METHOD OF ANALYSIS

M-073: HPLC-FLUORESCENCE METHOD FOR THE QUANTITATION OF AVERMECTIN B₁ AND 8,9-Z AVERMECTIN B₁ IN/ON FRUITS AND VEGETABLES:

(Unaudited Method Report)

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ATTACHMENTS:

Method Validation Results For Different Commodities
Precautions:

Skin contact and inhalation should be avoided when handling avermectins.

A. PRINCIPLE

The residues of concern in/on the fruit or vegetable commodity derived from plants treated with the acaricide/insecticide abamectin (or avermectin B₁) are the parent compound, avermectin B₁, and the photodegrade, 8,9-Z avermectin B₁. Abamectin consists of a major component, designated as avermectin B₁a, and a minor component, designated as avermectin B₁b. In Method of Analysis M-073, the total residues of avermectin B₁ (avermectin B₁a + avermectin B₁b) plus 8,9-Z avermectin B₁ (8,9-Z avermectin B₁a + 8,9-Z avermectin B₁b) in/on fruits and vegetables is determined by high-performance liquid chromatography (HPLC) with fluorescence detection using the external standardization technique. The structures of avermectin B₁a, B₁b and 8,9-Z avermectin B₁a, B₁b isomers are shown in Figure 1.

The avermectins are extracted from the sample matrix with acetonitrile+0.1% phosphoric acid and the avermectins partitioned into hexane. The hexane extract is concentrated/purified on an aminopropyl solid phase extraction (SPE) column and the purified extract derivatized with trifluoroacetic anhydride. The derivatized avermectins are analyzed by reversed-phase HPLC with fluorescence detection. The avermectin B₁a standard curve is used to calculate the concentration of avermectin B₁a + 8,9-Z avermectin B₁a and avermectin B₁b + 8,9-Z avermectin B₁b in/on the sample. The limit of detection (LOD) is 1 ng/g of avermectin B₁a + 8,9-Z avermectin B₁a or avermectin B₁b + 8,9-Z avermectin B₁b. The limit of quantitation (LOQ) is 2 ng/g.
Figure 1. Structures of avermectin B₁ and 8,9-Z avermectin B₁.
Avermectin acaricide/insecticide is a mixture of ≥90% avermectin B₁a and
≤10% avermectin B₁b
B. APPARATUS

Equipment from manufacturers other than those listed below may be substituted provided they are shown to be functionally equivalent.

1. **Homogenizers:**
   
   a. Hobart food processor; Model 84142 or 84186 (Hobart Corporation)
   
   b. Cuisinart food processor; Model DLC-X (Cuisinarts Inc.)
   
   c. Hand-held laboratory homogenizer and emulsifier; Bio Homogenizer Model 133/1281-0 (Biospec Products Inc., Purchased from Fisher Scientific)

2. **Balances:**
   
   a. Mettler AT261 DeltaRange or any analytical balance accurate to at least 0.1 mg
   
   b. Ohaus Brainweigh B300 or any toploading balance accurate to at least 0.01 g

3. **Sonicator:**
   
   Bransonic ultrasonic cleaner; Model Branson 5200 (Branson Ultrasoundics Corporation)

4. **Mechanical shaker, reciprocating box type:**
   
   Model 6000 (Eberbach Corporation)

5. **Centrifuge:**
   
   IEC Model HN-SII (International Equipment Company)

6. **Centrifuge tube, polypropylene:**
   
   50 mL conical tubes with graduation, Falcon Blue Max (Becton Dickinson Labware, catalog no. 2070)

7. **Disposable glass tube, borosilicate glass:**
   
   (Fisher Scientific, catalog no. 14-961-29)
8. **Micropipet:**
   100 and 1000 μL
   (EDP-2 electronic digital pipette, Rainin Instrument Co., Inc., catalog no. E2-100 and E2-1000)

9. **Volumetric Transfer/Mohr pipet:**
   0.5, 1, 2, 3, 4, 5, 10, 15 and 20 mL

10. **SAMCO transfer pipet, polyethylene:**
    15 mL
    (Saint-Amand Manufacturing Company, Inc., catalog no. 252 Sedi-Pet)

11. **Graduated cylinder:**
    25, 100, 250, 500 and 1000 mL.

12. **Volumetric flask:**
    10, 25, 100 and 1000 mL.

