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Determination of Residues of Spinosad in Water Using a Magnetic Particle-Based Immunoassay Test Kit

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A. Scope

This method is applicable for the determination of residues of spinosad in water samples over the concentration range of 0.10 ng/mL to 1.0 ng/mL, with validated limits of quantitation (LOQ) and detection (LOD) of 0.10 and 0.05 ng/mL, respectively. The range can be extended for analysis of samples containing higher concentrations of spinosad by dilution of a sample aliquot into the working range of the assay. The method is based upon using the Ohmicron Spinosad RaPID Assay® test kit and the Ohmicron RPA-1 RaPID Analyzer™.

The antibody used to develop the spinosad immunoassay test kit is sensitive to several individual spinosyns as well as some metabolites and degradates (Table I, Figure 1). The method is not capable of differentiating individual spinosyns. Therefore, the concentration of spinosad measured using this method should be expressed without reference to the individual compounds.

B. Principle

The Spinosad RaPID Assay applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of residues of spinosad in water samples. Immediately after collecting a water sample, a stabilizer solution is added. An aliquot of each sample is then pipeted into a disposable test tube. Enzyme-conjugated spinosad and paramagnetic particles coated with antibodies specific to spinosad are then sequentially added to the tube and the mixture is incubated. During the incubation period, the spinosad and the enzyme-conjugated spinosad compete for antibody sites on the magnetic particles. At the end of the incubation period, a magnetic field is applied to the particles. The spinosad and the enzyme-conjugated spinosad which are bound to the antibodies on the particles are held in the tube by the magnetic field while the unbound reagents are decanted. After decanting, the particles are washed to remove unbound enzyme conjugate. The presence of spinosad is detected after adding a color solution, consisting of the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The color solution is incubated in the presence of the antibody-bound enzyme conjugate to generate a colored product. After this incubation period, the reaction is stopped and stabilized by the addition of acid. The absorbance at 450 nm is then measured in each tube using the RPA-1.

Since the enzyme-conjugated spinosad was in competition with the unlabeled (sample) spinosad for the antibody binding sites, the level of color development will be inversely proportional to the concentration of spinosad in the sample (i.e., lower residue concentrations result in greater color development). A standard curve is constructed using linear regression after a ln/logit transformation of the concentration and absorbance values, respectively. The spinosad concentration in unknown samples is calculated from the regression equation using the preprogrammed software capabilities of the RPA-1.

C. Safety Precautions

1. Each analyst should be acquainted with the potential hazards of the reagents and products used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, PRODUCT LITERATURE AND OTHER RELATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents must be in compliance with local, state and federal laws and regulations.
2. Avoid contact of Stopping Solution (0.5% sulfuric acid) with skin and mucous membranes. Wear protective clothing and safety glasses when working with this material. If this reagent comes in contact with skin, flush the exposed area with water.

D. Equipment (Note M.1.)

1. Balance, analytical, Mettler AE50, cat. no. 01-909-402, Fisher Scientific, Pittsburgh, PA 15238.
2. Magnetic separator rack and base, 60 position, cat. no. A00004, Ohmicron Environmental Diagnostics Corp., Newtown, PA 18940.
3. Mixer, Vortex-Genie, cat. no. 12-812, Fisher Scientific.
4. Pipet, Eppendorf, repeater, cat. no. 21-380-8, Fisher Scientific.
5. Pipettor, Eppendorf, tri-volume (100 µL, 200 µL, 250 µL), cat. no. 21-278-38, Fisher Scientific.
6. Photoanalyzer, fixed wavelength spectrophotometer RPA-1, cat. no. A00003, Ohmicron Environmental Diagnostics Corp.
7. Timer, minutes and seconds with alarm, cat. no. 14-649-14, Fisher Scientific.

E. Glassware and Materials (Note M.1.)

1. Combitips for repeater pipet, 12.5 mL, cat. no. 21-380-8C, Fisher Scientific.
2. Pipet tips, Eppendorf disposable, 0.1-1.0 mL cat. no. 21-372-4, Fisher Scientific.
3. Pipet, 1.0 mL glass disposable, cat. no. 13-678-25B, Fisher Scientific.
4. Pipet, 5.0 mL glass disposable, cat. no. 13-678-25D, Fisher Scientific.
5. Pipet, 10 mL glass disposable, cat. no. 13-678-31J, Fisher Scientific.

