

MORSE LABORATORIES, INC.

**DETERMINATION OF ABAMECTIN RESIDUES IN FRUITS
AND VEGETABLES (RAW AGRICULTURAL COMMODITY)
BY LC-MS/MS**

Analytical Method# Meth-192, Revision #2

August 20, 2008

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Date:

Aug 20, 2008

TABLE OF CONTENTS

	<u>Page</u>
TITLE PAGE	1
TABLE OF CONTENTS	2
1 PRINCIPLE	3
2 EQUIVALENCE STATEMENT	3
3 APPARATUS AND EQUIPMENT	3
4 REAGENTS AND MATERIALS	5
5 REFERENCE STANDARDS	7
6 STANDARD PREPARATION	10
7 SAMPLE FORTIFICATION	14
8 SAMPLE EXTRACTION	14
9 AMINOPROPYL SPE CARTRIDGE CLEANUP (500mg/3mL)	15
10 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS	16
11 CALCULATIONS	18
12 REFERENCE	20
APPENDIX I Analysis Flowchart	21
APPENDIX II Silylation of Glassware.....	23
APPENDIX III Quality Control for SPE Cartridges	26
APPENDIX IV Mass Spectra for Parent and Product-ions	27

DETERMINATION OF ABAMECTIN RESIDUES IN FRUITS AND VEGETABLES (RAW AGRICULTURAL COMMODITY) BY LC-MS/MS

1 PRINCIPLE

The method described herein is capable of determining abamectin residues (avermectin B_{1a}, avermectin B_{1b}, and 8,9-Z avermectin B_{1a}) in a variety of raw agricultural commodity (RAC) fruits and vegetables. It is based on Novartis Crop Protection, Inc. Method M-073.1 (Reference 1).

Residues of avermectin B_{1a}, avermectin B_{1b}, and 8,9-Z avermectin B_{1a} are extracted from homogenized crop samples with acetonitrile:0.1% H₃PO₄ (25:75, v/v). The isolated residues are then partitioned into hexane. A suitable aliquot of the hexane extract is purified using aminopropyl solid phase extraction (SPE) cleanup. The purified extract is evaporated to dryness, reconstituted in acetonitrile, then submitted to HPLC analysis. During routine analysis, determination and quantitation of all targeted analytes are conducted using HPLC employing mass spectrometric (MS/MS) detection (LC-MS/MS). The limit of quantitation (LOQ) for all three analytes, in all matrices, is 0.002 ppm.

2 EQUIVALENCE STATEMENT

During the conduct of this analysis, comparable apparatus, solvents, glassware, and techniques (such as sample extract evaporation) may be substituted for those described in this method, except where specifically specified. In the event a substituted piece of equipment or technique is used, its use will be documented in the study records.

3 APPARATUS AND EQUIPMENT

Assorted laboratory glassware

Balances:	Analytical balance capable of weighing to ±0.1 mg Top-loading balance capable of weighing to ±0.01 g
Centrifuge:	Centrifric™ centrifuge (Fisher Scientific, Fair Lawn, NJ) IEC Clinical centrifuge (International Equipment Co., Needham Heights, MA)
Centrifuge bottles:	HDPE, 250-mL
Centrifuge tubes:	Polypropylene centrifuge tubes with screw cap closures, 50-mL (VWR Scientific, Bridgeport, NJ). For extraction and storage.

Evaporator:	N-Evap Laboratory Sample Evaporator, Model 115, attached to a nitrogen source (Organomation Associates, South Berlin, MA)
Graduated cylinders:	Glass; 1000, 100, and 50-mL Polypropylene, 100-mL
Graduated mixing cylinders:	Glass; 500, 250, 100, 50, and 25-mL
Homogenizer:	Omni Mixer Model 17105 with Generator Probe (Omni International, Waterbury, CT)
HPLC/MS system:	Applied BioSystems/MDS Sciex API 4000 LC/MS/MS system with a Shimadzu SIL-HTA autosampler, an integrated Shimadzu chromatograph consisting of (2) LC-10AD _{vp} Liquid Chromatograph units and a DGU-14A Degasser. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.
HPLC column:	50-mm × 2.0-mm i.d. Imtakt Cadenza CD-18, 3 μ particle size (Imtakt Corporation, Kyoto, Japan)
HPLC sample filter:	Nylon 66 filters, 4 mm, 0.45 μ m (Thomson Instrument Company, Oceanside, CA)
Microliter syringes:	Various sizes, (Hamilton Co., Reno, NV)
Pasteur pipets:	Glass, 9 inch and 5½ inch, disposable
Pipets:	Glass, graduated, serological; 25, 10, 5, 2 and 1 mL Glass, volumetric; 2.0, 1.0 and 0.5 mL
Pipets, adjustable:	<u>Finnpipette digital pipettors:</u> <i>Finnpipette</i> , 5-40 μ L: VWR Scientific Catalog #53515-038 <i>Finnpipette</i> , 40-200 μ L: VWR Scientific Catalog #53515-052 <i>Finnpipette</i> , 100-1000 μ L: Fisher Catalog #14-386-74

Pipets, adjustable (continued):	<u>Pipet tips:</u>
	1-200 μ L: VWR Scientific Catalog #53508-810
	100-1000 μ L: VWR Scientific Catalog #53516-164
Pipets, electronic:	EDP electronic pipets with 1000 μ L (1.00 mL) to 10.0 mL liquid ends and suitable pipet tips (Rainin Instrument Co., Inc., Ridgefield, NJ)
Pipets, transfer:	Polyethylene (VWR Scientific, Bridgeport, NJ)
Platform shaker:	Eberbach Model 6000 (Eberbach Corp., Ann Arbor, MI)
Solid Phase Extraction Apparatus:	Visiprep 12 or 24-port SPE vacuum manifold with disposable flow control liners (Supelco, Bellefonte, PA) Vac Elut SPS 24 (Varian Sample Preparation Products, Harbor City, CA)
Solid Phase Cartridge Reservoirs w/ Adaptors:	20 mL (Varian Sample Preparation Products, Harbor City, CA)
Syringe:	Glass, 2.5 mL, Hamilton Teflon [®] Luer-Lok (Hamilton Co., Reno, NV)
Test (culture) tubes:	Glass, silylated, 13 \times 100 mm and 16 \times 100 mm
Ultrasonic bath:	Branson Model 2210 ultrasonic bath (VWR Scientific, Bridgeport, NJ)
Vacuum pump:	Air Cadet Model 7530-40 vacuum pump, (Cole-Parmer Instrument Co., Chicago, IL)
Volumetric flasks:	Glass, various sizes
Vortex mixer:	VWR Scientific, Bridgeport, NJ