13. **Erlenmeyer flask:**
    125 mL.

C. **REAGENTS**

Reagents from manufacturers other than those listed below may be substituted provided they are shown to be functionally equivalent.

1. **Solvents, HPLC-Grade:**

   *Hexane*  
   (OmniSolv, EM Science, catalog no. HX0298-1)
   *Methanol*  
   (OmniSolv, EM Science, catalog no. MX0488-1)
   *Acetonitrile*  
   (OmniSolv, EM Science, catalog no. AX0142-1)
   *Ethyl acetate*  
   (OmniSolv, EM Science, catalog no. EX0241-1)

2. **Chemicals:**

   *Trifluoroacetic anhydride*  
   (Aldrich Chemical Co., catalog no. 10,623-2)
   *1-Methylimidazole*  
   (Fluka, catalog no. 67560)
   *Triethylamine*  
   (Aldrich Chemical Co., catalog no. 13,206-3)
   *Ethanolamine*  
   (Aldrich Chemical Co., catalog no. 11,016-7)
   *Sodium sulfate, anhydrous*  
   (Fisher Scientific, catalog no. S421)
   *Phosphoric acid, 85%*  
   (Fisher Scientific, catalog no. A260)

3. **Deionized water:**

   Prepared using the Millipore Milli-Q Reagent Water System (Millipore Corporation)

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4. **0.1% Phosphoric acid:**
Add about 500 mL water to a 1000 mL volumetric flask. Add 1 mL phosphoric acid (85%) and mix well. Dilute to the 1000 mL mark with water and mix well.

5. **Acetonitrile/0.1% Phosphoric acid (25+75):**
Mix together 125 mL acetonitrile and 375 mL 0.1% phosphoric acid.

6. **Ethyl acetate/Methanol (75+25):**
Mix together 75 mL ethyl acetate and 25 mL methanol.

7. **Acetonitrile/Triethylamine (95+5):**
Mix together 95 mL acetonitrile and 5 mL triethylamine.

8. **HPLC mobile phase:**
Mix together 920 mL methanol, 80 mL water and 0.5 mL ethanolamine.

**D. SOLID PHASE EXTRACTION (SPE) SYSTEM**

Equipment from manufacturers other than those listed below may be substituted provided they are shown to be functionally equivalent.

1. **SPE column:**
500 mg/3 mL Regular Bond Elut
NH₂ Aminopropyl
(Varian Sample Preparation Products, catalog no. 1210-2041)

2. **Regular Bond Elut accessories:**
Reservoir (empty), 75 mL capacity - catalog no. 1213-1012
Bond Elut adaptor - catalog no. 1213-1001
Luer stopcock - catalog no. 1213-1005
(Varian Sample Preparation Products)

3. **Vacuum manifold:**
(Supelco Inc.)
E. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) SYSTEM

Equipment from manufacturers other than those listed below may be substituted provided they are shown to be functionally equivalent.

1. **HPLC column:**
   Chromegabond MC18
   Column dimension = 150 x 4.6 mm; Particle size = 3 μm; Pore size = 60 Å
   (ES Industries, catalog no. 135111-MC18)

2. **HPLC guard column:**
   BDS Hypersil C18 guard column
   (Keystone Scientific, Inc., Catalog no. 88402-46-P)

3. **HPLC pump:**
   P200-300 Pump, Series Binary
   (Thermo Separation Products)

4. **Autosampler:**
   SpectraSYSTEM AS3000 with built-in column oven
   (Thermo Separation Products)

5. **Detector, fluorescence:**
   a. Model RF-551 Fluorescence HPLC Monitor - A Grating Monochromator
      Fluorescence Detector
      Excitation Wavelength, 365 nm; Emission Wavelength, 470 nm;
      Flow Cell, 12 μL
      (Shimadzu Corporation)
   b. Model FD-100 Filter Fluorimeter
      Excitation Filter, 365 nm; Emission Filter, 470 nm; Flow Cell, 24 μL
      (GTI/SpectroVision)

6. **Integrator:**
   Rainin Dynamax MacIntegrator I installed on a Macintosh Color Classic with
   ImageWriter II printer
   (Rainin Instrument Company)
F. ANALYTICAL STANDARD

Avermectin B₁
Analytical standard of avermectin B₁ of known purity.
Analytical standard L-676,863-038A005 (avermectin B₁ dissolved in glycerol formal) containing 0.819% w/w avermectin B₁₈ component and 0.054% w/w avermectin B₁₉ component is acceptable (source: Merck & Co., Inc., Rahway, NJ).

