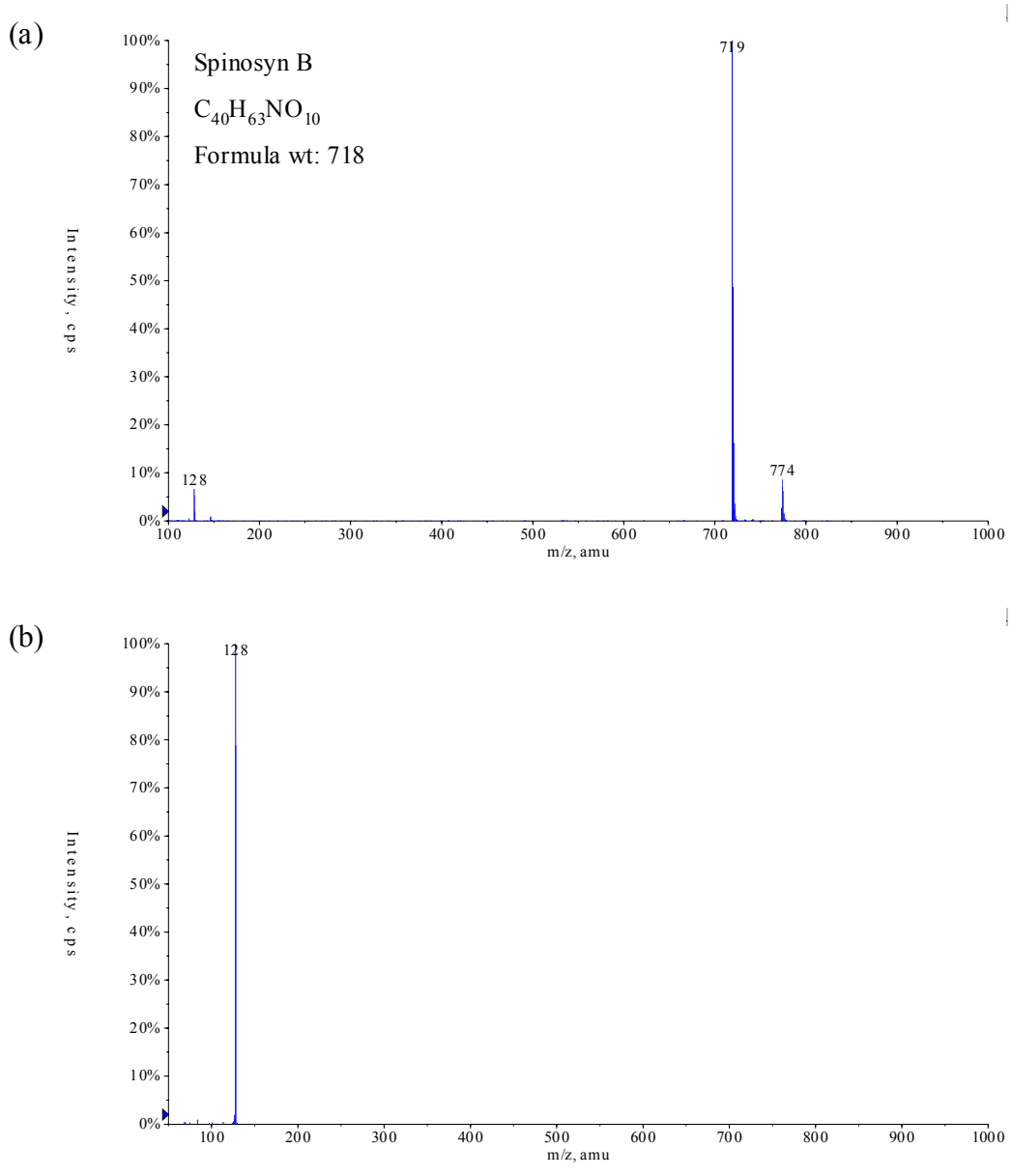


Figure 3. Mass Spectra for Spinosyn B: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 719 (b) Product-Ion Mass Spectrum of Spinosyn B Showing Fragment Ion at *m/z* 128

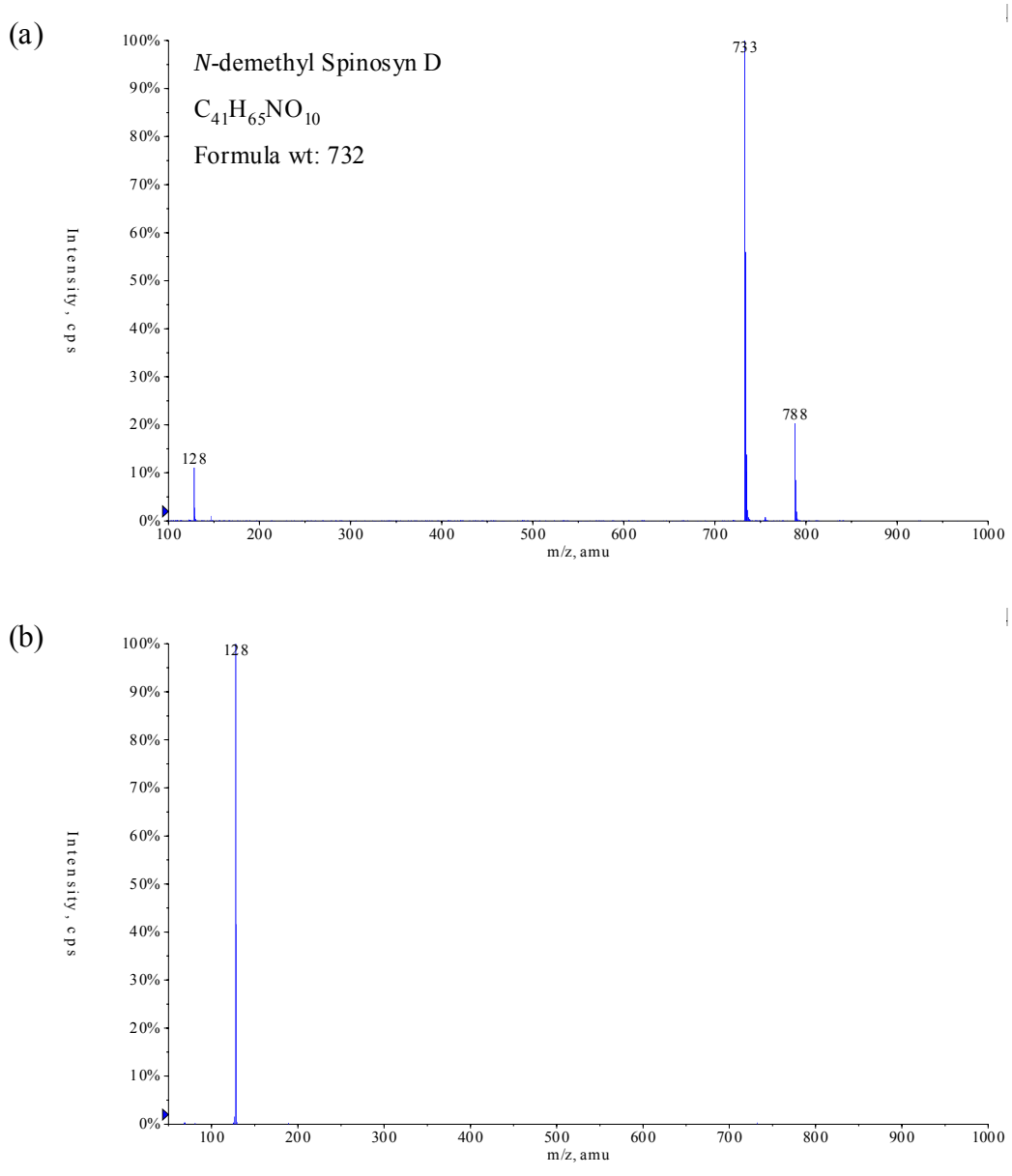


Figure 4. Mass Spectra for *N*-demethyl spinosyn D: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 733 (b) Product-Ion Mass Spectrum of *N*-demethyl spinosyn D Showing Fragment Ion at *m/z* 128