F. Reagents and Chemicals (Note M.1.)

1. Methanol, ChromAR HPLC grade, cat. no. UN1230, Mallinckrodt Specialty Chemicals, Paris, KY 40361.
2. Proficiency samples, Spinosad, cat. no. A00179, Ohmicron Environmental Diagnostics Corp (optional) (Note M.3.).
3. RaPID Assay 100 Tube Kit, Spinosad cat. no. A00178, Ohmicron Environmental Diagnostics Corp. (Notes M.2., M.3.).

   Kit contents include:

   a. Spinosad Antibody coupled to paramagnetic particles
   b. Enzyme Conjugate
   c. Standards (i.e., Calibration Standards) (Note M.4.)
   d. "Quality" Control Sample
   e. Diluent/Zero Standard
   f. Color Solution
   g. Stopping Solution
   h. Washing Solution
   i. Test Tubes
   j. Water Stabilizer

4. Sample diluent, Spinosad, cat. no. A00180, Ohmicron Environmental Diagnostics Corp (Note M.3.).
5. Standard, Spinosad (technical grade active ingredient). Obtain from Test Substance Coordinator, DowElanco, 9330 Zionsville Rd., Indianapolis, IN 46268.
6. Water stabilizer, Spinosad, cat. no. A00181, Ohmicron Environmental Diagnostics Corp.

G. Preparation of Standards

1. Preparation of Fortification Solutions

   a. Dissolve 0.050 g of spinosad technical grade analytical standard in methanol in a 100 mL volumetric flask. Dilute to volume with methanol to obtain a 500 µg/mL solution.
   b. Dilute 1.0 mL of the above 500 µg/mL spinosad solution with methanol in a 100-mL volumetric flask to obtain a 5.00 µg/mL solution.
   c. Dilute 10.0 mL of the above 5.00 µg/mL spinosad solution with methanol in a 100-mL volumetric flask to obtain a 500 ng/mL stock solution.

2. Preparation of Recovery Samples

a. Prepare the following fortification stock solutions in methanol. Begin by using the 500 ng/mL spinosad solution described in G.1.c. Prepare each solution by pipeting the appropiate volume of the stock solution into a 100-mL volumetric flask then filling to volume with methanol.

Stock Solution Concentration	Original Volume	Final Concentration
ng/mL	mL	ng/mL
500	10.0	50.0[a]
500	5.00	25.0
500	2.50	12.5
50.0	10.0	5.00
50.0	5.00	2.50

[a] Use the 50 ng/mL solution to prepare the 5.00 and 2.50 ng/mL fortification solutions.

b. Dispense 50 mL of the control water sample into a glass container. Use the fortification stock solutions from G.2.a. for preparation of fortified water samples as follows (see I.2.a.):

Stock Solution Concentraton	Stock Volume	Final Concentration
ng/mL	mL	ng/mL
2.50	1.00	0.05
5.00	1.00	0.10
12.5	1.00	0.25
25.0	1.00	0.50
50.0	1.00	1.00

3. Preparation of Standards

Using the stock solutions described in G.2.a., prepare calibration standards containing 0.05, 0.25 and 1.00 ng/mL of spinosad and a quality control sample containing 0.50 ng/mL of spinosad. The standards and the quality control sample must be prepared in Spinosad Sample Diluent (F.4.) using the fortification scheme below. This fortification scheme describes preparation of 50 mL of each standard.