G. PREPARATION OF AVERMECTIN B₁ STANDARD SOLUTIONS

1. Avermectin B₁ Standard Stock Solution

Weigh accurately about 310 mg of the avermectin B₁ analytical standard into a 100 mL volumetric flask. Dilute the flask to the 100 mL mark with acetonitrile and mix well. Label the flask “Avermectin B₁ Standard Stock Solution”.
The stock solution contains approximately 25 µg/mL of the avermectin B₁₈ component and 1.7 µg/mL of the avermectin B₁₉ component (see Notes 1 and 2).

Note 1: The weight of analytical standard can be adjusted depending on the concentration of avermectin B₁ in the HPLC working standards used for the analysis of avermectin in the samples.

Note 2: Solutions of avermectin B₁ in acetonitrile are stable for a least three (3) months when stored in the dark at -10 °C or lower.

2. Avermectin B₁ Intermediate Standard Stock Solution

Transfer a 1.0 mL aliquot of the “Avermectin B₁ Standard Stock Solution” to a 100 mL volumetric flask. Dilute the flask to the 100 mL mark with acetonitrile and mix well. Label the flask “Avermectin B₁ Intermediate Standard Stock Solution”. The intermediate standard stock solution contains approximately 250 ng/mL of the avermectin B₁₈ component and 17 ng/mL of the avermectin B₁₉ component (see Note 3).

Note 3: The “Avermectin B₁ Intermediate Standard Stock Solution” should be diluted with acetonitrile for use in the fortification of a known weight of control fruit or vegetable commodity for method recovery determinations.
3. **Avermectin B₁₈ HPLC Working Standard Solutions**
   (see Notes 4 and 5)

   a. Transfer a 1.0 mL aliquot of the "Avermectin B₁ Intermediate Standard Stock Solution" to a 25 mL volumetric flask. Dilute the flask to the 25 mL mark with acetonitrile and mix well. Label the flask "STD A". The solution contains approximately 10 ng/mL of the avermectin B₁₈ component.

   b. Transfer 1.0 mL, 2.0 mL, 3.0 mL and 4.0 mL aliquots of the "Avermectin B₁ Intermediate Standard Stock Solution" to separate 10 mL volumetric flasks. Dilute each flask to the 10 mL mark with acetonitrile and mix well. Label the flasks "STD B", "STD C", "STD D" and "STD E", respectively. The HPLC working standard solutions (STD B, STD C, STD D and STD E) contain approximately 25 ng/mL, 50 ng/mL, 75 ng/mL and 100 ng/mL of the avermectin B₁₈ component, respectively.

**Note 4:**
The concentration of avermectin B₁₈ in the HPLC working standard solutions can be adjusted depending on the concentration of avermectin B₁ in the samples.

**Note 5:**
The avermectin B₁₈ standard curve is used to calculate the amount of avermectin B₁₈ + 8,9-Z avermectin B₁₈ and avermectin B₁₆ + 8,9-Z avermectin B₁₆ in/on the sample.
H. SAMPLE PROCESSING

1. Transfer a representative amount of the fruit or vegetable sample into either a Hobart food processor, Cuisinart food processor or equivalent (see Notes 6 and 7).

   **Note 6:** Details about the fruit or vegetable portion to be processed are given in the method validation report for each commodity (see Attachments).

   **Note 7:** Untreated samples should be processed first before treated samples. For treated samples, samples suspected to contain the lowest residues should be processed first followed by samples suspected to contain the highest residues.

2. Process the sample until a homogenous blend is achieved and transfer to a suitable container (see Notes 8 and 9).

   **Note 8:** Dry ice may be added to the sample to achieve better processing and to prevent thawing of the frozen sample. Allow the dry ice to sublime before tightening the cap of the storage container.

   **Note 9:** A container made of HDPE (high density polyethylene) is acceptable.

3. Store the samples at -10 °C or colder (in a freezer) prior to analysis.

I. SAMPLE ANALYSIS

   **Note 10:** A method recovery sample should be assayed concurrently with the sample set. For example, fortify a 5 g test portion of the homogenized sample with 1 mL of "STD C".