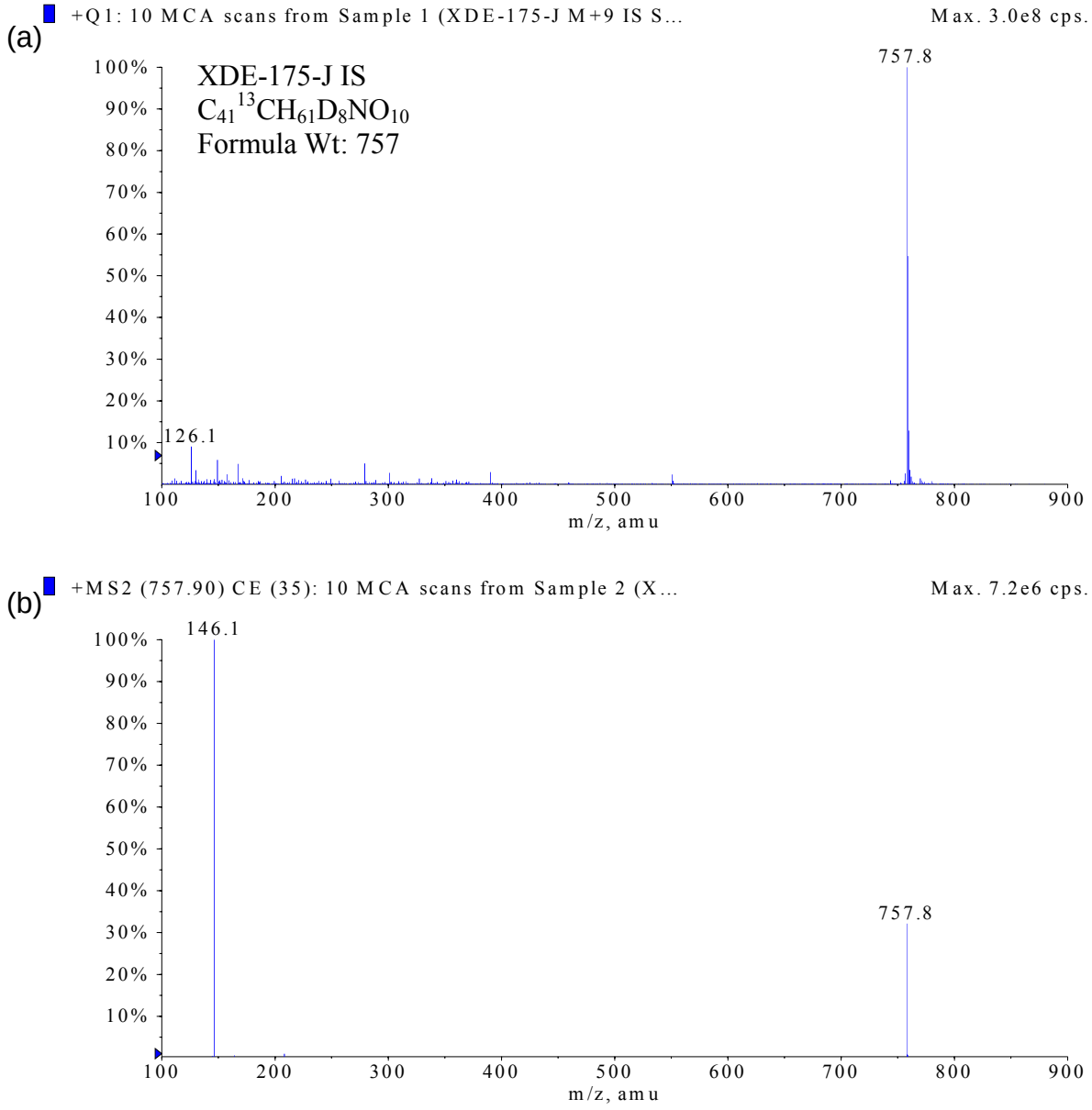


Figure 5. Mass Spectra for XDE-175-J Stable Isotope Internal Standard: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 757.8 (b) Product-Ion Mass Spectrum of XDE-175-J Stable Isotope Internal Standard Showing Fragment Ion at *m/z* 146.1

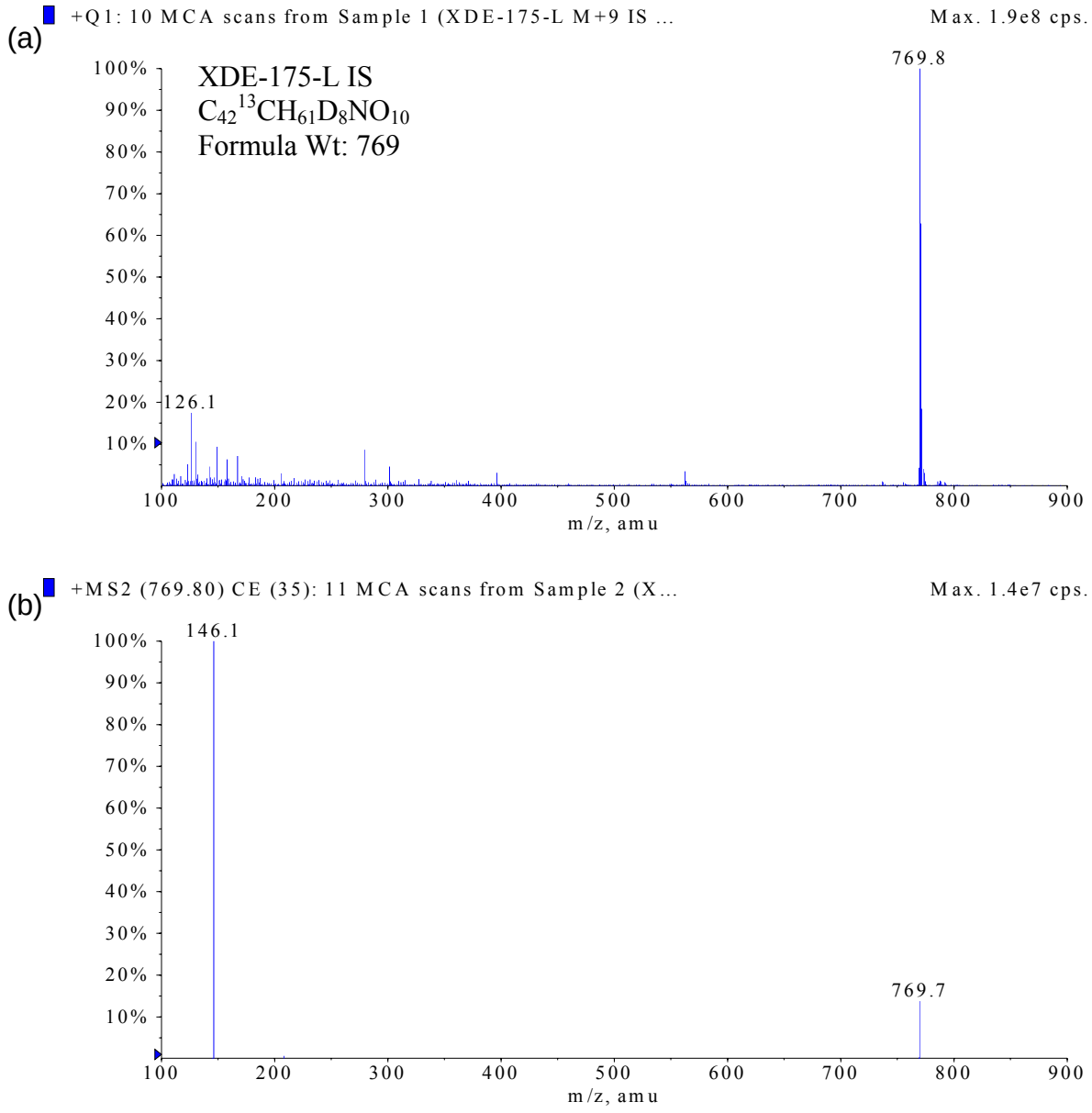


Figure 6. Mass Spectra for XDE-175-L Stable Isotope Internal Standard: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 769.8 (b) Product-Ion Mass Spectrum of XDE-175-L Stable Isotope Internal Standard Showing Fragment Ion at *m/z* 146.1

