I. INTRODUCTION/SUMMARY

A. Scope

This method is used for the determination of NOA-422601 (Avermectin B1a), NOA-421704 (Avermectin B1b), and NOA-427011 (8,9-Z Avermectin B1a) in water. The analytes are separated by high performance liquid chromatography (HPLC) with triple quadrupole mass spectrometry detection (LC-MS/MS). A pneumatically- and heat-assisted electrospray interface is used to introduce the HPLC effluent into the mass spectrometer. The analytes are detected in the Multiple Reaction Monitoring (MRM) mode by passing the positive molecular ion through quadrupole 1 (Q1), inducing fragmentation in the collision cell (Q2), and then monitoring a characteristic product ion fragment with quadrupole 3 (Q3). The chemical structures, chemical names, and Chemical Abstracts Registry numbers of the analytes are presented in Figure 1.

The analytical method was validated with a Limit of Quantification (LOQ) of 0.05 ppb for all analytes in water. The Limit of Detection (LOD), defined as the lowest standard concentration analyzed, was 0.1 ng/mL.

B. Principle

Acetonitrile is added to a sample container holding a water sample to ensure all analytes are desorbed from the surface of the storage container. A 75-mL aliquot of the sample is removed for analysis (equivalent to a 50-mL aliquot of sample water). The sample is partitioned with two 20-mL portions of methylene chloride. The organic is evaporated to dryness and reconstituted in 50% (v/v) acetonitrile/water until the proper dilution is reached. The samples are analyzed by LC-MS/MS. A flow diagram for this water method is presented in Figure 2.

II. MATERIALS AND METHODS

A. Apparatus

1.0 Balance, analytical (Mettler BB2400) or equivalent.

2.0 Beaker, glass, 150-mL (Fisher cat. #02-539J) or equivalent.

3.0 Cylinder, graduated, 100-mL (Fisher cat. #08-551D) or equivalent.

4.0 Mixer, Vortex-Genie 2 (Fisher cat. #12-812) or equivalent.

5.0 Pasteur pipet (Fisher cat. #13-678-7C) or equivalent.

6.0 Pipets, glass, class A certified, assorted volumes. These pipets are used when an exact addition of liquid is required (i.e., final addition of solvent to samples).

7.0 Pipetters, Oxford BenchMate adjustable, 40-200 µL volume range (Fisher cat. #21-231), 200-1000 µL volume range (Fisher cat. #21-229) or equivalent. (Note: These adjustable pipetters may only be used for addition of liquid where an exact volume added is not critical, i.e., addition of acid or base.)

8.0 Rotary evaporator, Buchi (Fisher cat. #09-548-105F) or equivalent.

9.0 Round bottom flask, 250 mL, (Fisher cat. #09-552B) or equivalent.

10.0 Separatory Funnels, 250-mL with polyethylene stopper (Fisher cat. #10-437-5C) or equivalent.

11.0 Vials, clear or amber, 1.5-mL with screw top caps (National Scientific Company cat. #C4013-15A) or equivalent.

B. Reagents and Analytical Standards

All reagents and polypropylene glycols are stored at room temperature. Solid analytical standards are stored in a freezer (temperature <-10°C).

1.0 Acetonitrile, HPLC grade (Fisher cat. #A998-4) or equivalent.

2.0 Ammonium formate, certified ACS grade (Fisher cat. #A666-500) or equivalent.

3.0 Methanol, HPLC grade (Fisher cat. #A452-4) or equivalent.

4.0 Methylene chloride, HPLC grade (Fisher cat. #D150SK-1) or equivalent.

5.0 Polypropylene glycol, M.W. 425 (Aldrich cat. #20,230-4).

6.0 Polypropylene glycol, M.W. 1000 (Aldrich cat. #20,232-0).

7.0 Polypropylene glycol, M.W. 2000 (Aldrich cat. #20,233-9).

8.0 PPG tuning solution (for mass calibration of the LC/MS system). Dissolve 0.0014 grams of PPG 425, 0.0100 grams of PPG 1000, 0.0400 grams of PPG 2000, and 0.0126 grams of ammonium formate in 50 mL of methanol, 50 mL water, and 0.1 mL of acetonitrile. Mix well. Store refrigerated in an amber bottle.

9.0 Sample diluent: 50% acetonitrile in water. Mix 500 mL of acetonitrile with 500 mL of purified water.

10.0 Test analytes tuning solution, 1 ng/µL. Mix one volume of a 10 ng/µL mixed solution of analytes in acetonitrile with nine volumes of 70% methanol/water. Store at refrigerated or frozen temperature.