4 REAGENTS AND MATERIALS

Acetone: OmniSolv[®] (EM Science, Gibbstown, NJ)

Acetonitrile:	OmniSolv [®] (EM Science, Gibbstown, NJ) HPLC grade, B&J (Burdick and Jackson) Brand [®] High Purity Solvent (VWR Scientific Products, Bridgeport, NJ)
Ammonium acetate:	HPLC grade (Fisher Scientific, Fairlawn, NJ)
Analytical standards:	Abamectin reference material (mixture of avermectin B _{1a} and avermectin B _{1b}) 8,9-Z avermectin B _{1a} reference material
Dimethyldichlorosilane:	Catalog # 3-3009 (Supelco, Inc., Bellefonte, PA). Also referred to as "DMDCS".
Ethyl acetate:	OmniSolv [®] (EM Science, Gibbstown, NJ)
Hexane:	(95% n-hexane), Ultra Resi-analyzed [®] (J.T. Baker Chemical Company, Phillipsburg, NJ)
Methanol:	HPLC Grade (Burdick and Jackson, Muskegon, MI) OmniSolv [®] (EM Science, Gibbstown, NJ)
Phosphoric acid:	85%, HPLC grade (Fisher Scientific, Fairlawn, NJ)
Sodium sulfate:	Anhydrous, granular (10-60 mesh), AR [®] (ACS) (Mallinckrodt Chemicals, Phillipsburg, NJ)
Solid phase extraction cartridges:	Bond Elut, Aminopropyl SPE cartridges, 500mg/3mL (Varian Sample Preparation Products, Harbor City, CA)
Water:	Deionized (DI) water (Polymetrics System, Morse Laboratories, Inc.) HPLC Grade water (Fisher Scientific, Fair Lawn, NJ) or B&J (Burdick and Jackson) Brand [®] High Purity Solvent (VWR Scientific Products, Bridgeport, NJ)

4.1 Reagents and Materials to be Prepared (including typical preparation instructions)

- 4.1.1 *0.1% phosphoric acid:* To a 500-mL mixing cylinder, add approximately 400 mL deionized water, then add 0.5 mL phosphoric acid (85%). Carefully swirl flask to mix contents. Bring to a final volume of 500 mL with deionized water. Transfer to a properly labeled secondary container. Mix well. Prepare as needed.

- 4.1.2 *Acetonitrile:0.1% phosphoric acid (25:75, v/v)*: To a 500-mL mixing cylinder, add 125 mL of acetonitrile and bring to a final volume of 500 mL with 0.1% phosphoric acid. Mix thoroughly. Prepare as needed.
- 4.1.3 *Ethyl acetate:methanol (75:25, v/v)*: To a 100-mL mixing cylinder, add 25 mL of methanol and bring to a final volume of 100 mL with ethyl acetate. Mix thoroughly. Prepare as needed.
- 4.1.4 *HPLC mobile phases*:

100 mM NH₄OAc in methanol. Add 0.77 g of ammonium acetate (NH₄OAc) to a 100-mL volumetric flask and dilute to the mark with HPLC grade methanol. Sonicate and shake thoroughly until all ammonium acetate is completely dissolved.

Component A: *Water:100 mM NH₄OAc in methanol (95:5, v/v)*. To a 1 liter graduated cylinder add 950 mL HPLC grade water and 50 mL of 100 mM NH₄OAc in methanol. Transfer entire solution to the HPLC solvent reservoir and once transferred, mix thoroughly.

Component B: *5 mM NH₄OAc in methanol*. To a 1 liter graduated cylinder add 50 mL of 100 mM NH₄OAc in methanol and 950 mL HPLC grade methanol. Transfer entire solution to the HPLC solvent reservoir and once transferred, mix thoroughly.

5 REFERENCE STANDARDS

5.1 Abamectin

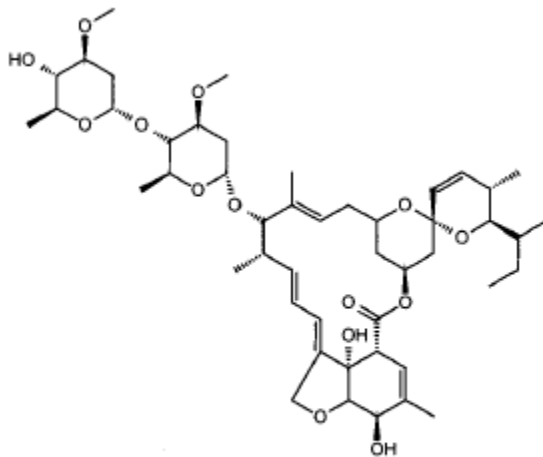
Common Name: Abamectin
Chemical Name: A mixture of avermectin B_{1a} and avermectin B_{1b}
CAS No.: 71751-41-2
Physical State: White powder
Source: Syngenta Crop Protection
Storage: in the dark typically at 1-8 °C

5.1.1 Avermectin B_{1a}

A targeted component in the above described reference material.