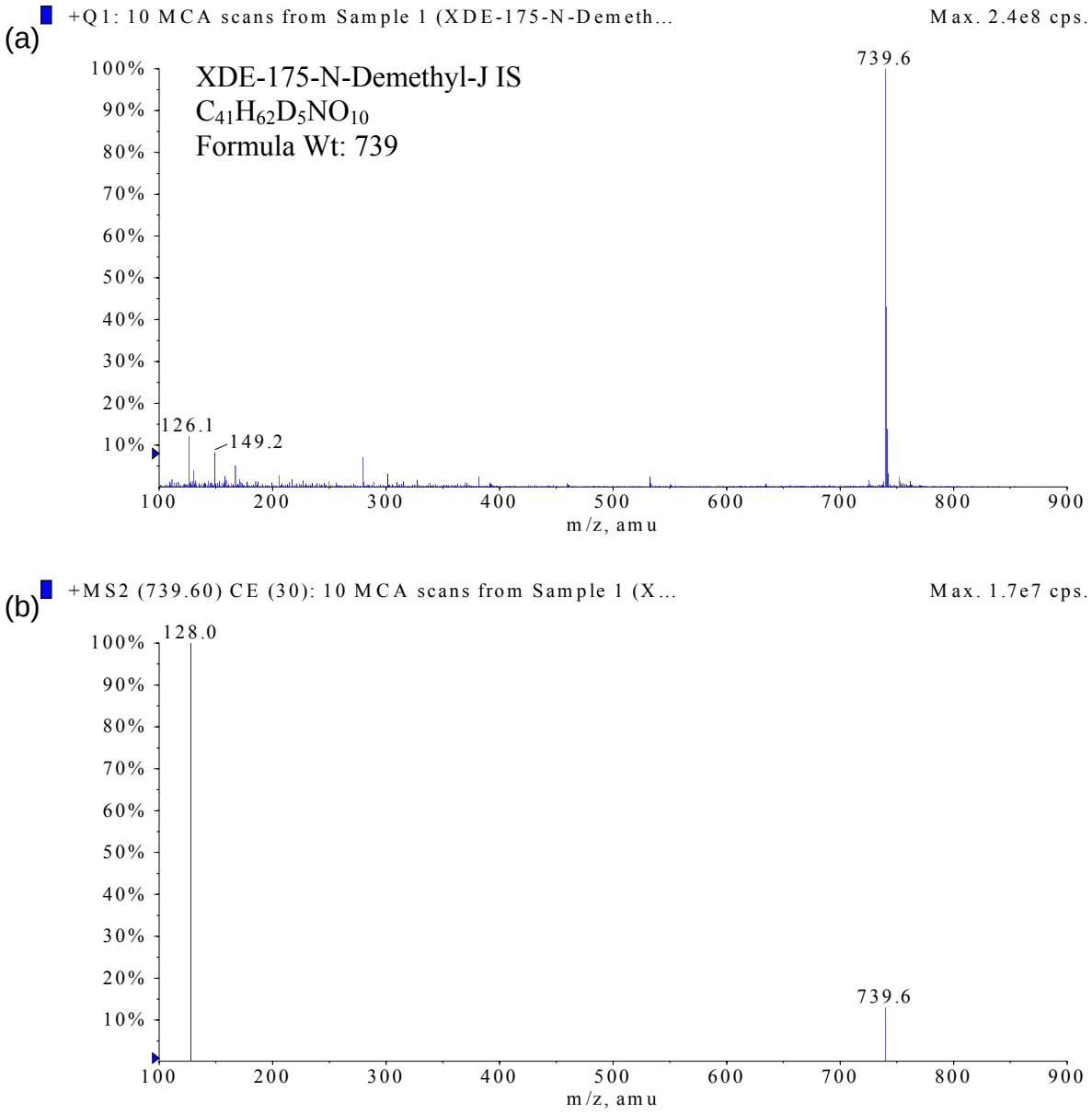


Figure 7. Mass Spectra for XDE-175-*N*-Demethyl-J Stable Isotope Internal Standard: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 739.6 (b) Product-Ion Mass Spectrum of XDE-175-*N*-Demethyl-J Stable Isotope Internal Standard Showing Fragment Ion at *m/z* 128.0

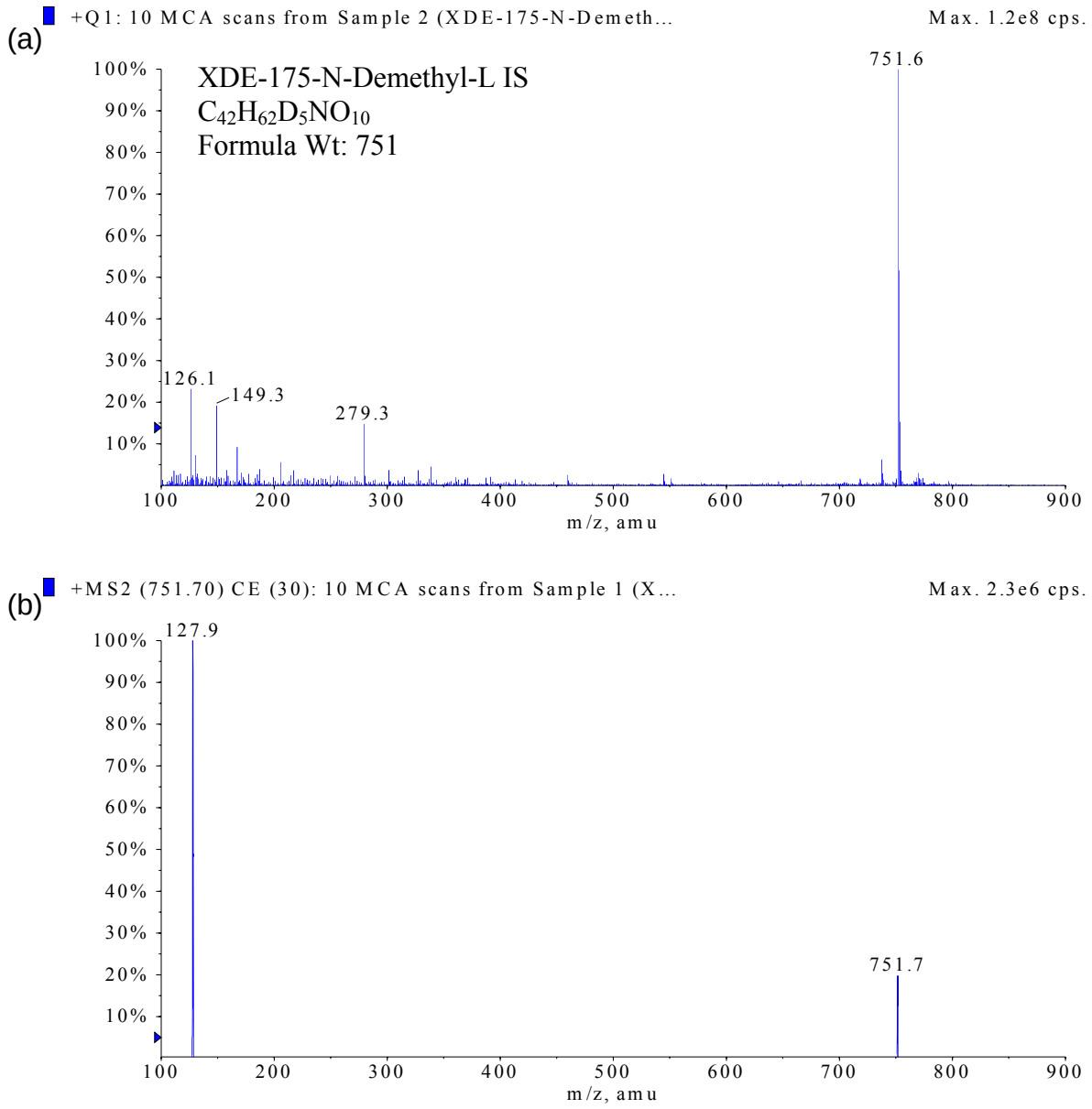


Figure 8. Mass Spectra for XDE-175-*N*-Demethyl-L Stable Isotope Internal Standard: (a) Q1 Scan using positive-ion Atmospheric Pressure Chemical Ionization showing $(M+H)^+$ at *m/z* 751.6 (b) Product-Ion Mass Spectrum of XDE-175-*N*-Demethyl-L Stable Isotope Internal Standard Showing Fragment Ion at *m/z* 127.9

Analytical Set I.D.: 040107 S02
Compound: Spinosyn A

Calibration Data

Linear with 1/x Weighting

Slope =	0.1627
Intercept =	-0.0077
r^2 =	0.9994

Standard Concentration (ng/mL)	Injection Number	Analyte Peak Area	ISTD Peak Area	Quantitation Ratio	Response Factor	Calculated Concentration	Percent of Theoretical
0.1	2	903	74130	0.012	0.1218	0.12197	122
0.5	15	5600	75420	0.074	0.1485	0.50338	101
1	28	9128	73258	0.125	0.1246	0.81277	81
5	41	63652	82486	0.772	0.1543	4.78887	96
10	54	139873	86048	1.626	0.1626	10.03561	100
20	67	271137	85262	3.180	0.1590	19.58782	98
35	80	489301	84601	5.784	0.1652	35.58631	102
50	87	702939	86188	8.156	0.1631	50.16327	100