11.0 Water, HPLC grade, purified in-house with a HYDRO™ purification system or equivalent.

12.0 NOA-422601, NOA-421704, and NOA-427011, Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

C. Safety and Health

Whereas most of the chemicals used and analyzed for in this method have not been completely characterized, general laboratory safety is advised (e.g., safety glasses, gloves, etc. should be used). Ammonium hydroxide is an irritant and should be used in a well-ventilated area (i.e., a fume hood).

D. Analytical Procedure

Note: All glassware should be thoroughly cleaned and followed with a rinse of acetonitrile or methanol prior to use. The analysis system is very sensitive and may detect contamination from previous samples if all glassware and extraction bottles are not properly cleaned prior to each use.

1.0 Water

(Note: Avermectin analytes in water adsorb to the surface of glass or plastic sample collection bottles. Acetonitrile must be added to desorb the analytes from the surface of the sample container prior to processing the sample.)

1.1 For lab-fortified water samples, or lab control samples, measure 50-mL of water in a graduated cylinder and transfer to a 250-mL separatory funnel. Sample fortification, if required for this particular sample, is to be done at this time (refer to Section II.K.2.0). Add 20 mL of acetonitrile. Swirl the contents to mix. The lab samples are now ready for analyte isolation (Step 1.4).

1.2 For field samples, decant the water sample into an appropriate-sized graduated cylinder and record the volume. Transfer the water sample back to the sample storage container. Determine the appropriate amount of acetonitrile to add to the water sample so that the final content will be 25% (v/v) in acetonitrile (see equation below).

volume ACN to add = 0.333 (volume water sample)

(Example: If the sample container has a volume of 150 mL of water, then you would add 50 mL of acetonitrile.) The volume of acetonitrile to be added should first be used to rinse the graduated cylinder used to measure the volume of water sample and then transferred to the sample container with the water sample. The sample (with acetonitrile added) should be mechanically shaken or sonicated for approximately five minutes to ensure the analytes are desorbed from the surface of the sample container. For sample processing, a 75-mL aliquot (equivalent to 50 mL of water sample) of the sample (with acetonitrile added) is measured into a 100-mL graduated cylinder.

1.3 Add 20-mL of methylene chloride and shake for approximately one minute.

1.4 Once phase separation has been obtained, drain the lower aqueous layer into a 250-mL roundbottom flask.

1.5 Repeat the partitioning with another 20-mLs of methylene chloride. Combine the second partitioning with the first in the same 250-mL roundbottom flask mentioned in step 1.4.

1.6 Evaporate the methylene chloride on a rotary evaporator with a bath temperature of not more than 40°C.

1.7 Pipette 2.5 ml of acetonitrile to the round bottom flask and swirl to dissolve the residue on the walls of the vessel.

1.8 Pipette 2.5 ml of water and add into the round bottom flask.

1.9 Additional dilution, if necessary, may be made using 50% acetonitrile in water. The sample should be stored refrigerated (<5°C) until the time of analysis. Samples should be stored frozen for long term storage (> 2 weeks).

1.10 Analyze the sample using LC-MS/MS.

E. Instrumentation

1.0 Description and Operating Conditions: HPLC

See Table 1 for a description of the reversed phase HPLC system and chromatographic conditions used for the analysis.

2.0 Description and Operating Conditions: LC-MS/MS

NOA-422601, NOA-421704, and NOA-427011 are monitored as positive ions. Triple quadrupole analysis (MS/MS) of the unique precursor/ product ion pair is suggested to achieve the low method LOQ. See Table 2 for a description of the mass spectrometer instrumentation and operating conditions.

3.0 Description and Operating Conditions: LC-MS/MS Electrospray Interface

The optimized values for the turboion interface may vary with time and may need to be periodically re-optimized. With the Sciex API-3000 LC-MS/MS system, typical electrospray operating conditions are described in Table 2.

4.0 Calibration and Standardization: LC-MS/MS

4.1 Calibrate and tune the mass spectrometer prior to analyzing samples. Check the calibration and tune by infusing a standard solution of polypropylene glycols (PPG), or another suitable mass calibration solution, into the mass spectrometer using the turbo ionspray interface while monitoring positive ions. The study director, or system

analyst, will determine how frequently mass calibration needs to be performed.