Common Name: Avermectin B_{1a}
Abbreviation: B_{1a}
Chemical Name: (1*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*,6*S*,6'*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-6'-[(*S*)-*sec*-butyl]-21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl- α -L-*arabino*-hexopyranosyl)-3-*O*-methyl- α -L-*arabino*-hexopyranoside

Structural Formula:

Avermectin B_{1a}

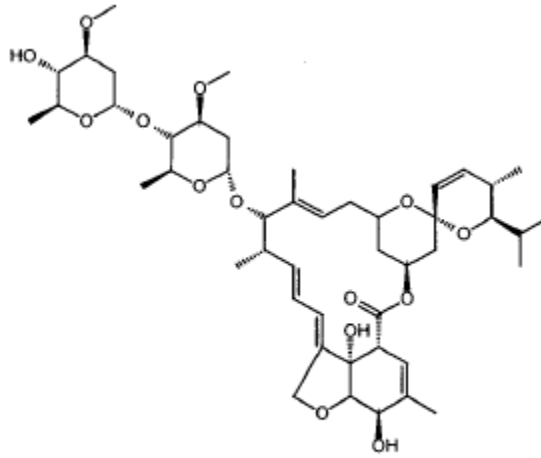
CAS No.: 65195-55-3
 Empirical Formula: C₄₈H₇₂O₁₄
 Molecular weight: 873.1 g
 Source: Syngenta Crop Protection
 Purity: Avermectin B_{1a} component: ≥80%
 Storage: in the dark typically at 1-8 °C

5.1.2 *Avermectin B_{1b}*

A targeted component in the above described reference material.

Common Name: Avermectin B_{1b}
 Abbreviation: B_{1b}
 Chemical Name: (1*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*,6*S*,6'*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-21,24-dihydroxy-6'-isopropyl-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl- α -L-*arabino*-hexopyranosyl)-3-*O*-methyl- α -L-*arabino*-hexopyranoside

Structural Formula:



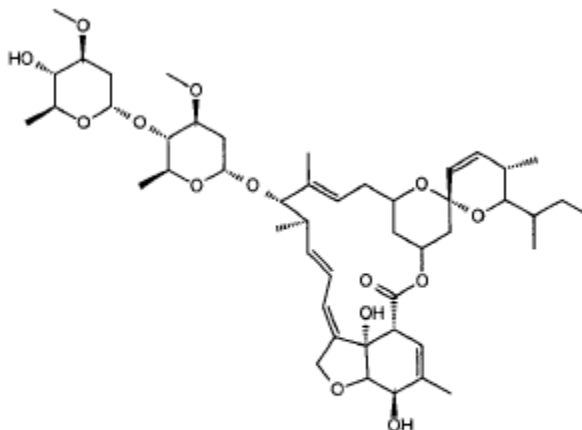
Avermectin B_{1b}

CAS No.: 65195-56-4
Empirical Formula: C₄₇H₇₀O₁₄
Molecular weight: 859.1 g
Source: Syngenta Crop Protection
Purity: Avermectin B_{1b} component: ≤20%
Storage: in the dark typically at 1-8 °C

5.1.3 8,9-Z avermectin B_{1a}

Common Name: 8,9-Z avermectin B_{1a}
Abbreviation: B_{1a} Z-isomer

Structural Formula:

8,9-Z Avermectin B_{1a}

CAS No.: 113665-89-7
Empirical Formula: C₄₈H₇₂O₁₄
Molecular weight: 873.1 g
Source: Syngenta Crop Protection
Storage: in the dark typically at 1-8 °C

6 STANDARD PREPARATION

All standard solutions prepared in this section are to be stored in the freezer and in the dark (typically at -8 to -22°C) when not in use. Avermectin B_{1a} and avermectin B_{1b} have been shown to be stable for at least 111 days down to concentrations of 0.1 µg/mL when stored as such in glass. HPLC calibration standards have been shown to be stable for at least 12 days when stored under the same conditions.

6.1 Stock Standard Solutions

6.1.1 *Abamectin (avermectin B_{1a} and avermectin B_{1b})*

Abamectin analytical standard is provided as a mixture of homologs, avermectin B_{1a} and avermectin B_{1b}. A stock standard solution is prepared which is a mixture of both compounds and contains specific concentrations of each.

Typically, 25.0 mg (corrected for purity), based on avermectin B_{1a}, of analytical standard is accurately weighed and quantitatively transferred to a 25-mL volumetric flask. The contents are brought to a final volume of 25 mL with acetonitrile. The resulting concentration of the solution is 1000 µg/mL with respect to avermectin B_{1a}.

The concentration of avermectin B_{1b} in the prepared solution is determined as follows:

$$\text{Concentration of avermectin B}_{1b} = \frac{\text{actual wt. of anal. std. used} \times \frac{\% \text{ avermectin B}_{1b} \text{ in anal. std.}}{100}}{\text{volume of stock soln.}}$$

For example:

A Certificate of Analysis for a lot of abamectin primary standard states the abamectin concentration is 90.7% (85.6% avermectin B_{1a} and **5.1%** is avermectin B_{1b})

The actual amount of reference material weighed out (equivalent to 25 mg avermectin B_{1a}) to prepare 25 mL of a 1000 µg/mL avermectin B_{1a} stock standard was **29.2 mg** (25/85.6 × 100).

$$\begin{aligned} \text{Concentration of avermectin B}_{1b} &= \frac{29.2 \text{ mg} \times \frac{5.1\%}{100}}{25 \text{ mL}} \\ &= 0.0596 \text{ mg/mL or } 59.6 \text{ } \mu\text{g/mL} \end{aligned}$$

In this example, the concentration of the stock standard solution is:

avermectin B _{1a} :	1000 µg/mL
avermectin B _{1b} :	59.6 µg/mL

6.1.2 8,9-Z avermectin B_{1a}

Typically, 25.0 mg (corrected for purity) of analytical standard is accurately weighed and quantitatively transferred to a 25-mL volumetric flask. The contents are brought to a final volume of 25 mL with acetonitrile. The resulting concentration of the solution is 1000 µg/mL.

6.2 Fortification/Intermediate Concentration Standard Solutions

The stock standard solution concentration established for avermectin B_{1b} as an example in Section 6.1.1 above, is used for demonstration purposes for the following preparations.