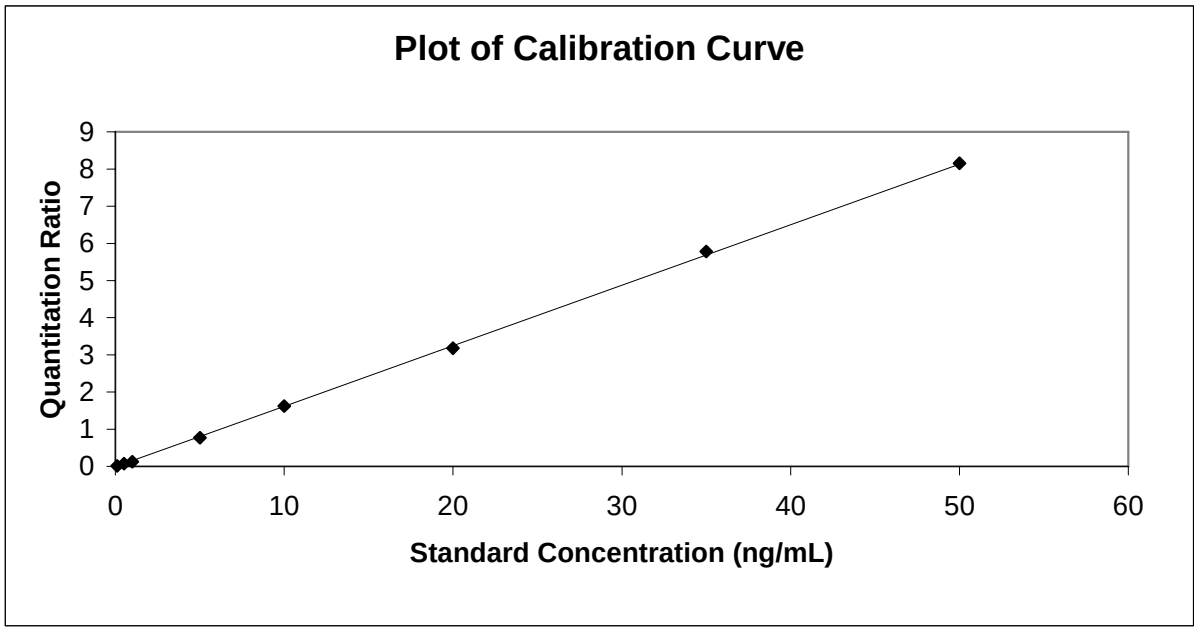


Figure 9. Typical Calibration Curve for the Determination of Spinosyn A in Wet Crops (Lettuce Leaves)

Analytical Set I.D.: 040107 S02
Compound: Spinosyn D

Calibration Data

Linear with 1/x Weighting

Slope =	0.1770
Intercept =	-0.0079
r^2 =	0.9993

Standard Concentration (ng/mL)	Injection Number	Analyte Peak Area	ISTD Peak Area	Quantitation Ratio	Response Factor	Calculated Concentration	Percent of Theoretical
0.1	2	915	82902	0.011	0.1104	0.10720	107
0.5	15	7277	85827	0.085	0.1696	0.52380	105
1	28	12537	84289	0.149	0.1487	0.88505	89
5	41	80862	93536	0.865	0.1729	4.92828	99
10	54	172127	96949	1.775	0.1775	10.07402	101
20	67	343307	98824	3.474	0.1737	19.66849	98
35	80	621118	96944	6.407	0.1831	36.23685	104
50	87	869457	99965	8.698	0.1740	49.17630	98

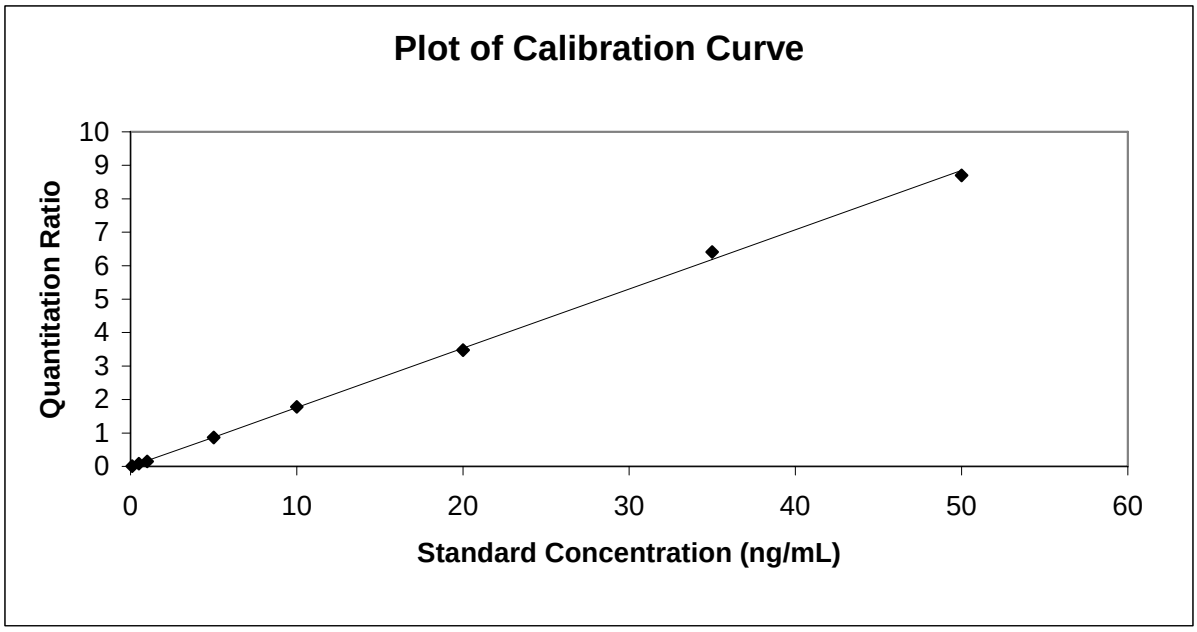


Figure 10. Typical Calibration Curve for the Determination of Spinosyn D in Wet Crops (Lettuce Leaves)

Analytical Set I.D.: 040107 S02
Compound: Spinosyn B

Calibration Data

Linear with 1/x Weighting

Slope =	0.3061
Intercept =	-0.0073
r^2 =	0.9994

Standard Concentration (ng/mL)	Injection Number	Analyte Peak Area	ISTD Peak Area	Quantitation Ratio	Response Factor	Calculated Concentration	Percent of Theoretical
0.1	2	3170	108159	0.029	0.2931	0.11967	120
0.5	15	15302	102531	0.149	0.2985	0.51145	102
1	28	24546	97372	0.252	0.2521	0.84740	85
5	41	138586	95465	1.452	0.2903	4.76610	95
10	54	304561	101540	2.999	0.2999	9.82198	98
20	67	597921	100322	5.960	0.2980	19.49322	97
35	80	1053968	97740	10.783	0.3081	35.24946	101
50	87	1576162	101420	15.541	0.3108	50.79072	102

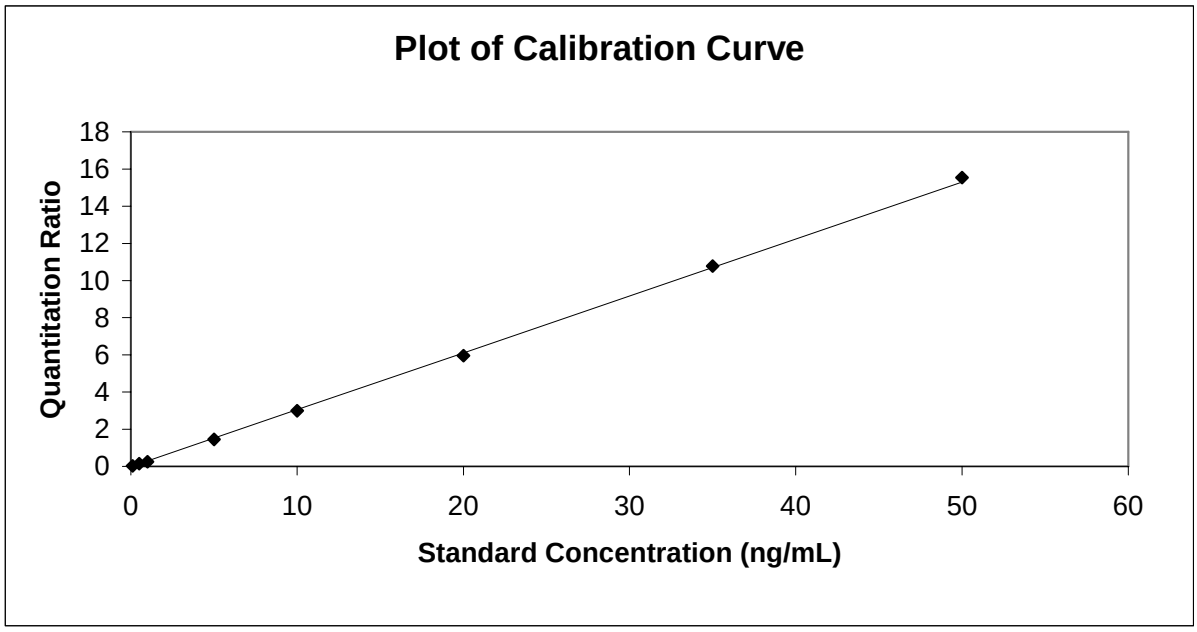


Figure 11. Typical Calibration Curve for the Determination of Spinosyn B in Wet Crops (Lettuce Leaves)