Stock Solution Concentraton	Stock Volume	Final Concentration
ng/mL	mL	ng/mL
2.50	1.00	0.05
5.00	1.00	0.10
12.5	1.00	0.25
25.0	1.00	0.50
50.0	1.00	1.00

H. Instrument Settings

To obtain results from the Spinosad RaPID Assay use the following parameter settings on the RPA-1 Photoanalyzer:

Parameter	RPA -1 Abbreviation	Setting
Protocol Name	Protocol Nam	SPINOSAD
Data Reduction	Data Reduct	Linear Regression
Transformation	Xformation	Ln/LgtB
Number of Standards	# of Standard	4
Number of Standard Replicates	# of Reps:	2
Standard #1 Concentration	Cal #1 Conc	0.00
Standard #2 Concentration	Cal #2 Conc	50
Standard #3 Concentration	Cal #3 Conc	250
Standard #4 Concentration	Cal #4 Conc	1000
Minimum Correlation	Correlation Flag	0.990
Maximum concentration (pg/mL)	Normal Range Hi	1000
Minimum concentration (pg/mL)	Normal Range Low	20
Number of Controls	# of Controls	1
Number of Control Replicates	Ctrl Replicates	2
Number of Reagent Blanks	# Rgt Blk	0
Wavelength	Wavelength	450 nm
Read Mode	Read Mode	Absorbance
Units	Units	pg/mL
Precision of Standards	Rep %CV Flag	100

I. Determination of Spinosyn in Water

1. Reagent Preparation

Remove all samples and kit reagents from refrigerated storage and allow them to equilibrate to room temperature. A minimum of 30 minutes is recommended for warming.

2. Assay Procedure

Each test is conducted in an individual test tube. Duplicate analyses of a sample or standard constitutes two tests. A standard curve and the appropiate control and recovery samples must be included with each analytical set. For further details consult the Spinosad RaPID Assay Kit Insert (Appendix A).

a. **Critical step: spinosad adsorbs to glass and plastic surfaces in aqueous solution. Collect water samples in glass vessels. Immediately after collection, add Water Stabilizer at a 1:100 dilution.** For example, add 0.5 mL of the stabilizer solution to a 50 mL water sample. Add the Water Stabilizer prior to transferring sample aliquots to other containers to prevent adsorbtive loss to the container.

b. Turn on the RPA-1 Photoanalyzer at least 30 minutes prior to measuring absorbance in the completed assays.

c. Label test tubes for standards, controls and samples and place the tubes in the proper rack position. Be sure that the rack is removed from the Magnetic Separator.

d. **Critical step: Accurate delivery of the correct sample volume and proper pipeting technique in this step are critical to obtaining accurate and precise data.** Add 0.20 mL of the standard or sample to each test tube using an Eppendorf pipetter. Pipet each sample or standard directly to the bottom of the tube, avoiding having liquid adhere to the sides of the test tube. Use a fresh pipet tip for each standard and sample.

e. Using a repeater pipet equipped with a 12.5 mL combitip, add 0.25 mL (Dial Setting =1) of Enzyme Conjugate down the inside wall of each tube.

f. Thoroughly mix the Antibody Coupled Paramagnetic Particles by swirling the bottle. Avoid vigorous shaking and foaming.

g. Using a repeater pipet equipped with a 12.5 mL combitip, add 0.50 mL (Dial Setting = 2) of the Antibody Coupled Paramagnetic Particles down the inside wall of each tube.

h. When dispensing of the magnetic particles is completed, mix the samples by either gently vortexing (Vortex setting = 3-4) each tube for 1-2 seconds or by gently swirling the entire rack for a few seconds.

i. Incubate for 30 minutes at ambient temperature.

j. After the incubation period, combine the rack and the magnetic base. Seat all tubes by pressing them into the base. Allow 2 minutes for the particles to separate.

k. Do not separate the tube rack from the magnetic base. Using a smooth motion, invert the combined rack assembly over a collection container and pour out the tube contents. Keep the rack inverted and gently blot the test tube rims on several layers of paper toweling.

l. Using the repeater pipet equipped with a 12.5 mL combitip, add 1 mL (Dial Setting = 4) of Washing Solution to each tube. Wait 2 minutes. Decant the Washing Solution into an appropriate collection container using the technique described in Step I.4.k.

m. Repeat Step I.4.l.

n. Remove the tube rack from the magnetic separator then add 0.50 mL of Color Solution using a repeater pipet equipped with a 12.5 mL combitip (Dial Setting = 2).

o. Gently vortex each tube for 1-2 seconds.

p. Incubate for 20 minutes at room temperature. During this incubation, dispense approximately 1 mL of Washing Solution into a clean tube for use as an instrument blank.

q. At the end of the incubation period, add 0.5 mL of Stopping Solution using the repeater pipet (Dial Setting = 2).

r. Measure the absorbance in each tube using the RPA-1 Photoanalyzer within 10 minutes after adding the Stopping Solution.