6.2.1 Avermectin B_{1a}/avermectin B_{1b}:

10 µg/mL B_{1a}/
0.596 µg/mL B_{1b}: Transfer 250 µL of the "1000 µg/mL B_{1a}/59.6 µg/mL B_{1b}" stock standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

1.0 µg/mL B_{1a}/
0.0596 µg/mL B_{1b}: Transfer 2.5 mL of the "10 µg/mL B_{1a}/0.596 µg/mL B_{1b}" standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

0.1 µg/mL B_{1a}/
0.00596 µg/mL B_{1b}: Transfer 2.5 mL of the 1.0 µg/mL B_{1a}/0.0596 µg/mL B_{1b} standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

6.2.2 8,9-Z avermectin B_{1a}:

10 µg/mL: Transfer 250 µL of the 1000 µg/mL stock standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

1.0 µg/mL: Transfer 2.5 mL of the 10 µg/mL standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

0.1 µg/mL: Transfer 2.5 mL of the 1.0 µg/mL standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

6.2.3 Solutions prepared as mixtures:

Avermectin B_{1a} + Avermectin B_{1b} + 8,9-Z avermectin B_{1a}:

25 µg/mL B_{1a}/
1.49 µg/mL B_{1b}/
25 µg/mL B_{1a} Z-isomer: Transfer 625 µL of the "1000 µg/mL B_{1a}/59.6 µg/mL B_{1b}" stock standard solution and 625 µL of the 1000 µg/mL Z-isomer stock standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile.

2.5 µg/mL B_{1a}/
0.149 µg/mL B_{1b}/
2.5 µg/mL B_{1a} Z-isomer: Transfer 2.5 mL of the "25 µg/mL B_{1a}/1.49 µg/mL B_{1b}/25 µg/mL B_{1a} Z-isomer" standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile.

250 ng/mL B_{1a}/
14.9 ng/mL B_{1b}/
250 ng/mL B_{1a} Z-isomer: Transfer 250 µL of the "25 µg/mL B_{1a}/1.49 µg/mL B_{1b}/
25 µg/mL B_{1a} Z-isomer" standard solution to a 25-mL
volumetric flask. Bring to volume with acetonitrile.

25 ng/mL B_{1a}/
1.49 ng/mL B_{1b}/
25 ng/mL B_{1a} Z-isomer: Transfer 2.50 mL of the "250 ng/mL B_{1a}/14.9 ng/mL B_{1b}/
250 ng/mL B_{1a} Z-isomer" standard solution to a 25-mL
volumetric flask. Bring to volume with acetonitrile.

6.3 HPLC (Calibration) Standard Solutions

Typically the following concentrations of mixed standard solutions containing all three analytes (avermectin B_{1a}, avermectin B_{1b}, and 8,9-Z avermectin B_{1a}) are prepared. Due to the very low concentrations of avermectin B_{1b} contained in the solutions, both avermectin B_{1a} and avermectin B_{1b} residues are calculated using the calibration curve generated for avermectin B_{1a}. The highest concentration of standard solution analyzed does, however, produce an avermectin B_{1b} response that is sufficient for avermectin B_{1b} peak identification.

0.2 ng/mL B_{1a}/
0.0119 ng/mL B_{1b}/
0.2 ng/mL B_{1a} Z-isomer: Transfer 200 µL of the "25 ng/mL B_{1a}/1.49 ng/mL B_{1b}/
25 ng/mL B_{1a} Z-isomer" standard solution to a 25-mL
volumetric flask. Bring to volume with acetonitrile. Mix well.

0.4 ng/mL B_{1a}/
0.0238 ng/mL B_{1b}/
0.4 ng/mL B_{1a} Z-isomer: Transfer 400 µL of the "25 ng/mL B_{1a}/1.49 ng/mL B_{1b}/
25 ng/mL B_{1a} Z-isomer" standard solution to a 25-mL
volumetric flask. Bring to volume with acetonitrile. Mix well.

1.0 ng/mL B_{1a}/
0.0596 ng/mL B_{1b}/
1.0 ng/mL B_{1a} Z-isomer: Transfer 1000 µL of the "25 ng/mL B_{1a}/1.49 ng/mL B_{1b}/
25 ng/mL B_{1a} Z-isomer" standard solution to a 25-mL
volumetric flask. Bring to volume with acetonitrile. Mix well.

2.0 ng/mL B_{1a}/

0.119 ng/mL B_{1b}/

2.0 ng/mL B_{1a} Z-isomer: Transfer 200 µL of the "250 ng/mL B_{1a}/14.9 ng/mL B_{1b}/250 ng/mL B_{1a} Z-isomer" standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

4.0 ng/mL B_{1a}/

0.238 ng/mL B_{1b}/

4.0 ng/mL B_{1a} Z-isomer: Transfer 400 µL of the "250 ng/mL B_{1a}/14.9 ng/mL B_{1b}/250 ng/mL B_{1a} Z-isomer" standard solution to a 25-mL volumetric flask. Bring to volume with acetonitrile. Mix well.

7 SAMPLE FORTIFICATION

1. Weigh 5.00 g of prepared sample (macerated or ground, as necessary) into a 50-mL polypropylene centrifuge tube.
2. Fortify the sample with the appropriate amount of standard solution(s). Disperse solution(s) over as much of the sample as possible. Use a volume of fortification solution ≤0.5 mL.
3. Proceed with Step 8.1.2.

8 SAMPLE EXTRACTION

1. Weigh 5.00 g of prepared sample (macerated or ground, as necessary) into a 50-mL polypropylene centrifuge tube.
2. Add 20 mL acetonitrile:0.1% phosphoric acid (25:75, v/v) solution to the sample in the centrifuge tube.
3. Blend using a high-speed homogenizer at high speed for ~1 minute. Rinse the blender probe by blending with 20 mL hexane contained in another 50 mL centrifuge tube (*see Note below*). Add the hexane rinse solution to the original extract and shake for ~5 minutes in a reciprocating (platform) shaker. Centrifuge the extract for ~10 minutes at about 2500 rpm.

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

4. Using a disposable polyethylene transfer pipet, transfer the hexane layer containing the avermectins to a 100-mL polypropylene graduated cylinder.

5. Re-extract the aqueous remainder two additional times with 20 mL of hexane each time. Combine the hexane extracts in the same 100-mL polypropylene graduated cylinder. Bring to a final volume of 100 mL with hexane. Transfer to a 250-mL HDPE centrifuge bottle and mix well (see Note 1 below). Add ~1 g of anhydrous sodium sulfate. Briefly shake to remove traces of aqueous solution. Proceed to Section 9 for aminopropyl SPE cleanup.

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

STOPPING POINT. Hexane extracts contained in HDPE centrifuge bottles can be stored up to 11 days in the freezer (typically -8 to -22°C).