   **Note 11:** About 10-12 samples can be prepared for HPLC analysis in a typical 8 hour working day.

1. **Extraction**

   a. Transfer a 5 g portion of the processed sample to a clean 50 mL conical, polypropylene centrifuge tube.

   b. **For raw agricultural commodity (RAC) samples:**
      Add 20 mL acetonitrile/0.1% phosphoric acid (25+75) solution to the sample in the centrifuge tube.

      **For dried samples (processed commodity):**
      Add 15 mL 0.1% phosphoric acid solution to the sample in the centrifuge tube and shake for 1 hr. Add 5 mL acetonitrile to the sample.
c. Blend the sample using the hand-held laboratory homogenizer. Rinse the blender probe by blending with 20 mL hexane contained in another 50 mL centrifuge tube (see Note 12). Add the hexane rinse solution to the original extract and shake for about 5 min in a reciprocating shaker. Centrifuge the extract for 10 min at about 2500 rpm.

*Note 12:* After each sample, rinse the blender probe with water followed by methanol.

d. Using a disposable polyethylene transfer pipet, transfer the hexane layer containing the avermectins to a 125 mL Erlenmeyer flask.

e. Extract the avermectins from the aqueous extract using two more portions of 20 mL hexane. Combine the hexane extracts in the 125 mL Erlenmeyer flask.

f. Add approximately 1 g of anhydrous sodium sulfate to the combined hexane extracts. Mix well (see Note 13).

*Note 13:* Samples should not be stored in hexane overnight and processed the next day since avermectins tend to be adsorbed on to glass surfaces when stored in hexane for extended periods of time.

2. **Clean-up of Final Extract on SPE Column**

a. Attach a 500 mg/3 mL aminopropyl SPE cartridge, fitted with an adaptor and a 75 mL reservoir, to the vacuum manifold (see Note 14).

*Note 14:* Recoveries of avermectins have been validated using only Varian Regular Bond Elut SPE columns (aminopropyl, 500 mg/3 mL). Avermectin method recoveries should be confirmed if SPE columns from other manufacturers are substituted for the Varian SPE columns.

b. Apply a mild vacuum and condition the SPE column with 10 mL methanol followed by 10 mL hexane. Discard the rinse solutions (see Notes 15 and 16).

*Note 15:* The SPE column should not be allowed to run dry during the clean-up procedure.

*Note 16:* The flow rate through the SPE column should not exceed 3-4 drops per second.

c. Transfer the entire hexane extract to the SPE column. Apply mild vacuum and allow the hexane solution to drain from the column. Discard the hexane.
d. Rinse the flask and sodium sulfate with 10 mL of hexane. Add the rinse solution to the SPE column. Apply a mild vacuum and allow the hexane solution to drain from the column. Discard the hexane.

e. Rinse the SPE column with 3 mL of ethyl acetate using a mild vacuum. Discard the ethyl acetate rinse.

f. Elute the avermectins from the column with 2 mL of ethyl acetate/methanol (75+25) solution using mild vacuum. Collect the eluate in a test tube (see Note 17).

**Note 17:** Residual ethyl acetate/methanol (75+25) solution remaining in the SPE column should be drained and collected using moderate to high vacuum.

g. Evaporate the ethyl acetate/methanol solution to dryness under a stream of nitrogen using a heat lamp or heating block at a temperature not to exceed 35 °C.

h. Add 0.5 mL of acetonitrile to the tube, allow to stand for 5 min and sonicate the mixture for about 2 min to dissolve all the residue (see Note 18).

**Note 18:** All residue should dissolve in the acetonitrile added.

**STOPPING POINT!** If required, samples may be stored overnight at 0 °C or colder (in freezer).

3. **Derivatization of Samples and Standards**

a. To 0.5 mL of each sample (step I-2h) and working standards (step G-3) contained in a glass tube add 0.5 mL of acetonitrile/triethylamine (95+5) solution. Sonicate the mixture for about 1 min.

b. Add 50 µL of 1-methylimidazole to each sample and standard tube. Mix well.

c. Add 50 µL of trifluoroacetic anhydride to each sample and standard tube. Mix well after each addition. Allow the solution to stand for approximately 3 min (see Note 19).