3. Operating Procedure for the RPA-1 Photoanalyzer

The RPA-1 is pre-programmed with the protocols for several RaPID Assay procedures. The following steps describe how to set up and run the analyzer to measure absorbance in the tubes for the Spinosad RaPID Assay.

a. Switch on the instrument and allow it to warm up at least 30 minutes prior to use. The RPA-1 will perform a self test. If all parameters are satisfactory, the "SELECT COMMAND" prompt will appear.

b. At the "SELECT COMMAND" prompt press "RUN".

c. At the "RUN PROTOCOL" prompt, scroll through the protocols using the arrow keys until "SPINOSAD" appears. Press "ENTER".

d. At the "SPL. REPLICATES" (sample replicates) prompt, press "2" to indicate the number of replicates for each sample, then press "ENTER".

e. At the "BLANK TUBE/INSERT TUBE" prompt, insert the tube with 1 mL of Washing Solution. The display will briefly read "'EVALUATING TUBE" then "REMOVE TUBE" and the instrument will produce an audible beep indicating that the absorbance has been measured. After hearing the beep remove the tube.

f. At the "CAL. #1 REP. #1/INSERT TUBE" prompt, insert the first replicate of the first standard (0.00 µg/L). Remove the tube after the beep.

g. Follow the prompts on the instrument display until all of the standards have been measured. The tube order is important. The RPA-1 has been programmed to evaluate the standards in ascending order, in duplicate, starting with 0.00 µg/L.

h. After all the standards have been evaluated, the instrument will report the equation of the line, the transformed data and the standard data.

i. Insert the control tubes at the "CNTRL. #1 REP. #1" and "CNTRL. #1 REP. #2" prompts. The instrument will report the calculated values for each replicate of the control sample.

j. Evaluate the results for the standard curve and the control samples. At the "EDIT CALIBRATORS YES/NO" prompt press "NO".

k. At the "SPL. #1 REP #1/INSERT TUBE" prompt insert the first sample tube. Remove the tube after the beep.

l. Continue sample analysis following the prompts on the instrument display. Press "STOP" after all the samples have been evaluated and the results have been reported by the RPA-1.

J. Calculations (Note M.5.)

1. Standard Curve

The RPA-1 RaPID Analyzer contains preprogrammed data reduction capabilities which calculates a standard curve for each analytical set using the absorbances measured during the assay. The calibration curve is constructed using linear regression after performing a ln (C)/Logit (B/B_o) data transformation of the concentration and absorbance values respectively.

The regression equation is :

$$\text{Logit}\frac{B}{B_o} = \text{slope} * \text{Ln(Conc)} + \text{Y intercept}$$

Where:

$$\text{Logit}\frac{B}{B_o} = \text{Ln}\frac{B/B_o}{1-B/B_o}$$

B = the absorbance measured at a specific spinosad concentration
B_o = the absorbance measured for the 0.00 ng/mL standard
Conc = the spinosad concentration of the standard

EXAMPLE: Calculation of the standard curve from Analytical Set 081194DDLY.

Concentration, pg/mL	ln (Conc)	Absorbance, 450 nm	B/B_o	Logit (B/B_o)
0.00	N/A	1.281	N/A	N/A
50	3.91	1.024	0.799	1.381
250	5.52	0.667	0.521	0.084
1000	6.91	0.358	0.279	-0.949

Using these ln (conc) and Logit (B/B_o) values to calculate a linear regression, the results are:

Slope = -0.778
Y Intercept = 4.412

The results are presented graphically in Figure 2.

2. Calculation of Spinosad in Samples

a. The RPA-1 RaPID Analyzer will calculate a predicted concentration of spinosad in each unknown sample using the regression equation calculated from the standards which are analyzed as part of the analytical set. It will report the absorbance value and calculated spinosad concentration for each replicate of each sample as well as the mean absorbance, the mean spinosyn concentration and the percent coefficient of variation of the duplicate measurements for each sample. The mean values are the final result for each sample.

b. The following information may also appear as part of the data report:

"**nd**" indicates the absorbance measured is greater than or equal to the absorbance of the 0.00 ng/mL standard; therefore, a concentration cannot be calculated. The result should be reported as "not detected" and the LOD should be noted.