4.2 Determine the specific ion to monitor for each analyte by infusion of an analyte test solution (approx. 1 ng/µL in 70% methanol/water) or flow injection analysis (FIA) while scanning the Q1 quadrupole mass analyzer to find the optimum ion. For the avermectin analytes, the predominant ion is the sodium ion adduct of the molecular ion. Determine the specific product ion fragment to monitor for each analyte in the MS/MS mode by passing the characteristic precursor ion through Q1, fragmenting the ion in Q2, and scanning the resulting ion fragments in Q3. The selected product ion chosen to monitor will depend on the intensity of the ion fragment along with the possibility that an interference also has the same fragment ion. Table 3 lists the precursor/product ion pairs that are monitored ion for each analyte. Typical MS/MS mass fragmentation spectra will be presented for each analyte in the final report.

4.3 Determine the retention time of the analytes by injecting a standard solution into the HPLC. During a series of analyses, the analyte retention time should vary no more than 2% from its mean value, on a daily basis.

4.4 Calibrate the instrument by constructing a calibration curve from detector response (chromatographic peak height or area) and the amount of analyte injected. Typical calibration data and chromatograms of calibration standards will be presented in the final report.

F. Interferences

1.0 There are no known interferences originating from the sample cleanup procedure. However, interferences can originate from impure chemicals, solvents, contaminated glassware, and the HPLC water supply.

G. Confirmatory Techniques

1.0 No confirmatory analysis procedure is included in this method. This method employs highly specific LC-MS/MS for the detection mode, coupled with the characteristic retention time observed for the analyte on the appropriate HPLC column.

H. Time Required

1.0 The sample extraction and cleanup procedure can be completed for a set of twenty water samples in an eight-hour work day.

2.0 Each HPLC analysis requires approximately 11 minutes.

I. Modifications and Potential Problems

1.0 Contaminants from chemicals, solvents, glassware, and the HPLC water supply can interfere with the analysis. It is recommended that a reagent blank be run with an analysis set to verify that no interferences are originating from the chemicals and reagents used in this procedure. MS techniques are very sensitive. All glassware should be solvent rinsed before use to prevent inadvertent contamination of control or low level samples.

2.0 No analyte stability or solubility problems have been observed when standard solutions have been prepared and stored as detailed in Section II.J.

3.0 All of the avermectin analytes in water samples will adsorb to the surface of glass or plastic containers. Acetonitrile must be added to desorb the analytes from the sample container surface prior to sample processing and analysis. When acetonitrile is added to water samples (25% by volume) prior to sample storage, no analyte losses are observed. It may be advantageous to add the appropriate volume of acetonitrile to water samples as they are collected, prior to refrigerated storage.

4.0 LC-MS/MS is required to achieve the method LOQ for all analytes.

5.0 Reversed phase columns from other manufacturers may be substituted for the column used in this study provided that the analyst demonstrates acceptable peak shape and sensitivity with the substituted column. The mobile phase gradient may need to be altered if a different column is used.

6.0 To achieve the water method LOQ of 0.05 ppb, the author extracted and processed 50 mL of water, used a final sample volume of 5 mL, and injected 10 µL of sample onto the HPLC column. Analysts must carefully optimize instrument sensitivity and obtain good, sharp eluted peaks from the HPLC to achieve the target LOQ.

7.0 Several injections (3-4) of samples or standards should be made into the LC/MS prior to initiation of the sample run sequence. This is necessary to stabilize analyte retention times and to stabilize the MS response to the analytes.

8.0 The product insecticide Abamectin consists of NOA-422601 (≥ 80%) and NOA-421704 (≤ 20%).

J. Preparation of Standard Solutions

All stock solutions are stored in amber bottles in a freezer (< -10°C) when not in use. Mixed standards may be stored in a freezer or refrigerated (< 5°C). No analyte stability or solubility problems have been observed in the standard solutions used in this study. The mixed standards are used for fortifications and as HPLC standards.

1.0 Prepare individual 100 ng/µL stock solutions for each analyte. Weigh approximately 10.0 mg of analyte. Determine the appropriate volume of acetonitrile to add using the equation presented below. The concentration of the analytical standard is corrected for its chemical purity.

$$V\,(mL) = \frac{w\,(mg) \times P}{C\,(ng/uL)} \times 10^3$$

Where "V" is the volume of acetonitrile needed; "w" is the weight, in mg, of the solid analytical standard; "P" is the purity, in decimal form, of the analytical standard; "C" is the desired concentration of the final solution, in ng/µL; and 10^3 is a conversion factor.