**Note 19:** Caution! Trifluoroacetic anhydride (TFAA) produces irritation and necrosis of tissues. Avoid contact with skin and eyes. The reaction of TFAA with each sample and standard is extremely exothermic and should be performed under a hood.
d. Add 1 mL of HPLC mobile phase to each tube. Mix well (see Notes 20 and 21).

**Note 20:** The approximate concentration of derivatized avermectin B₁₄ in the standards range from 2.4 ng/mL to 24 ng/mL after dilution with the HPLC mobile phase.

**Note 21:** The derivatized avermectins are stable for at least 24 hours when stored in the freezer.

*STOPPING POINT!* If required, samples may be stored overnight at 0 °C or colder (in freezer).

J. HPLC ASSAY CONDITIONS

The HPLC assay conditions cited below are provided as a guide in establishing operating conditions. Conditions should be adjusted as required to obtain chromatographic peak shape, resolution and sensitivity equivalent to or better than those shown in Figure 2 and in the attached representative chromatograms.

1. **Mobile phase:**
   Methanol/Water/Ethanolamine (92+8+0.05)

2. **Flow rate:**
   0.80 mL/min

3. **Column temperature:**
   40 °C

4. **Sample injection volume:**
   50 μL

5. **Detector:**
   Fluorescence
   Excitation wavelength = 365 nm
   Emission wavelength = 470 nm
Figure 2. Sample chromatograms for control fresh prune RAC (Top), control fresh prune RAC fortified with avermectin B₁₈ at the 2 ppb level (Middle) and 100 ng/mL avermectin B₁₈ HPLC standard (Bottom). Amounts of analyte injected for the bottom chromatogram: 1.2 ng avermectin B₁₈ and 0.08 ng avermectin B₁₁₈.
6. Approximate retention times for avermectin derivatives:
   (see Note 22)
   Avermectin B\(_{1b}\)/8,9-Z Avermectin B\(_{1b}\) = About 9.5 min
   Avermectin B\(_{1a}\)/8,9-Z Avermectin B\(_{1a}\) = About 11.0 min

   Note 22: The retention times indicated are approximate and will change from column to column, and with variations in the composition of the mobile phase and column temperature.

7. HPLC Run Time:
   Integrator = 15 min
   Autosampler = 17 min

K. HPLC ANALYSIS OF SAMPLES AND STANDARDS

   Note 23: Derivatized sample solutions (from Section I.3.d) exceeding 24 ng/mL of avermectin B\(_{1a}\) or avermectin B\(_{1b}\) should be diluted to approximately 10 ng/mL of avermectin B\(_{1a}\) or avermectin B\(_{1b}\) with HPLC mobile phase prior to analysis.

Before beginning the analyses of derivatized samples and standards, inject a derivatized avermectin standard and note the retention time and separation of the avermectin B\(_{1a}\) and avermectin B\(_{1b}\) peaks. If reproducibility of the autosampler is not known, make at least two injections of the derivatized standard to determine the precision of the injection system.

The following injection sequence is recommended for the analysis of sample and standard solutions:

1. Inject aliquots of the derivatized avermectin working standards. Inject aliquots of the derivatized sample solutions followed by injection of aliquots of the derivatized avermectin working standards.

2. The sample set should include a derivatized control sample and at least one avermectin B\(_{1a}\) method recovery sample. The method recovery sample should yield a value between 70-120% of the theoretical fortification level.

L. CONFIRMATORY TECHNIQUE

Identification of the avermectin B\(_{1a}\)/8,9-Z avermectin B\(_{1a}\) and avermectin B\(_{1b}\)/8,9-Z avermectin B\(_{1b}\) peaks is confirmed by direct comparison of HPLC retention times obtained for the sample peaks versus retention times observed for the avermectin B\(_{1a}\) and avermectin B\(_{1b}\) HPLC working standards.
M. CALCULATIONS

Note 24: Since both avermectin B₁₄ and 8,9-Z avermectin B₁₄ or avermectin B₁₇b and 8,9-Z avermectin B₁₇b yield identical products after derivatization, the method quantitates the sum of avermectin B₁₄+8,9-Z avermectin B₁₄ and avermectin B₁₇b+8,9-Z avermectin B₁₇b in the sample. The avermectin B₁₄ standard curve is used to quantify avermectin B₁₇b residues since the response factors for derivatized avermectin B₁₄ and avermectin B₁₇b have been shown to be equivalent.