"**x.xx nd**" (i.e., 0.01nd) indicates that the calculated concentration is below the limit of detection. The result should be reported "not detected" and the LOD should be noted (Note M.6.).

"**x.xx Hi**" (i.e., 1.36Hi) indicates that the calculated value exceeded the upper range of the assay. The sample should be diluted 1:10 with Spinosad Sample Diluent then re-analyzed.

c. Example Calculation

To manually calculate the concentration of spinosad in a water sample use the following equation:

Concentration = e^a, where:

$$a = \left(\frac{\text{Logit}\frac{B}{Bo} - \text{Y intercept}}{\text{slope}} \right)$$

Sample 14909802 X11 from analytical set 081194DDLY was fortified with 0.10 ng/mL of spinosad:

Mean absorbance (450 nm) = 0.926

$$\frac{B}{Bo} = \frac{0.926}{1.281} = 0.723$$

$$\text{Logit}\ \frac{B}{Bo} = \text{Ln}\frac{0.723}{1-0.723} = \ln(2.61) = 0.959$$

$$\text{Concentration} = e^{\left(\frac{0.959\ -\ 4.412}{-0.778}\right)}$$

$$= 0.085 \text{ ng/mL}$$

3. Calculation of the Final Concentration in Diluted Samples

Final Concentration = Measured Concentration x Dilution Factor

4. Calculation of Percent Recovery

The percent recovery for the quality control sample or for fortified samples is calculated as follows (Note M.6.):

$$\%\ \text{Recovery} = \frac{\text{Calculated Concentration - Blank Concentration}}{\text{Fortified Concentration}} \times 100$$

K. Quality Control

1. Analytical Set Definition

An analytical set is defined as a group of 60 tubes. The size of the set is based on the capacity of the magnetic separator rack. Analytical sets of less than 60 tubes can be analyzed. The first 10 tubes (rack positions 1-10) are used for duplicate analysis of the four standards and a quality control solution. Following the control solution, up to 25 samples (recovery or study samples) may be analyzed in duplicate (2 tubes). If more samples are to be analyzed than can be accommodated in one rack, the remaining samples should be analyzed as a distinct analytical set with a new standard curve, control and recovery samples.

2. Quality Control Solution

A quality control solution containing 0.50 ng/mL of spinosad should be analyzed at the beginning of every batch of samples (rack positions 9 and 10). The quality control sample should be assayed in the same manner as all other samples. Use the results obtained from analysis of the control solution to determine whether the assay was properly executed. Additional recovery samples may be analyzed to further ensure proper execution of the method.

3. Study Samples

All study samples should be analyzed in duplicate. If the spinosad concentration exceeds the range of the assay, dilute the sample 1:10 with Spinosad Sample Diluent (e.g., 1.0 mL sample + 9.0 mL Spinosad Sample Diluent), then perform the analysis on the diluted sample aliquots. Multiply the result by the dilution factor to obtain the final result (see J.3.).

4. Criteria for Acceptance of an Analytical Set

The correlation coefficient for the linear regression of the calibration curve should be greater than 0.990. The coefficient of variation should be less than 10% for each replicate pair of standards and the quality control sample. The recovery value for the quality control sample should be within ± 20% of the expected value (i.e., 0.04-0.06 ng/mL). If the data fail to meet these performance criteria, the analyst should evaluate the results, determine the potential source of the variation and, if necessary, repeat the analysis. The calibration and quality control data generated during validation of this method are tabulated and summarized in Table II.

5. Modifications and Potential Problems

a. No modificatons to the assay procedure are recommended.

b. Gross particulate material should be removed prior to analysis by allowing the material to settle or by centrifugation at approximately 2200 rpm for five minutes.

c. Spinosad adsorbs to glass and plastic surfaces in aqueous solution. Water samples should be collected in glass vessels (with PTFE cap liners). Water Stabilizer should be added to the sample immediately after collection or fortification.

d. The Water Stabilizer (Triton X-100) may interfere with High Performance Liquid Chromatography (HPLC) determination of spinosyns in water (1) when the optional solid phase extraction clean-up step is used. If a sample is to be analyzed

by HPLC and immunoassay, split the sample prior to adding Water Stabilizer. Partitioning of aliquots for HPLC and immunoassay should be performed immediately upon collection of the sample.

e. Samples which have been preserved with monochloroacetic acid, or other acid, should be neutralized with strong base (e.g., 6N NaOH) prior to assay.