9 AMINOPROPYL SOLID PHASE EXTRACTION (SPE) CARTRIDGE (500mg/3mL) CLEANUP

Note: Check or calibrate the SPE cartridges prior to use in order to ensure optimum method performance. In general, check one tube per lot number per box. This assessment should be conducted well in advance of needing the tubes for sample analysis. Recovery of $>90\%$ is desired to ensure that a box of tubes is suitable for use. The analyses are conducted on a reagent spike basis. See Appendix III for detailed instructions on assessment of the SPE tubes.

Procedure:

1. Set up SPE processing system and support apparatus and proceed with aminopropyl SPE cleanup. In general, set vacuum to produce a flow rate of approximately 2-3 distinct drops/second (not continuous flow) for all elutions. Attach a 20-mL reservoir with adaptor to the SPE cartridge.
2. Condition a 500 mg/3 mL aminopropyl SPE cartridge by passing 10 mL of methanol, followed by 10 mL of hexane, through the cartridge. Do not let the cartridge go to dryness after conditioning. (Stop elution when conditioning solvent reaches top of frit.) Discard eluate.
3. Load the sample (10 mL aliquot of sample extract from Step 8.5, equivalent to 0.50 g of sample) onto the cartridge. Stop elution when loading solvent reaches top of frit. Discard the eluate to waste.

Note: Store remaining sample extract from Step 8.5 at -8 to -22°C .

4. Wash the sample-laden SPE cartridge sequentially with 10 mL of hexane (2 times), followed by 3 mL of ethyl acetate. Stop elution when last washing solvent reaches top of frit. Discard all eluates.
5. Place a silanized 13 × 100-mm test tube (calibrated at 2.5 ml) under the SPE cartridge.

Note: Polypropylene tubes **cannot** be substituted for silanized test tubes.

6. Using mild vacuum, elute the abamectin-related residues from the cartridge with 2.0 mL of ethyl acetate:methanol (75:25, v/v).

Note: Residual ethyl acetate:methanol (75:25, v/v) solution remaining in the SPE cartridge should be drained and collected using moderate to high vacuum.

7. Evaporate the eluate to near dryness on an N-Evap evaporator @ ≤ 35 °C. Continue evaporation to just-dry using manual nitrogen blowdown.

Note: Do not over-evaporate as abamectin residues have a tendency to irreversibly adsorb onto glass surfaces, even when silanized. Handle manual evaporation and redissolving of residue one sample at a time.

8. Immediately redissolve the residue in acetonitrile and bring to a final volume of 2.5 mL with acetonitrile. Cap, sonicate (mandatory) and mix well. Final sample concentration: 1 mL = 0.2 g sample. Submit to LC/MS/MS analysis. Store sample extracts in freezer (−8 to −22°C) if not analyzed immediately.

10 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS

Note: The column and conditions stated in the method have been satisfactory for the matrices being analyzed. The specific column packing, mobile phase, column temperature and flow rate listed are typical conditions for this analysis. Alternate columns may be used depending on the need to resolve analyte and/or interfering responses. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

10.1 Operating Conditions

Instrument: Applied BioSystems/MDS Sciex API 4000 LC/MS/MS system with a Shimadzu SIL-HTA autosampler, an integrated Shimadzu chromatograph consisting of (2) LC-10ADvp Liquid Chromatograph units and a DGU-14A Degasser. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.

HPLC column: 50-mm × 2.0-mm i.d. Imtakt Cadenza CD-18, 3µ particle size

Mobile phase: Fisher water, Burdick and Jackson methanol, and Fisher ammonium acetate

Component A: Water:100 mM NH₄OAc in methanol (95:5, v/v)

Component B: 5 mM NH₄OAc in methanol

Gradient:

<u>Time (min)</u>	<u>Percent of Mobile Phase A</u>	<u>Percent of Mobile Phase B</u>
0.00-0.50	35	65
10.0-10.4	5	95
10.5-13.0	35	65

Divert Valve: Programmed to divert LC flow from column to waste (bypassing detector) from 0.00 to 6.50 minutes and again from 10.0 to 12.9 minutes. LC flow is directed to detector during the 6.50 to 10.0 minute window. Diversion time settings can be adjusted as necessary depending on the retention times of the analytes.

Flow Rate: 300 µL/min. (or 0.300 mL/min.)

Interface: TIS (Turbo Ion Spray)

Ionization Mode: Positive (+)

Acquisition Mode: MRM

Source Temperature: 500 °C

Curtain Gas: Nitrogen @ 10

Collision Gas: Nitrogen @ setting of "12"

Injection Volume: 5 µL

Column Temperature: 40 °C

Resolution: Q1-Unit, Q3-Low (Note: Unit is equivalent to medium)

Transitions Monitored:	<u>Ion, m/z</u>		<u>Time, ms</u>	<u>CE, v</u>	
	<u>Q1</u>	<u>Q3</u>			
Avermectin B _{1a} and 8,9-Z avermectin B _{1a} :	895.5	751.5	300	60	(quantitation)
	895.5	449.2	300	63	(confirmation)
Avermectin B _{1b} :	881.2	737.0	300	59	(quantitation)
	881.2	449.2	300	63	(confirmation)

Note: Parent ions (Q1) represent corresponding Na⁺ adducts (M + Na)⁺. M = parent mass.

Retention Times:	Avermectin B _{1b}	~8.2 minutes
	Avermectin B _{1a} :	~8.8 minutes
	8,9-Z avermectin B _{1a} :	~9.2 minutes

10.2 Sample Analysis

Prepare a five-point standard curve by injecting constant volumes of standard solutions. Use constant volume injections for sample extracts as well. Sample responses greater than those produced by the highest concentration of standard curve require dilution and reinjection. Inject a curve check standard every 3-4 sample injections.

11 CALCULATIONS

Calculations for instrumental analysis are conducted using a validated software application to create a standard curve based on linear regression. The regression functions are used to calculate a best fit line (from a set of standard concentrations in ng/mL versus peak response) and to determine concentrations of the analyte found during sample analysis from the calculated best fit line. **Weighting (1/x) is used.**

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response
x	=	ng/mL found for peak of interest
m	=	slope
b	=	y-intercept

Note: The calibration curve generated for Avermectin B_{1a} is also used to quantify residues found for Avermectin B_{1b} in field samples and fortified samples.