For example:

The volume required to dilute 9.9 mg of an analyte, of 98.0% purity, to a final concentration of 100 ng/µL is:

$$V\,(mL) = \frac{9.9\ mg \times 0.98}{100\ ng/uL} \times 10^3 = 97.0\ mL$$

2.0 A 1.0 ng/µL mixed standard is prepared by combining an aliquot of each analyte stock solution and diluting the solution to volume with acetonitrile. Pipette 1.0 mL of each 100 ng/µL stock solution into a 100-mL volumetric flask and dilute to the calibration mark with acetonitrile.

3.0 Fortification and calibration standards are prepared by subsequent dilutions of the 1.0 ng/µL solution with 50% acetonitrile/water. Fortification standards should be prepared such that no more than 1.0 mL of the fortification solution is added to a sample. (Example: For a 50-g (50 mL) water sample, the addition of 0.5 mL of a 0.01 ng/µL fortification solution will result in a fortification level of 0.1 ppb.)

K. Methods of Calculation

1.0 Determination of Residues in Samples

1.1 Inject the sample solution from Step II.D.1.9 into the analysis system. The sample solution may be diluted if the analyte response exceeds the range of the calibration curve. The amount of analyte injected (ng) is determined by entering the value of the chromatographic peak height, or area, in the calibration response curve (Step II.E.4.4) and calculating the corresponding value of nanograms injected. Typical chromatograms for control and fortified control water are presented in Figures 3-15.

2.0 Determination of Residues in Fortified Samples

Validate the method for each set of samples analyzed by including a control sample and one or more control samples fortified prior to the extraction procedure with 0.05 ppb or more of each analyte.

2.1 Add an appropriate volume of a fortification solution to the sample prior to any of the cleanup steps. The total volume of the added fortification solution should not exceed 1.0 mL.

2.2 Proceed with the sample cleanup procedure.

3.0 Calculations

Calculations may be performed by computer program or manually as follows.

3.1 Calculate the analyte concentration (in ppb) from equation (1):

$$(1)\ \text{ppb analyte} = \frac{\text{ng analyte found}}{\text{g sample injected}}$$

The grams of sample injected is calculated from equation (2).

$$(2)\ \text{grams water injected} = g \times \frac{V_i}{V_f}$$

where, g is the grams of water (assume density of water = 1.0 g/mL) extracted, V_i is the volume (mL) injected onto the HPLC column, and V_f is the final volume (mL) of the cleaned-up sample (from Step II.D.1.9).

The amount (ppb) of analyte found is calculated from equation (3)

$$(3)\ \text{ppb analyte found} = \frac{\text{ng analyte found}}{\text{g sample injected}}$$

3.2 The accuracy of the method is determined by the average recovery of the analytes fortified into the test substrate. The precision is estimated by the relative standard deviation of the determined concentration.

V. TABLES

TABLE 1. HPLC SYSTEM AND OPERATING CONDITIONS

Instrumentation:

Shimadzu LC-10Atvp HPLC System

Operating Conditions:

Injection Volume: 10 µL
Mobile Phase Flow Rate: 0.3 mL/min
Column: Phenomenex Luna 5µ phenyl-hexyl 150 x 2 mm.
Mobile Phase A: Methanol
Mobile Phase B: Water

Mobile Phase Gradient Program:

Time (min.)	% A	% B
0.0	70	30
0.2	70	30
2.0	95	5
6.4	95	5
6.5	70	30
9.0	Stop	

Total Analysis Time: 11 min.

Analyte Retention Times:

NOA-421704	7.10 min
NOA-422601	7.30 min
NOA-427011	7.60 min

TABLE 2. MASS SPECTROMETRY SYSTEM AND OPERATING CONDITIONS

Instrumentation:

PE Sciex API 3000 LC-MS/MS

Software:

MacQuan Version 1.7.1

Instrument Parameters

(IS) Ion Source:	5000	(IQ2) Inter Quad 2 Lens:	-58
(TEM) Temperature:	500°C	(R02) Quad 2 Rod Offset:	-63
(OR) Orifice Plate:	90	(ST3) Stubbies:	-78
(RNG) Focusing Ring:	350	(R03) Quad 3 Rod Offset:	-68
(Q0) Quad 0 Rod Offset:	-10	(DF) Deflector:	-400
(IQ1) Inter Quad 1 Lens:	-10.9	(CEM) Channel Electron Multiplier:	2400
(ST) Stubbies (Prefilter):	-17	(NEB) Nebulizer Gas (N_2)	12
(R01) Quad 1 Rod Offset:	-12	(CUR) Curtain Gas (N_2)	8
(CAD) Collisionally Activated Dissociation Gas (N_2)			8

*Note: State file values will vary slightly from instrument to instrument. The values often will be changed slightly during instrument optimization procedures.