6. Interferences

Thirty pesticides, 16 inorganic compounds and eight additional organic compounds were tested for potential interference with conjugate binding in the assay (2) (Table III). The only compound which exhibited an I_{50} concentration below 10 µg/mL was carbendazim (I_{50} = 5.56 µg/mL). The I_{50} concentration is the concentration of a specific compound which results in 50% inhibition of conjugate binding to the available antibodies. The I_{50} for spinosyn A is approximately 0.0003 µg/mL.

7. Specificity/Sensitivity

Several factors, metabolites and degradates of spinosad have been tested to determine whether the Spinosad RaPID Assay Test Kit will detect their presence in a water sample (2). In general, the assay is sensitive (I_{50} < 2 ng/mL) to factors which have little or no modification to the trimethylpyranosyl ring. The assay is relatively insensitive (I_{50} > 50 ng/mL) to spinosyns or degradates of spinosyns where the trimethylpyranosyl (forasamine) ring portion of the molecule has been modified or is missing. One exception is that the assay is sensitive to spinosyn K (Table I, Figure 1). The level of sensitivity is not equivalent for all spinosyns. Thus, when a mixture of spinosyns and degradates are present in a sample, the assay response generated by the individual components may not be additive.

3. False Positive/False Negative Rate

Twenty-one control samples were analyzed during the validation study. The samples included water from five distinct sources and analyses by two analysts. There were no false positives (false positive is defined as a measured concentration of spinosad at or above the proposed or calculated LOD) (Table IV). Five samples from a single water source were fortified with 0.05 ng/mL of spinosad to determine the false negative rate. Spinosad was detected in each of the samples (Table IV).

5. Confirmatory Method

The detection and/or quantitation of spinosyn A, spinosyn B, spinosyn D, and *N*-demethyl spinosyn D in water can be confirmed by HPLC using DowElanco analytical method GRM 94.12 (1).

6. Assay Time

The time required to analyze a typical analytical set (25 samples, four standards and the quality control sample in duplicate) is approximately two hours. The analysis time does not include the time required for the kit reagents to equlibrate to room temperature.

7. Correlation to a Non-Immunochemical Method

The immunochemical method reports a "total" of spinosad and related spinosyn residue, while the HPLC method quantifies the four analytes separately. The degree of correlation in results obtained using HPLC and immunoassay techniques can be variable. Some of the photolytic and anaerobic aquatic degradates of spinosad (5,6) cross-react with the antibody used to develop the spinosad test kit (Table I). Additionally, the immunoassay is sensitive to spinosyn A and spinosyn D which are the major components of spinosad; it will also detect many of the spinosyns which are minor components of this material. The HPLC method (1) is equally sensitive to the four spinosyns it measures (spinosyn A, spinosyn B, spinosyn D, and *N*-demethyl spinosyn D). The HPLC method was not designed to detect the aquatic degradates or minor components of spinosad.

Good agreement between the methods can be obtained from freshly fortified samples. However, due to the ability of the immunochemical method to detect several spinosyns, metabolites and degradates that are not measured when using the HPLC method, the immunochemical method generally measures higher concentrations of residue than the HPLC technique in aged water samples (7).

M. Notes

1. Equipment, glassware, materials, reagents and chemicals 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 not listed here.

2. Only the Ohmicron Spinosad RaPID Assay Test kit may be used for performing this method. Other test kits should not be substituted.

3. Store all kit reagents, sample diluent and proficiency samples at 2-8 °C. Do not freeze. Reagents may be used until the expiration date. The test tubes require no special storage conditions and may be stored separately from the kit reagents.