The ng/g of avermectin B₁₄+8,9-Z avermectin B₁₄ and avermectin B₁₇b+8,9-Z avermectin B₁₇b in the sample is determined from the avermectin B₁₄ standard curve.

1. At the completion of the HPLC run, determine the linear regression coefficients (slope and y-intercept) for the avermectin B₁₄ standard curve by plotting the concentrations of avermectin B₁₄ HPLC working standards (ng/mL) vs. the response (avermectin B₁₄ peak area) of the standards. Determine the coefficient of determination (r²) for the standard curve. The r² value should be greater than 0.97 (see Note 25).

Note 25: The 10–100 ng/mL avermectin B₁₄ HPLC working standards are plotted since the standards and samples are derivatized in an identical manner.
2. Use Eqn. 1 to calculate the concentration of avermectin B$_{1a}$+8,9-Z avermectin B$_{1a}$ and avermectin B$_{1b}$+8,9-Z avermectin B$_{1b}$ in the sample (see Note 23):

\[
C = \frac{(R - I) \times V \times DF}{S \times W}
\]  
(Eqn. 1)

- **C** = Concentration (ng/g) of avermectin B$_{1a}$+8,9-Z avermectin B$_{1a}$ or avermectin B$_{1b}$+8,9-Z avermectin B$_{1b}$ in/on the sample.
- **R** = Response (peak area) of avermectin B$_{1a}$ or avermectin B$_{1b}$ in the sample.
- **I** = y-Intercept of the avermectin B$_{1a}$ standard curve.
- **V** = Volume (mL) of sample before derivatization (0.5 mL).
- **DF** = Sample dilution factor. The dilution factor is equal to 1 if no further dilution of the derivatized sample is made.
- **S** = Slope of the avermectin B$_{1a}$ standard curve.
- **W** = Weight of sample (g).

**N. REPORTING RESULTS**

Reporting of residue values of avermectin B$_{1a}$+8,9-Z avermectin B$_{1a}$ and avermectin B$_{1b}$+8,9-Z avermectin B$_{1b}$ in the samples should follow the guidelines shown below:

a. Residue values less than 1 ng/g (LOD) should be reported as ND (not detected).

b. Residue values between 1 ng/g and 1.9 ng/g (LOQ equals 2 ng/g) should be reported as NQ (not quantitated).
O. METHOD VALIDATION RESULTS

1. Introduction:

The method validation performed for each commodity included the determination of the accuracy, precision, linearity, specificity and limits of detection (LOD) and quantitation (LOQ). The method was validated by Merck Research Laboratories, Three Bridges, NJ. Method validation results obtained for each commodity can be seen in the attachments to the report.

2. Fortifications (Method Recovery Samples):

An aliquot of the homogenized sample was fortified with the desired analyte (avermectin B\textsubscript{1a}, avermectin B\textsubscript{1b} or 8,9-Z avermectin B\textsubscript{1a}) at the following levels (see Notes 23 and 26):

- 2, 10, 50 and 100 ppb avermectin B\textsubscript{1a}
- 2 ppb avermectin B\textsubscript{1b}
- 2, 10 and 50 ppb 8,9-Z avermectin B\textsubscript{1a} (see Note 27)

Note 26: The active ingredient in the acaricide/insecticide abamectin (avermectin B\textsubscript{1}) is a mixture of at least 80% avermectin B\textsubscript{1a} and not more than 20% avermectin B\textsubscript{1b} (typically 90% avermectin B\textsubscript{1a} and 10% avermectin B\textsubscript{1b}).

Note 27: Solutions of 8,9-Z avermectin B\textsubscript{1a} in acetonitrile should be used for fortification of the control fruit or vegetable commodity for method recovery determinations. Preparation of the 8,9-Z avermectin B\textsubscript{1a} fortification solutions used for method validation can be seen in Section O.3 of the report.