The calculations for ppm found and percent recovery (for fortified samples) are:

1. The amount of analyte (in ppm) found in the sample is calculated according to the following equation:

$$ppm = ng/mL \times \frac{HPLC \text{ final vol. (mL)}}{sample \text{ wt. (g)}} \times \frac{mL \text{ ext. solv.}}{mL \text{ aliq.}} \times \frac{1}{1000} \times HPLC \text{ dil. factor}$$

where:

ng/mL found	=	ng/mL of analyte found in sample injected
sample wt. (g)	=	gram weight of sample extracted (typically 5.00 g)
mL ext. solv.	=	final volume of extraction solvent (typically 100 mL)
mL aliq.	=	volume of sample extract processed through method (typically 10 mL)
1/1000	=	conversion factor from ng to µg
HPLC final vol. (mL)	=	volume of final extract submitted to HPLC (typically 2.5 mL)
HPLC dil. factor	=	dilution of sample extract required to produce an analyte response bracketed by standards

2. The percent recovery for fortified control samples is calculated as follows:

$$\% \text{ Recovery} = \frac{ppm \text{ found in fortified control} - ppm \text{ found in control}}{ppm \text{ added}} \times 100$$

12 REFERENCE

1. "HPLC-Fluorescence Method for the Quantitation of Avermectin B₁ and 8,9-Z Avermectin B₁ in/on Fruits and Vegetables," Method No. M-073.1, Novartis Crop Protection, Inc., Greensboro, NC, August 7, 1998.

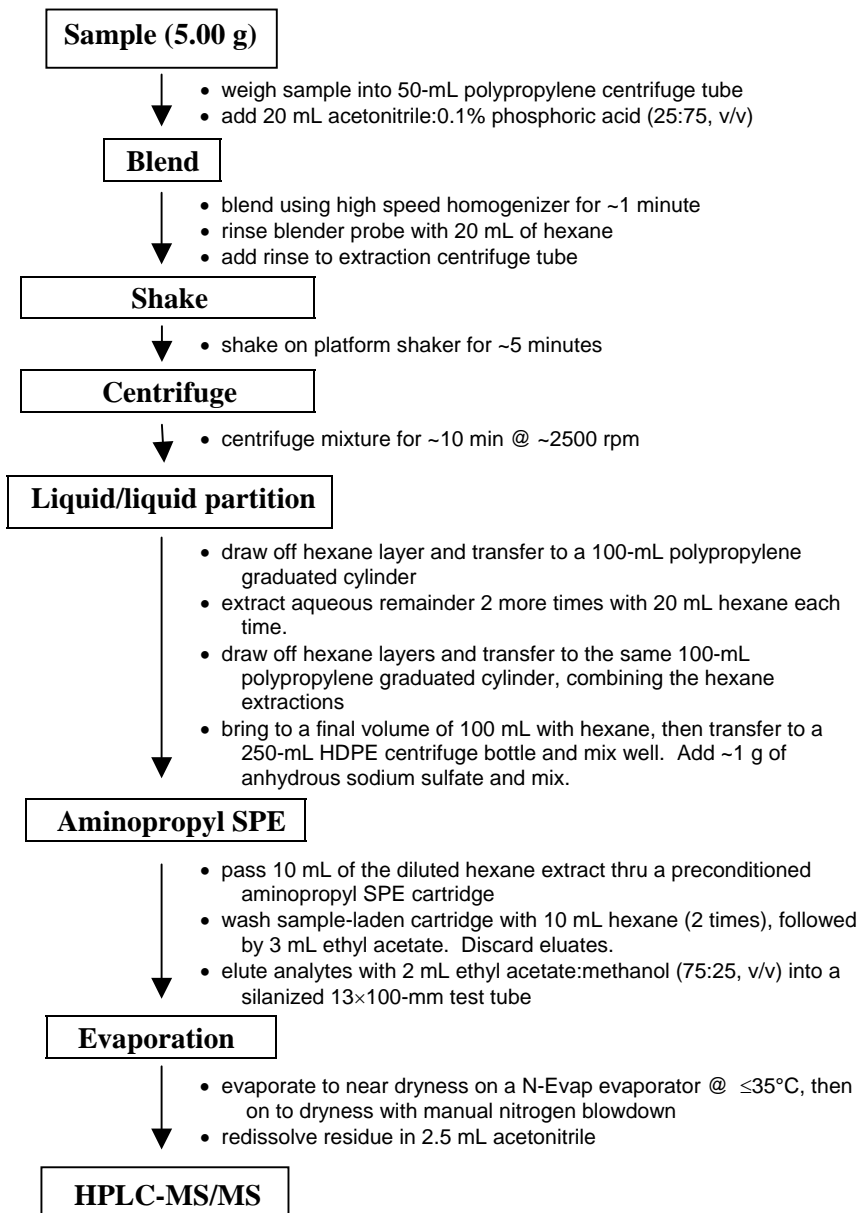
Method authors: Gary L. Westberg
Frances M. Brookey

APPENDIX I

Analysis Flowchart

ANALYSIS FLOWCHART

(RAC Fruits and Vegetables)



APPENDIX II

Silylation of Glassware

Preparation of Glassware to be Silylated

All glassware to be silylated must be clean and free of organic (i.e., oils/gums) and/or inorganic (i.e., salts) residues and aged layers (>5 silylation treatments) of preexisting silylated coating residues. Treatment with a cleaning agent such as Chem-Solv[®] will produce a properly clean/prepared surface for silylation.

Glassware with unknown history or that requires the removal of aged layers of silylated coating should be treated by exposing (maintaining reagent contact) the glass surfaces to 100% Chem-Solv[®] a minimum of 10 minutes. For maintenance treatment, glassware previously properly prepared and silylated should be similarly exposed to 50% Chem-Solv[®] (in water) a minimum of 5 minutes. Insure that all surfaces which can come in contact with the analytes are properly treated. Rinse thoroughly with DI water, followed by acetone. Allow to air dry.