4. The standards and "quality control" sample provided with the kit are not used for this method.

5. The data reduction software of the RPA-1 Photometric Analyzer utilizes seven significant digits but only three are reported (8). Therefore, attempts to use the reported absorbance values to recalculate transformations, regressions or sample concentrations may result in rounding differences between recalculated and RPA-1 reported results. The difference between the recalculated and the RPA-1 reported results are small and will not impact the study results.

6. The blank concentration should only be subtracted if the measured concentration is greater than the LOD. Due to the sigmoidal dose-response curve inherent to competitive immunoassays, it is possible to calculate concentrations below the LOD; however, they are not distinguishable from zero (or blanks) with any degree of certainty. Measured concentrations in blank samples which are between the LOD and LOQ may be subtracted from the sample results. However, the analyst should recognize that a spinosad concentration measured between the LOD and LOQ may be inaccurate.

N. References

1. West, S.D., "Determination of XDE-105 and Metabolites in Water by High Performance Liquid Chromatography with Ultraviolet Detection," GRM 94.12, 1994, unpublished method of DowElanco.

2. Itak, J.A., "A Study to Determine the Behavior of Various Pesticides and Other Non-Pesticide (Organic and Inorganic) Compounds in the XDE-105 RaPID Assay," study number 1003, 1994, unpublished report of Ohmicron Environmental Diagnostics.

3. Keith, L.H.; Crummett,W.; Deegan, J.; Libby, R.A.; Taylor, J .T.; Wentler, G., "Principles of Environmental Analysis," *Anal. Chem.* 1983, 55:2210-2218.

4. OPP-00385 "Publication of Addenda for Data Reporting; Requirements for Pesticide Assessment Guidelines (N, E, and K), *Federal Register and Addenda*, Vol. 59, No. 128, 1994.

5. Reeves, G.L. "The Anaerobic Aquatic Metabolism of [^{14}C]-XDE-105," GHE-P-2989, 1993, unpublished report of DowElanco.

6. Saunders, D.G.; McGibbon, A.S., "Photodegradation of XDE-105 Factors A and D in pH 7 Buffer," GH-C 3044, 1994, unpublished report of DowElanco.

7. McGibbon, A.S., "Aquatic Microcosm Dissipation of XDE-105", ENV94057, 1994, unpublished protocol of DowElanco.

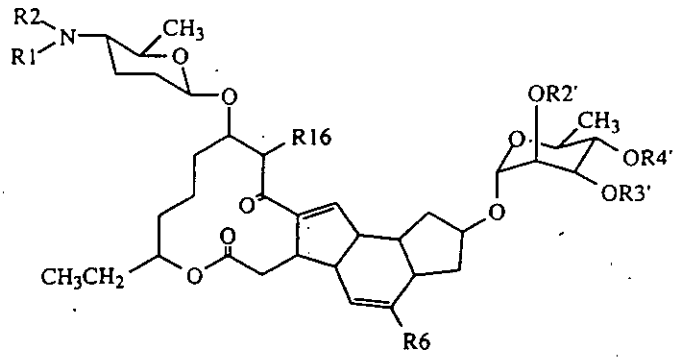
8. RPA-1 RaPID Photometric Analyzer Owner's Manual, Ohmicron Corporation, Newtown, PA, 1992.

Table I. Sensitivity of the Spinosad Immunoassay Test Kit to Individual Spinosyns, Metabolites and Degradates.

High Sensitivity (I_{50} < 2.0 ng/mL)	Low Sensitivity (I_{50} > 50 ng/mL)
Spinosyn A	Spinosyn H
Spinosyn B	Spinosyn J
Spinosyn C	Spinosyn L
Spinosyn D	Spinosyn A reverse pseudoaglycone
N-Demethyl Spinosyn D	Spinosyn A aglycone
Spinosyn E	
Spinosyn F	
Spinosyn K	
Spinosyn A Pseudoaglycone	