For each sample matrix, controls (0 ppb) and fortified samples are extracted and assayed for the desired analyte according to Method of Analysis M-073. Analyte recovery for each sample matrix is determined by comparing the amount of analyte added to the amount of analyte found.
3. Preparation of 8,9-Z Avermectin B₁₄ Fortification Solutions:

**Note 28:** An 8,9-Z avermectin B₁₄ of known purity should be used to prepare the fortification solutions used for the determination of the method recoveries for 8,9-Z avermectin B₁₄. Analytical standard L-652,280-002T002 (8,9-Z avermectin B₁₄) dissolved in glycerol formal containing 0.26% w/w 8,9-Z avermectin B₁₄ is acceptable (source: Merck & Co., Inc., Rahway, NJ).

**Note 29:** Solutions of 8,9-Z avermectin B₁₄ in acetonitrile are stable for at least three (3) months when stored in the dark at -10 °C or lower.

a. Weigh accurately about 240 mg of the 8,9-Z avermectin B₁₄ analytical standard into a 25 mL volumetric flask. Dilute the flask to the 25 mL mark with acetonitrile and mix well. Label the flask "8,9-Z Avermectin B₁₄ Stock Solution". The stock solution contains approximately 25 μg/mL of the 8,9-Z avermectin B₁₄ component.

b. Transfer a 1.0 mL aliquot of the "8,9-Z Avermectin B₁₄ Stock Solution" to a 100 mL volumetric flask. Dilute the flask to the 100 mL mark with acetonitrile and mix well. Label the flask "8,9-Z Avermectin B₁₄ - 250 ng/mL Solution". The solution contains approximately 250 ng/mL of the 8,9-Z avermectin B₁₄ component.

c. Transfer a 5.0 mL aliquot of the "8,9-Z Avermectin B₁₄ - 250 ng/mL Solution" to a 25 mL volumetric flask. Dilute the flask to the 25 mL mark with acetonitrile and mix well. Label the flask "8,9-Z Avermectin B₁₄ - 50 ng/mL Solution". The solution contains approximately 50 ng/mL of the 8,9-Z avermectin B₁₄ component.

d. Transfer a 5.0 mL aliquot of the "8,9-Z Avermectin B₁₄ - 50 ng/mL Solution" to a 25 mL volumetric flask. Dilute the flask to the 25 mL mark with acetonitrile and mix well. Label the flask "8,9-Z Avermectin B₁₄ - 10 ng/mL Solution". The solution contains approximately 10 ng/mL of the 8,9-Z avermectin B₁₄ component.
4. **Determination of the Accuracy of the Method:**

The accuracy of the analytical method is the statistical agreement of the test results obtained by the analytical method to the theoretical value. The accuracy (recovery) for each analyte at each fortification level is calculated using Eqn. 2.

\[
\text{Recovery (\%)} = \frac{\text{ng/g Analyte Found}}{\text{ng/g Analyte Added}} \times 100 \quad \text{(Eqn. 2)}
\]

5. **Determination of the Precision of the Method:**

The precision of the analytical method is the statistical agreement among individual test results on multiple sampling of a homogeneous sample or samples. The precision of the analytical method is expressed as the relative standard deviation of the test results. The precision of the analytical method is calculated from the standard deviation and the average recovery value using Eqn. 3.

\[
\text{Precision (\%)} = \frac{\text{Standard Deviation}}{\text{Average Recovery}} \times 100 \quad \text{(Eqn. 3)}
\]

6. **Determination of the Linearity of the Method:**

Linear regression analysis of the plot of avermectin B\textsubscript{1a} peak area versus avermectin B\textsubscript{1a} concentration (ng/mL) should yield a coefficient of determination (r\textsuperscript{2}) greater than 0.97 in all cases.

7. **Determination of the Specificity of the Method:**

Interferences from the control commodity matrix and reagents subjected to the analytical procedure should be less than the limit of detection (LOD) for apparent residues of avermectin B\textsubscript{1a}+8,9-Z avermectin B\textsubscript{1a} or avermectin B\textsubscript{1b}+8,9-Z avermectin B\textsubscript{1b}.
8. **Determination of the Limits of Detection (LOD) and Quantitation (LOQ) of the Method:**

a. The LOD of the method is defined as the lowest concentration of avermectin that the analytical method can reliably detect (signal-to-noise ratio, S/N > 3).

b. The LOQ of the method is defined as the lowest concentration of avermectin that the analytical method can quantitate with acceptable recovery (S/N > 10).
Fax

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Number of Pages (Including Cover Page)  24

Date  11-11-97

Subject  Abamectin Crop Residue Method

Message