Analytical Set I.D.: 040107 S02
Compound: Spinosyn N-Demethyl D

Calibration Data

Linear with 1/x Weighting

Slope =	0.1448
Intercept =	-0.0035
r^2 =	0.9987

Standard Concentration (ng/mL)	Injection Number	Analyte Peak Area	ISTD Peak Area	Quantitation Ratio	Response Factor	Calculated Concentration	Percent of Theoretical
0.1	2	1861	145247	0.013	0.1281	0.11287	113
0.5	15	10820	137869	0.078	0.1570	0.56622	113
1	28	15725	131560	0.120	0.1195	0.84960	85
5	41	87554	130211	0.672	0.1345	4.66651	93
10	54	194114	139578	1.391	0.1391	9.62563	96
20	67	381905	136809	2.792	0.1396	19.29642	96
35	80	676647	134045	5.048	0.1442	34.87402	100
50	87	1018981	136375	7.472	0.1494	51.60872	103

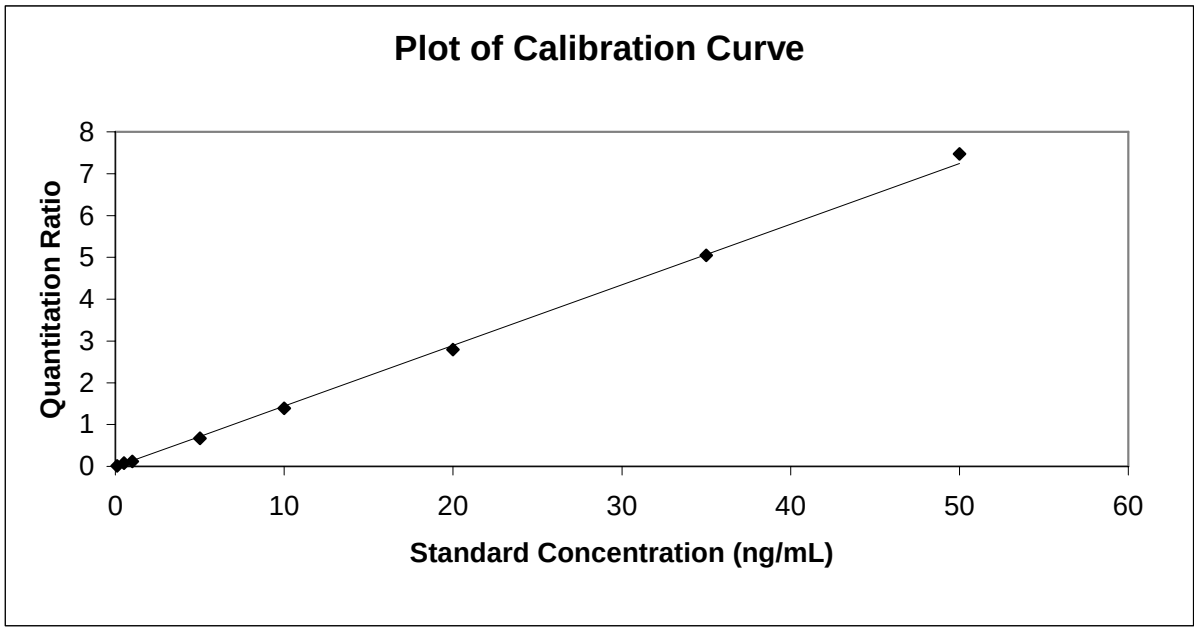


Figure 12. Typical Calibration Curve for the Determination of *N*-Demethyl Spinosyn D in Wet Crops (Lettuce Leaves)

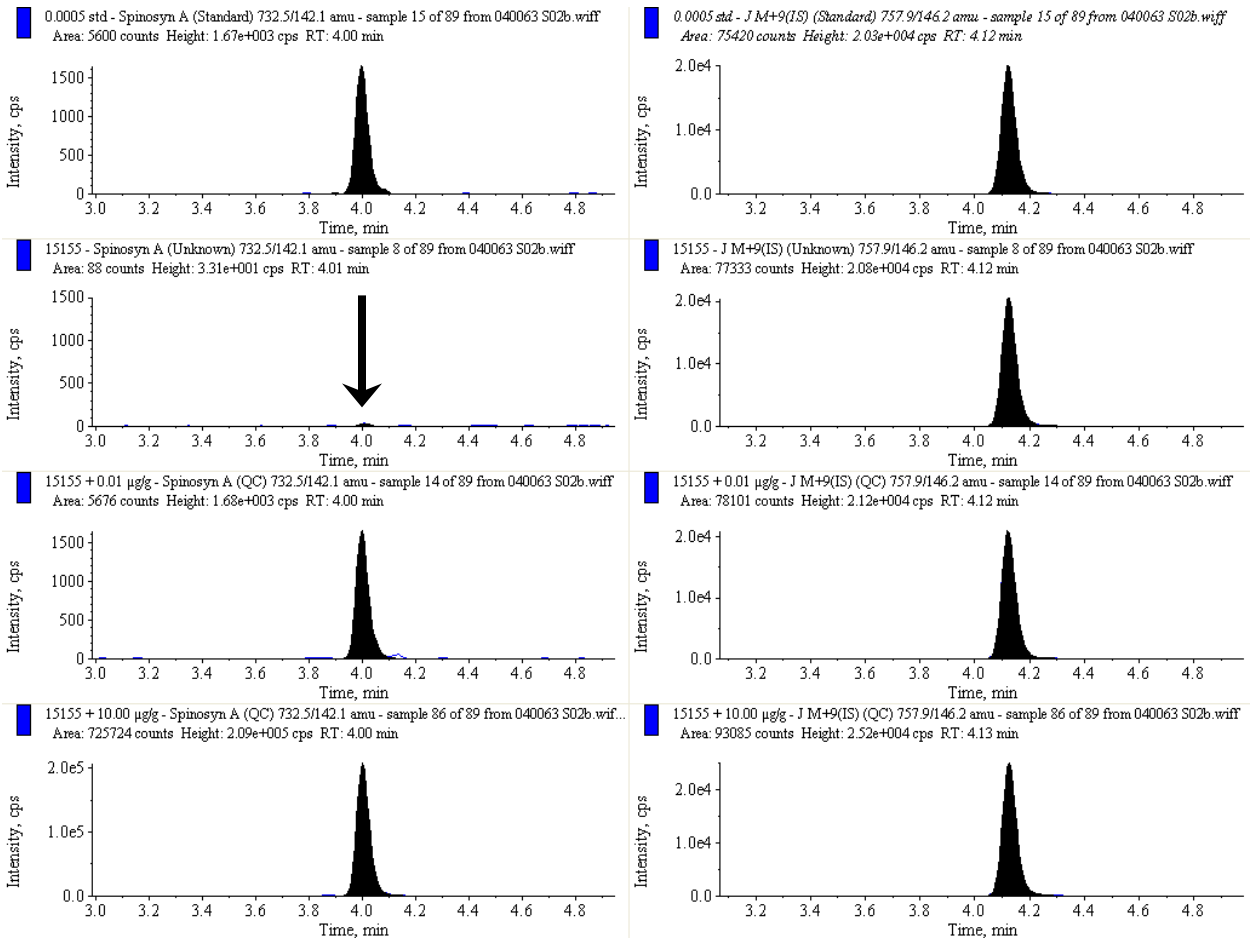


Figure 13. Typical MRM Chromatograms for the Determination of Spinosyn A in Lettuce Leaves