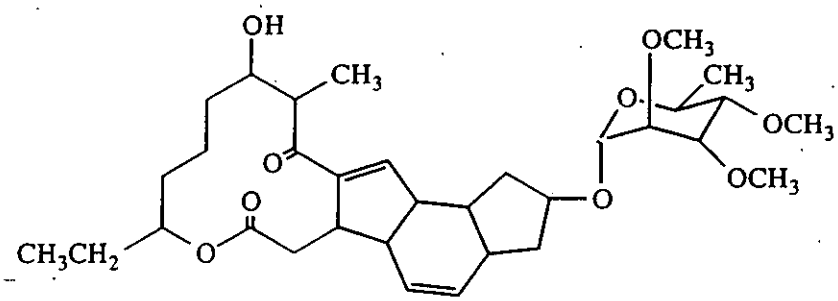
Table III. Compounds Tested for the Potential to Interfere in the Spinosad RaPID Assay Test Kit

Pesticides	Organic compounds	Inorganic compounds
Alachlor	N-Acetylglucosamine	Calcium (chloride dihydrate)
Aldicarb	Aflatoxin B1	Copper (chloride)
Azinphos methyl	Aflatoxin G1	Iron (chloride hexahydrate)
Carbaryl	Humic acid	Magnesium (chloride hexahydrate)
Carbendazim	β-Lactose	Manganese (chloride)
Carbofuran	Methyl oleate	Mercuric (chloride)
Chlorothalonil	Polyoxin D	Nickel (sulfate hexahydrate)
Chlorpyrifos	L(+) Rhamnose	Nitrate (sodium)
Chlorpyrifos methyl		Peroxide (hydrogen)
Cyanazine		Phosphate (sodium, heptahydrate)
2,4-D		Silicates (sodium meta-)
Dicamba		Sodium chloride
Dinoseb		Sulfate (sodium)
EPN		Slufite (sodium)
Iprodine		Thiosulfate (sodium, pentahydrate)
Malathion		Zinc (chloride)
Metalaxyl		
Methamidophos		
Methiocarb		
Methomyl		
Metribuzin		
Parathion		
Parathion methyl		
Phosmet		
Picloram		
Procymidone		
Propachlor		
Thiabendazole		
Triclopyr		
Vinclozolin		



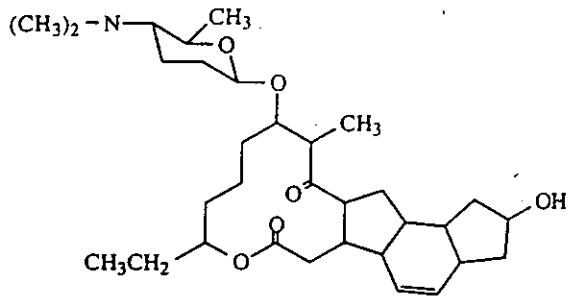
Spinosyn	R1	R2	R6	R16	R21	R2'	R3'	R4'
A	Me	Me	H	Me	Et	Me	Me	Me
B	H	Me	H	Me	Et	Me	Me	Me
C	H	H	H	Me	Et	Me	Me	Me
D	Me	Me	Me	Me	Et	Me	Me	Me
N-Demethyl D	H	Me	Me	Me	Et	Me	Me	Me
E	Me	Me	H	Me	Me	Me	Me	Me
F	Me	Me	H	H	Et	Me	Me	Me
H	Me	Me	H	H	Et	H	Me	Me
J	Me	Me	H	H	Et	Me	H	Me
K	Me	Me	H	H	Et	Me	Me	H
L	Me	Me	Me	H	Et	Me	H	Me

Me = Methyl
Et = Ethyl
H = Hydrogen

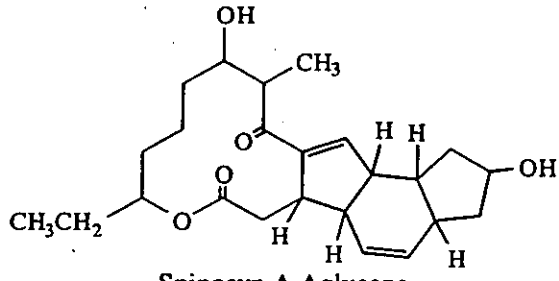


Spinosyn A Pseudoaglycone

Figure 1. Structures of Some Spinosyns and Spinosyn Metabolites and Degradates.



Spinosyn A Reverse Pseudoaglycone



Spinosyn A Aglycone

Figure 1 (Cont.) Structures of Some Spinosyns, and Spinosyn Metabolites, and Degradates