Silylation of Glassware

Silylation is a process used to chemically treat glassware or other products in order to prevent or minimize binding of analyte residues to the glass surface.

Caution: **DO NOT ALLOW DIMETHYLDICHLOROSILANE TO COME IN CONTACT WITH WATER. CHLORINE GAS AND HYDROGEN CHLORIDE GAS WILL BE PRODUCED.**

THIS PROCEDURE MUST BE CONDUCTED INSIDE AN EFFICIENT FUME HOOD. HEAVY LATEX GLOVES MUST BE WORN.

1. Pour a small amount of the 5% **DMDCS** solution (5 mL DMDCS + 95 mL hexane) into the glassware to be treated. Stopper bulk container. Rotate the glassware to thoroughly coat the inside surfaces. Pour excess solution into the next piece of glassware to be treated.

Note: Moisture in the air tends to inactivate this reagent. To insure maximum activity of the silylating agent during the coating process, limit the exposure (to the atmosphere) of the silylating agent to approximately 5 minutes.

2. Allow the treated glassware to dry (approximately 20 minutes). Rinse thoroughly with hexane, then reagent acetone. Again allow to dry.
3. Glassware is now ready for use.

- Notes:**
- Any glassware that is cleaned with a brush after it has been silylated, must be resilylated.
 - Store pure DMDCS at room temperature.

- 5% solutions of DMDCS in hexane are stable for 5 days when stored well-stoppered at room temperature. Choose a storage container with minimum air space above the surface of the solution.

APPENDIX III

Quality Control for SPE Cartridges

Quality Control for SPE Cartridges

Aminopropyl SPE Cartridges

1. Transfer 10 μL of a "2.5 $\mu\text{g}/\text{mL}$ B_{1a}/0.149 $\mu\text{g}/\text{mL}$ B_{1b}/2.5 $\mu\text{g}/\text{mL}$ B_{1a} Z-isomer" mixed standard solution (in acetonitrile) to a 16 \times 100-mm silanized test tube containing 10.0 mL of hexane. Mix well.
2. Follow Steps 9.1 through 9.7 of the procedure.
3. For B_{1b} evaluation:
Redissolve residue in 1.0 mL of acetonitrile. Final concentration of the analytes are **1.49 ng/mL B_{1b}** and 25 ng/mL for both B_{1a} and B_{1a} Z-isomer (analyte of interest in bold font).
4. For B_{1a} and B_{1a} Z-isomer evaluation:

Transfer 100 μL of the solution from Step 3 to a silanized 13 \times 100-mm test tube containing 900 μL of acetonitrile. Mix well. Final concentration of the analytes are 0.149 ng/mL B_{1b} and **2.5 ng/mL for both B_{1a} and B_{1a} Z-isomer** (analytes of interest in bold font).
5. Submit both solutions to HPLC for analysis.

APPENDIX IV

Mass Spectra for Parent and Product-ions

FIGURE 1. Full Scan MS Spectrum of ~1 µg/mL Avermectin B_{1a} or 8,9-Z Avermectin B_{1a}

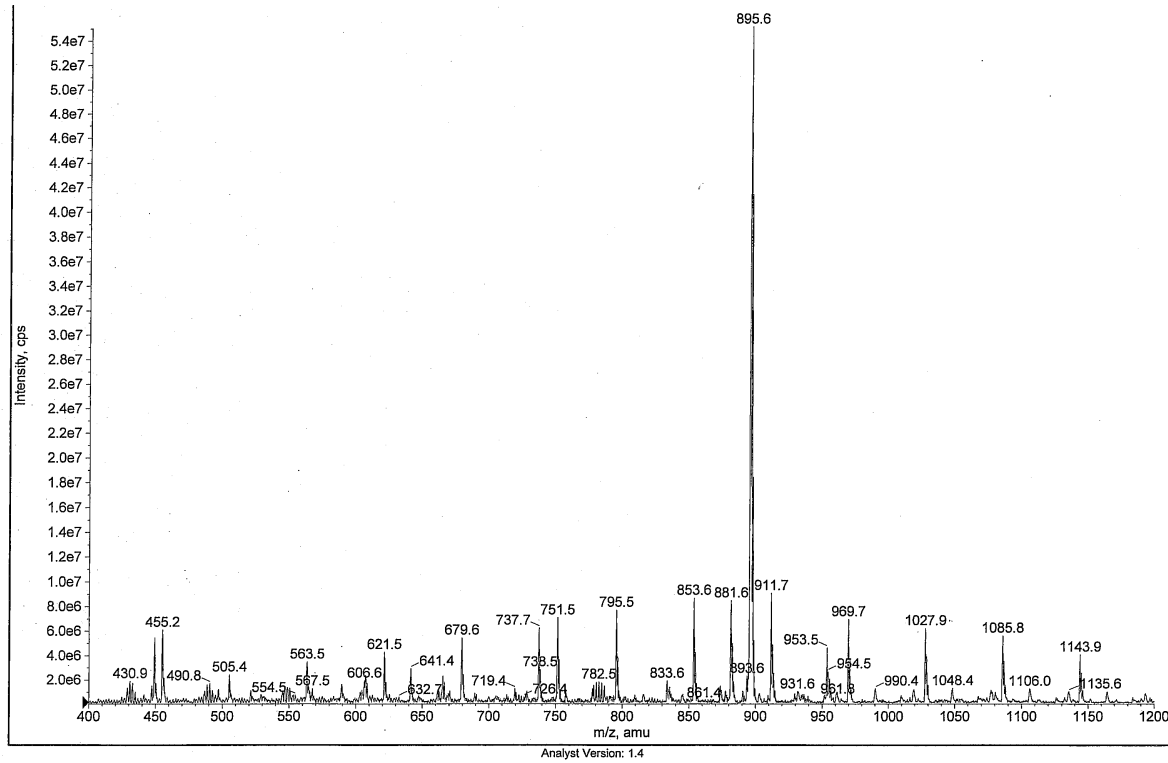


FIGURE 2. MS/MS Full Scan (Q3 Scan) of an ~0.2 µg/mL Avermectin B_{1a} or 8,9-Z Avermectin B_{1a} (Collision Energy Set to 60V)