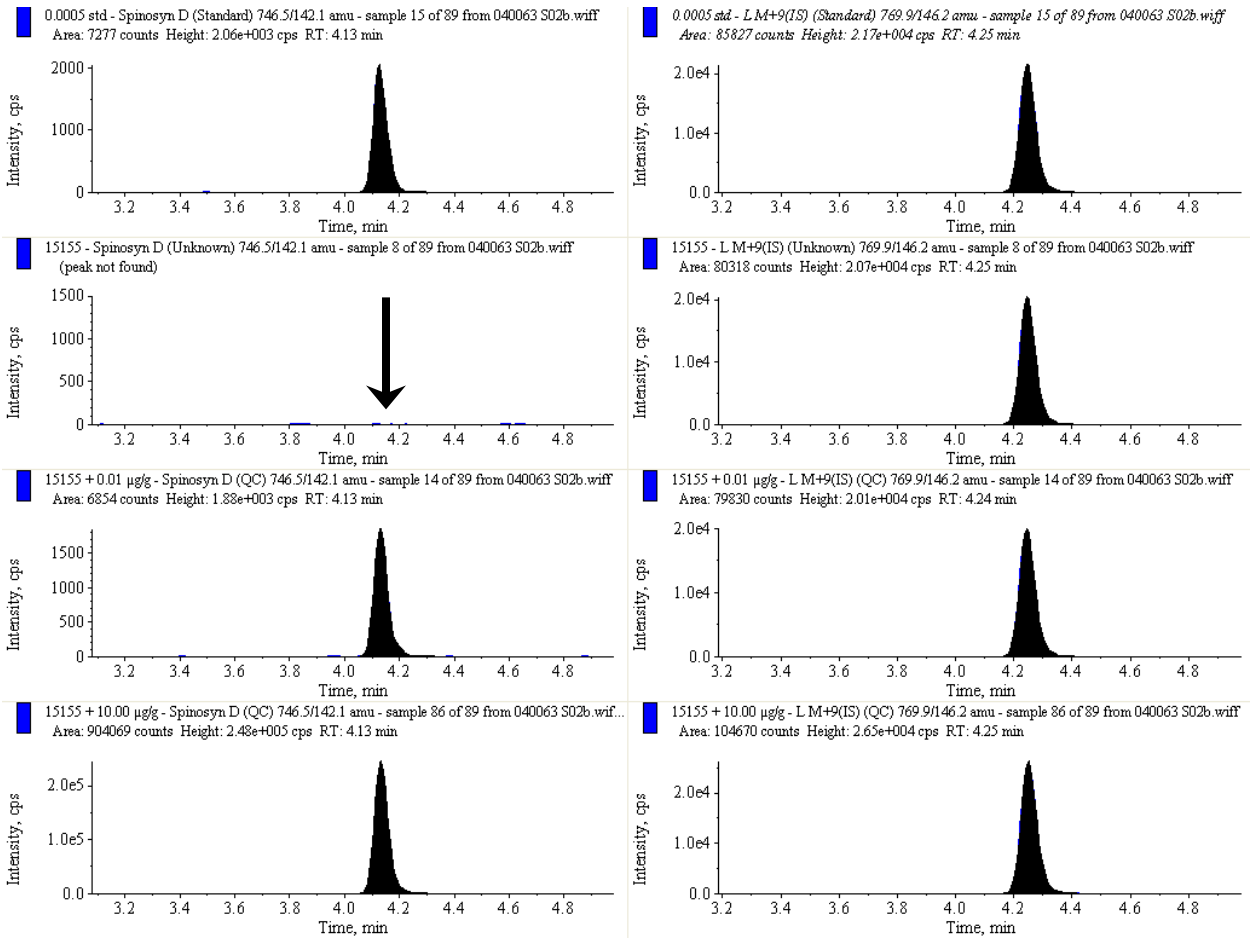


Figure 14. Typical MRM Chromatograms for the Determination of Spinosyn D in Lettuce Leaves

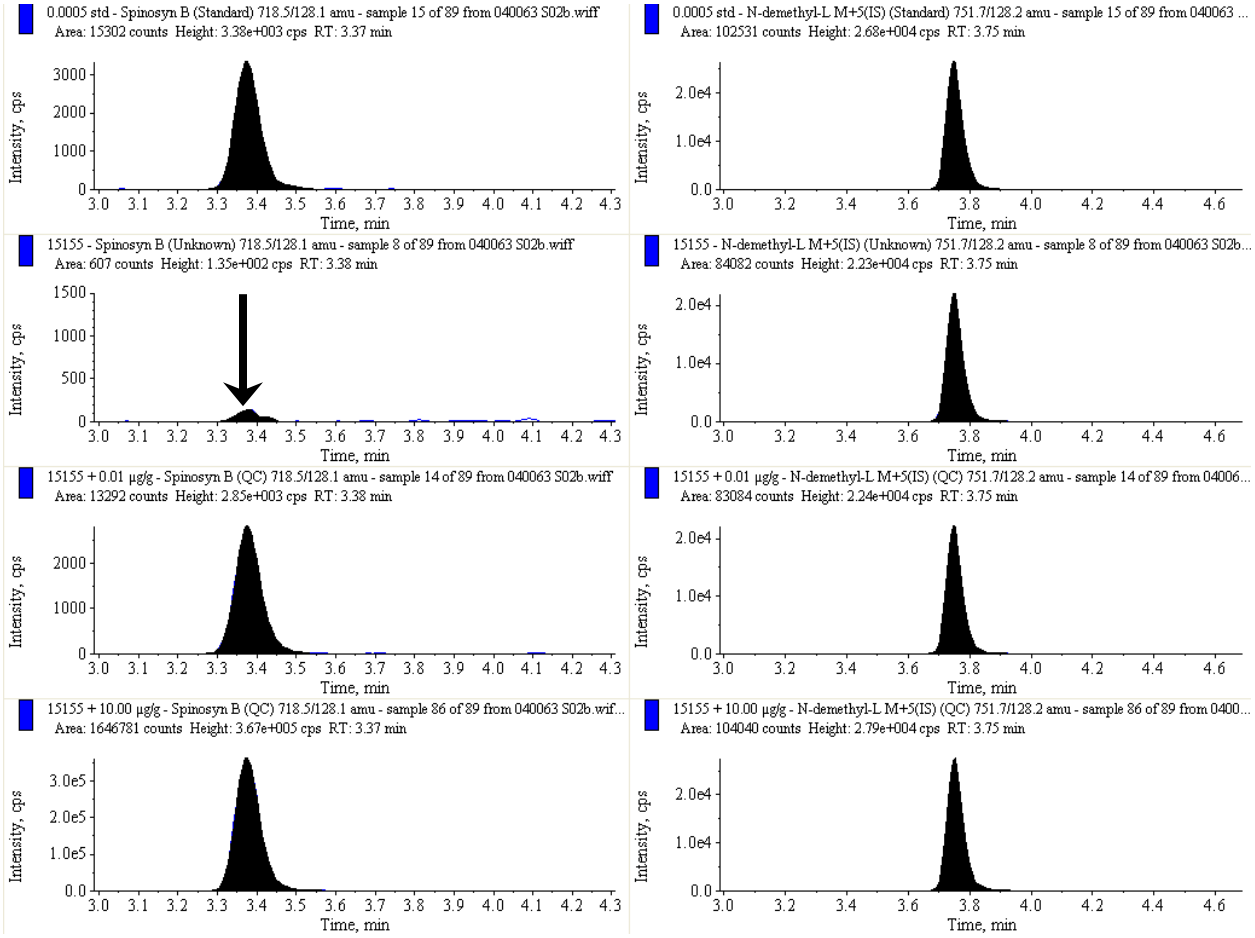


Figure 15. Typical MRM Chromatograms for the Determination of Spinosyn B in Lettuce Leaves