Data Collection

Time 0 → 4.0 minutes, pause, no data collected
Time 4.0 → 10.0 minutes, collect data

Mode: MRM on positive detection

Dwell Time: 0.30 sec per ion transition

Ion Transitions Monitored

895.5 > 751.5 NOA-422601
881.5 > 737.5 NOA-421704
895.5 > 751.5 NOA-427011

TABLE 3. TYPICAL ANALYTE MONITORING IONS: LC-MS/MS

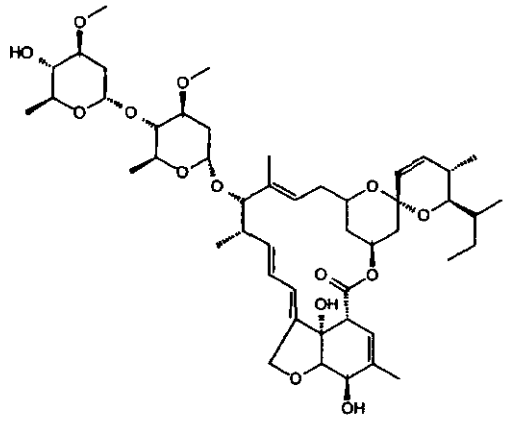
Analyte	Exact Molecular Weight	Q1 Molecular Ion*	Q3 Product Ion
NOA-422601	872.5	895.5	751.5
NOA-421704	858.5	881.5	737.5
NOA-427011	872.5	895.5	751.5

* All analytes form sodium adducts as the primary molecular ion.

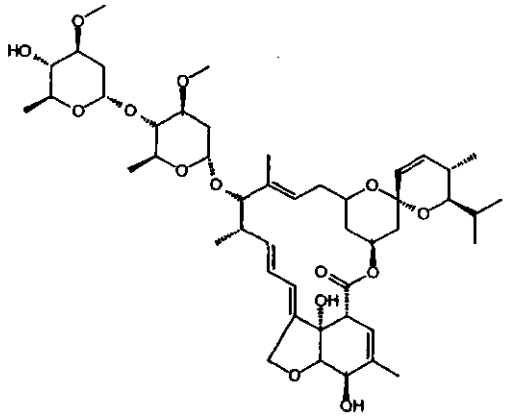
Note: The masses set for Q1 and Q3 in data acquisition should be based on the center of the observed precursor ion mass in Q1 and the center of the observed product ion mass in Q3. These masses may vary as much as 0.1-0.2 amu from the exact molecular mass obtained by manual calculations.

VI. FIGURES

FIGURE 1. CHEMICAL NAMES AND STRUCTURES

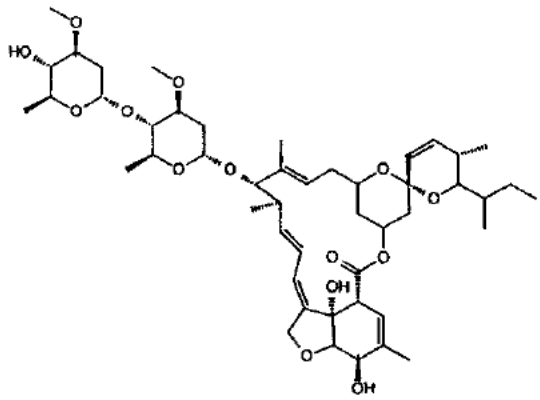


NOA-422601 (Avermectin B1a)
CAS No.: 65195-55-3



NOA-421704 (Avermectin B1b)
CAS No.: 65195-56-4

FIGURE 1. CHEMICAL NAMES AND STRUCTURES (Continued)



NOA-427011 (8,9-Z Avermectin B1a)
CAS No.: 113665-89-7

FIGURE 2. FLOW DIAGRAM FOR WATER

Add acetonitrile to water so that the final content will be 25% by volume.
Ensure analytes are desorbed from the surface of the water storage container by mechanically shaking the sample for several minutes.
Measure a 75-mL aliquot of the water sample containing acetonitrile.

↓

Partition with Methylene Chloride.

↓

Evaporate the Methylene Chloride

↓

Add 2.5 mL of Acetonitrile
Add 2.5 mL water.
Vortex mix sample.
Perform additional dilution of the sample, if required.

↓

Analyze by LC-MS/MS using reversed phase HPLC.