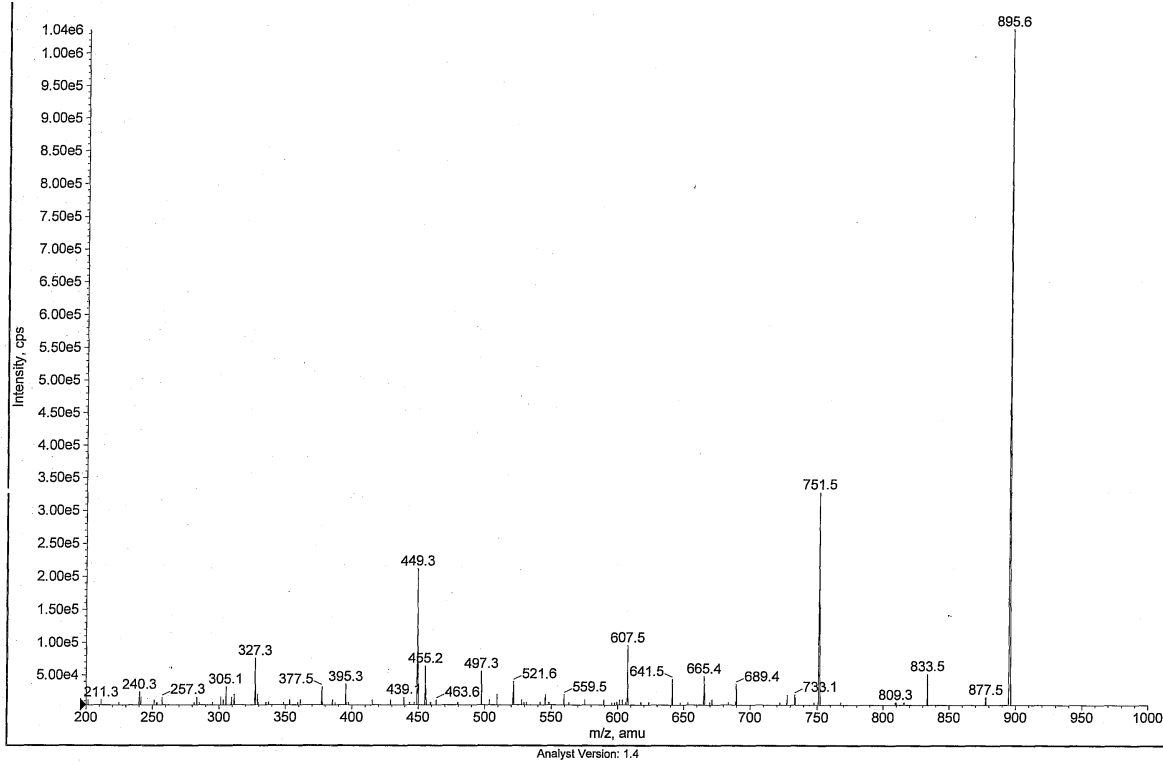


FIGURE 3. Full Scan MS Spectrum of ~0.2 µg/mL Avermectin B_{1b} Standard

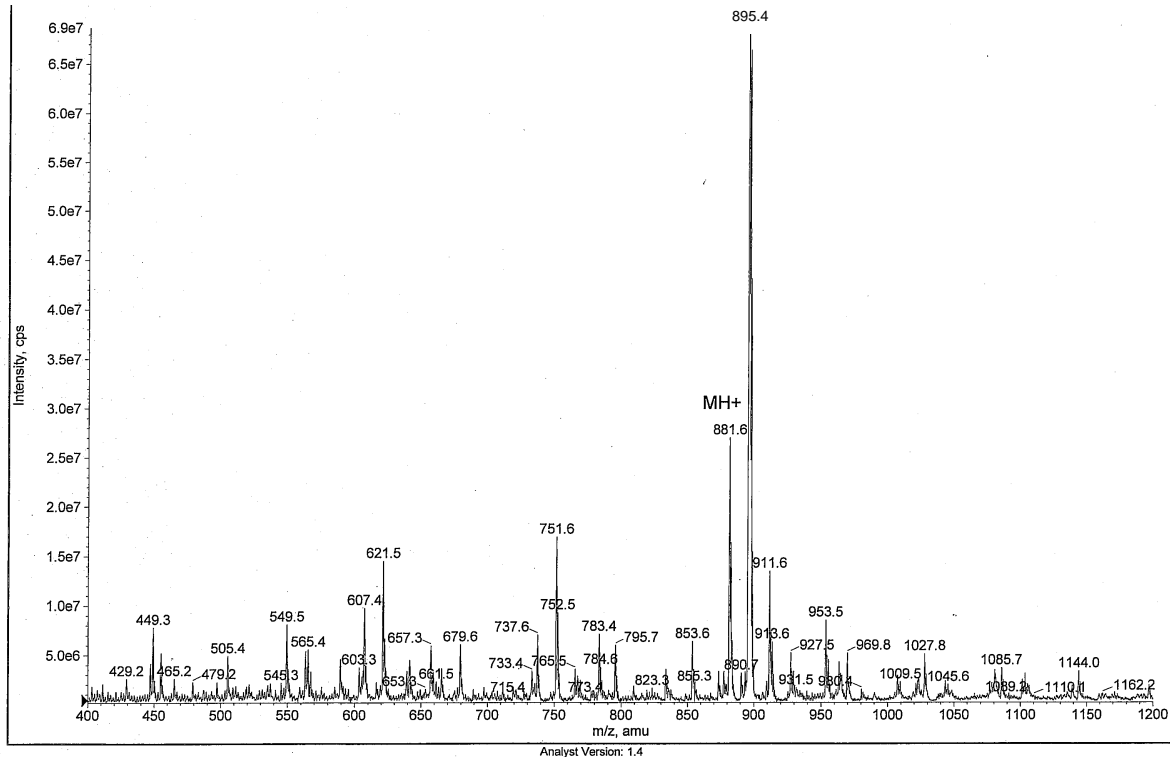


FIGURE 4. MS/MS Full Scan (Q3 Scan) of an ~0.2 µg/mL Avermectin B_{1b} Standard (Collision Energy Set to 59V)