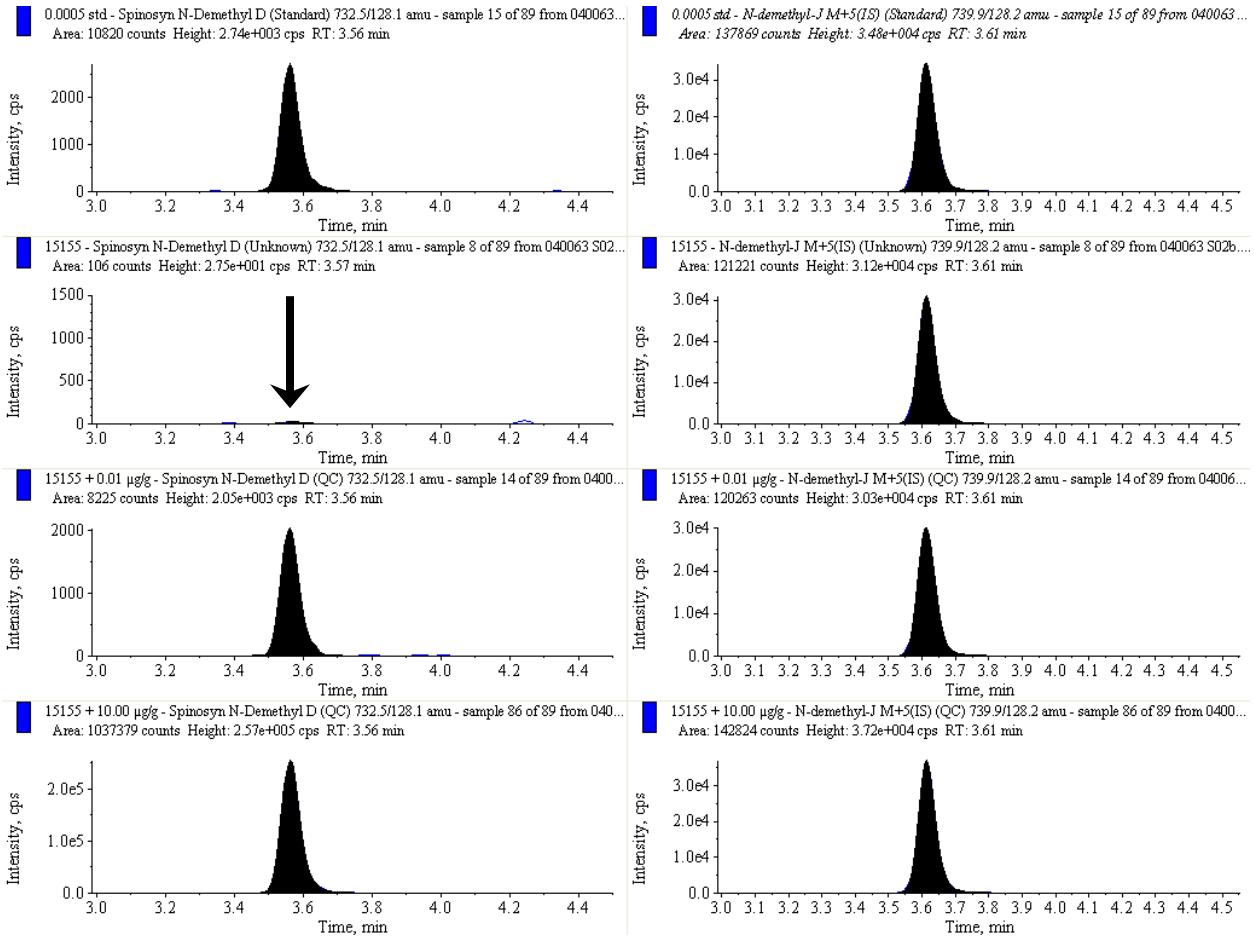


Figure 16. Typical MRM Chromatograms for the Determination of *N*-Demethyl Spinosyn D in Lettuce Leaves

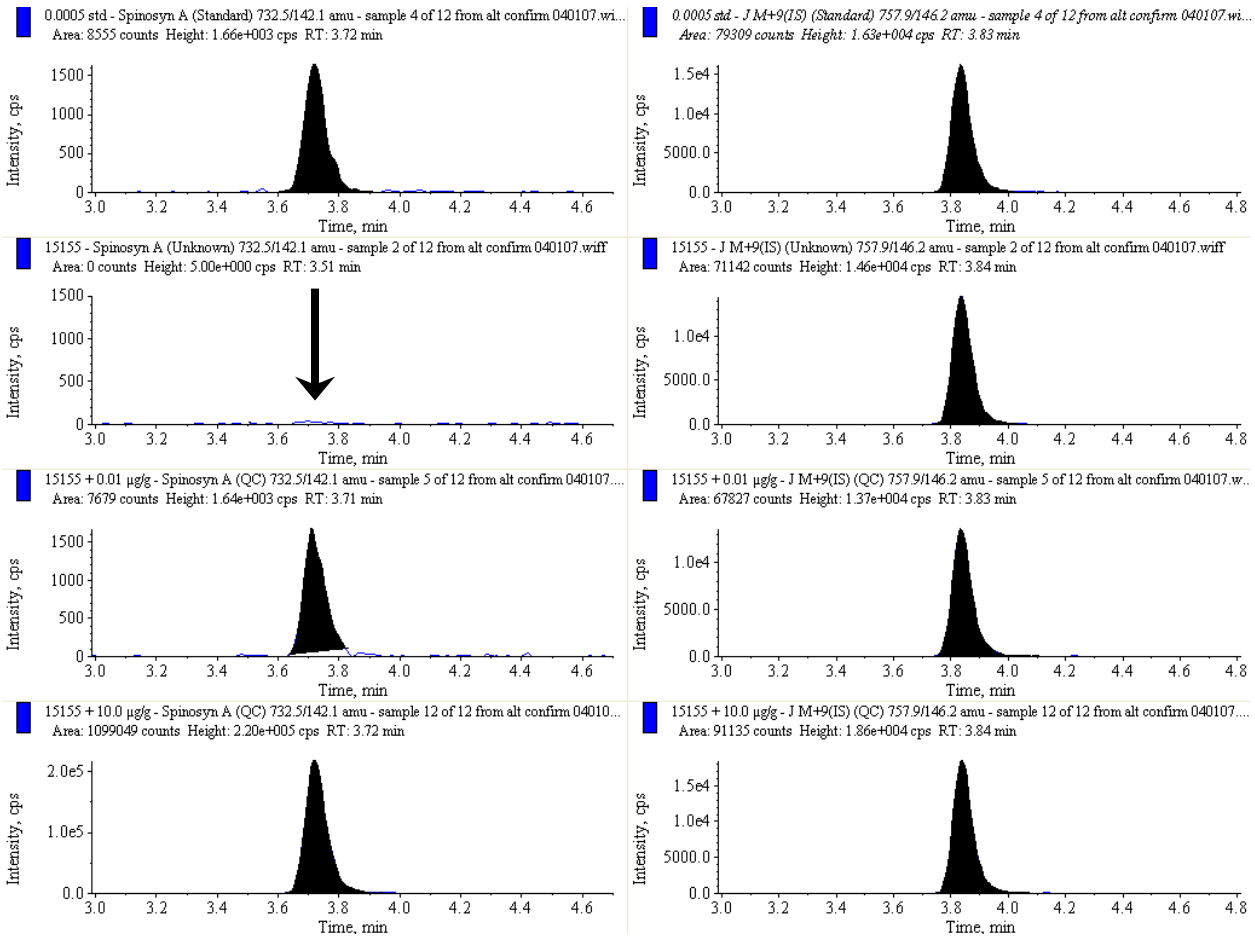


Figure 17. Typical MRM Chromatograms for the Confirmation of Spinosyn A Residues in Lettuce Leaves

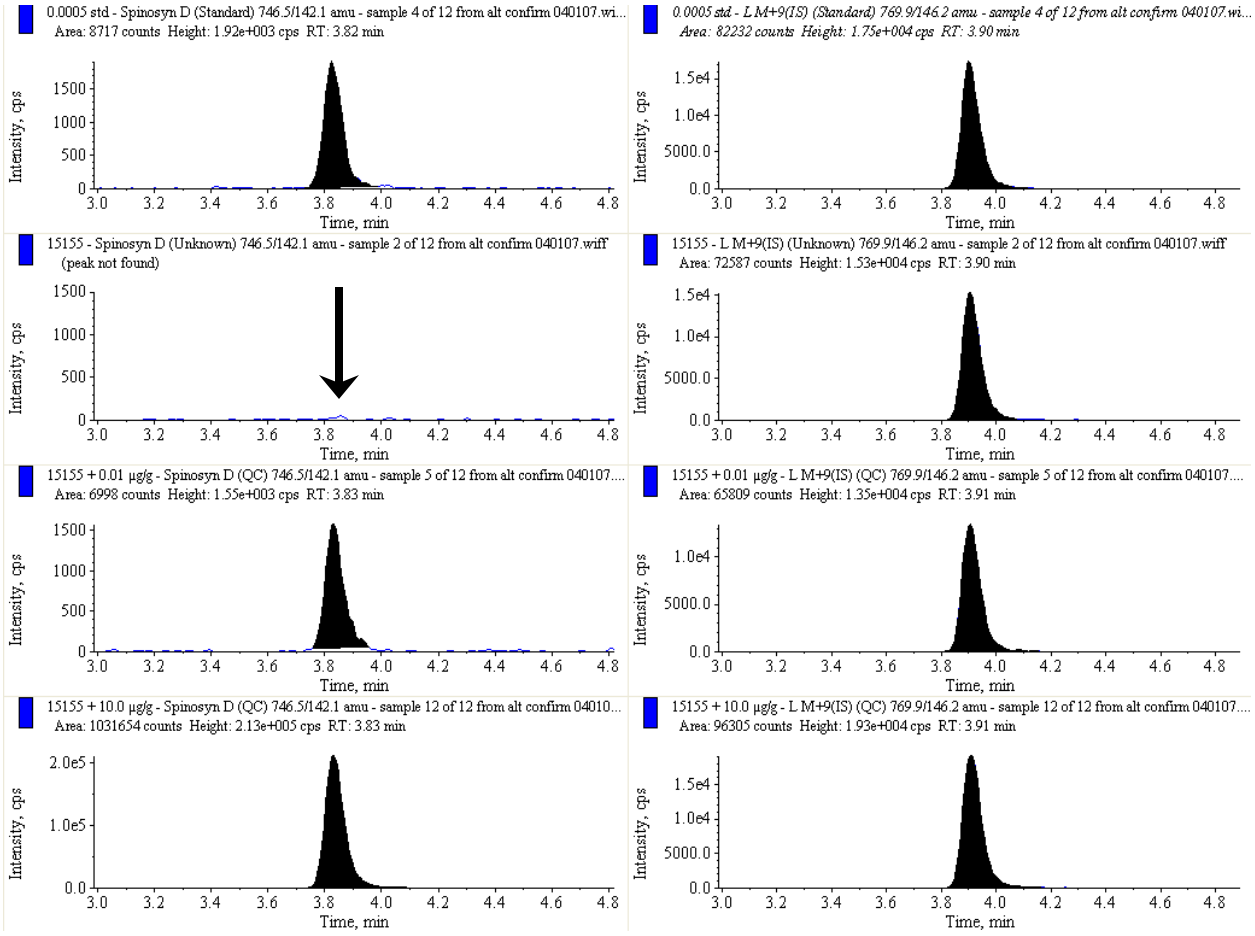


Figure 18. Typical MRM Chromatograms for the Confirmation of Spinosyn D Residues in Lettuce Leaves

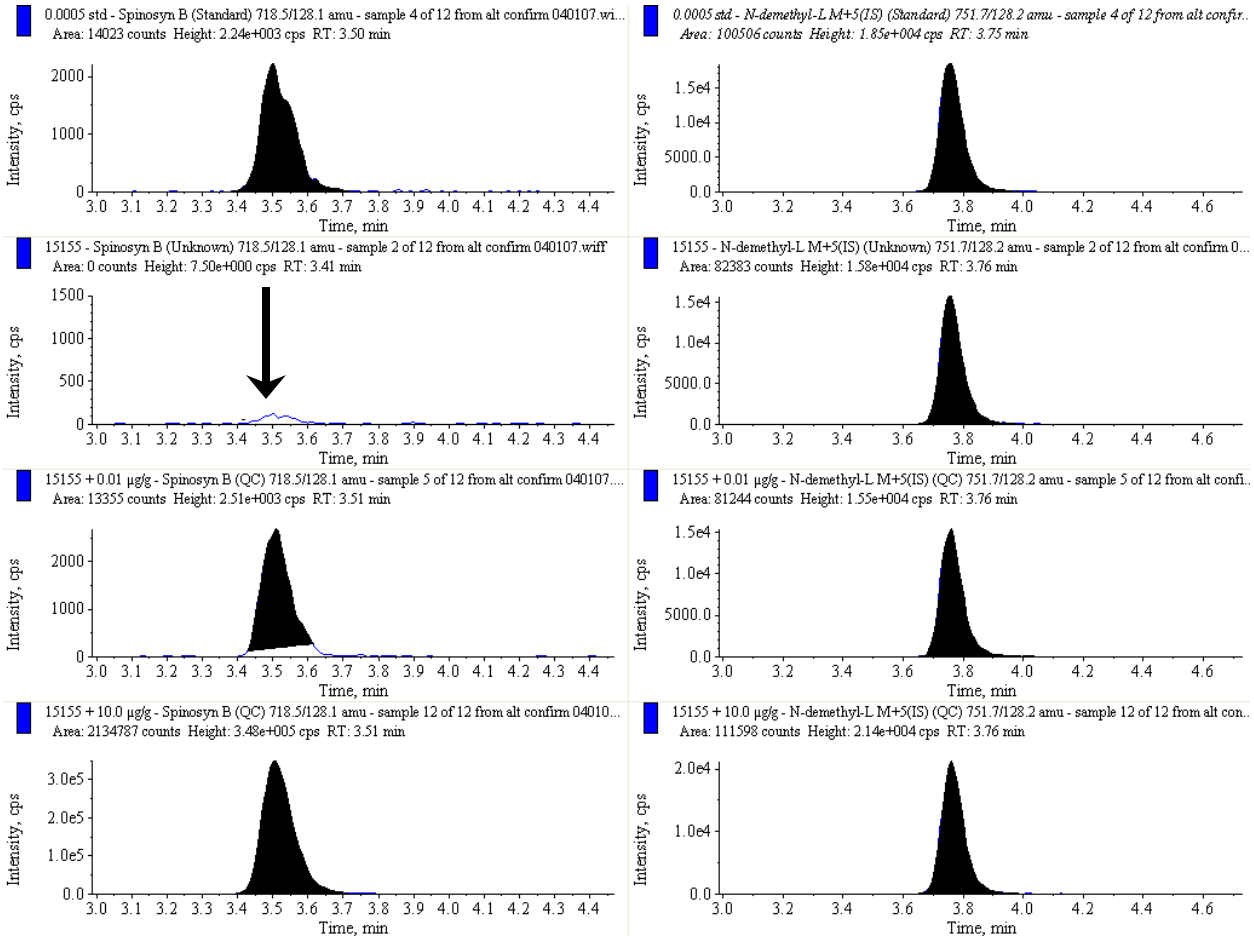


Figure 19. Typical MRM Chromatograms for the Confirmation of Spinosyn B Residues in Lettuce Leaves

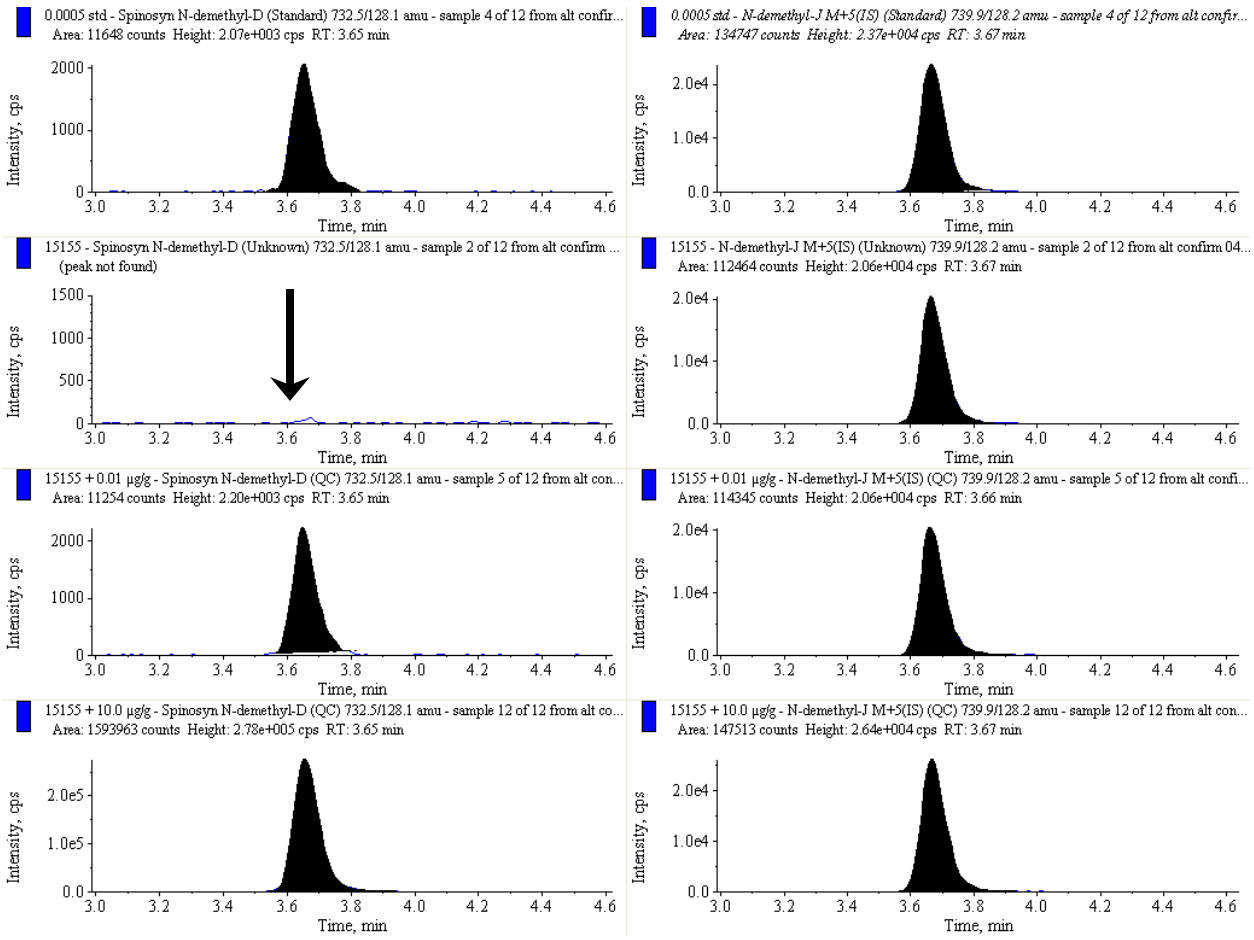


Figure 20. Typical MRM Chromatograms for the Confirmation of *N*-Demethyl Spinosyn D Residues in Lettuce Leaves