Analytical method for avermectin B1a, avermectin B1b, and 8,9-Z avermectin B1a in water

Reports:	ECM: MRID 45906203. Cassidy, P., Y. Li, J. Vargo, and N. Chamkasem. 2002. Analytical Method for the Determination of NOA-422601 (Avermectin B1a), NOA-421704 (Avermectin B1b), and NOA-427011 (8,9-Z Avermectin B1a) in Water by High Performance Liquid Chromatography with Mass Spectrometric Detection. Lab study ID: 14410 (Ricerca), 115-00 (Syngenta). Unpublished study performed by Ricerca, LLC, Concord, OH; submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Jun. 12, 2002. ILV: MRID 45906204. Robinson, N. 2002. Independent Laboratory Validation of Syngenta Analytical Method 115-00 for the Determination of NOA-422601 (Avermectin B1a), NOA-421704 (Avermectin B1b), and NOA-427011 (8,9-Z Avermectin B1a) in Water. Lab study ID: RJ3338B, 2235-02. Unpublished study performed by Syngenta, Bracknell, Berkshire, UK; submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Nov. 26, 2002.
Document No.:	MRIDs 45906203 & 45906204
Guideline:	850.6100
Statements:	The method validations were conducted in compliance with FIFRA GLP or UK GLP standards. Signed and dated Data Confidentiality, GLP Compliance, Quality Assurance, and Report Approval (ECM report only) statements were provided for the ECM and ILV reports.
Classification:	This analytical method is classified as **acceptable**. It was independently validated on the initial attempt, with minor modifications to more closely reproduce the method. However, the LOQ is greater than toxicological levels of concern for estuarine/marine invertebrates.
PC Code:	122804
Primary Reviewer:	Gregory Orrick Environmental Scientist
Secondary Reviewer:	James Lin Environmental Engineer

Signature: Gregory Orrick
Date: Mar. 19, 2014
Signature:
Date: Mar. 19, 2014

Executive Summary

This analytical method, 115-00, is designed for the quantitative determination of avermectin B1a, avermectin B1b, and 8,9-Z avermectin B1a in water using LC-MS/MS (see Table 1). The method is quantitative for the analytes at the stated LOQ of 0.05 µg/L. The LOQ is greater than toxicological levels of concern for estuarine/marine invertebrates at acute (0.01 µg/L) and chronic (0.00035 µg/L) exposure durations. The independent laboratory was successful at validating the method at the first attempt, following minor modifications to more closely reproduce the method.

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Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Avermectin B1a, Avermectin B1b, & 8,9-Z Avermectin B1a	45906203	45906204	X	Water	6/12/02	Syngenta Crop Protection, Inc.	LC-MS/MS	0.05 µg/L

I. Principle of the Method

Fortified water samples are treated with acetonitrile to desorb analytes from container surfaces. An aliquot (75-mL) is removed and partitioned with two 20-mL portions of dichloromethane. The organic portion is evaporated to dryness and reconstituted in 50% (v/v) acetonitrile/water for analysis with LC-MS/MS. The method quantifies avermectin B1a, avermectin B1b, and 8,9-Z avermectin B1a in water at the stated LOQ of 0.05 µg/L.

II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) (*i.e.*, the method is quantitative) for each analyte.

Table 2. Initial Validation Method Recoveries for Analytes in Surface and Ground Water

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Avermectin B1a	0.05 µg/L	10	73-104	91	11	12
	0.5 µg/L	10	80-105	91	6.7	7.4
	10 µg/L	10	85-102	92	6.2	6.8
Avermectin B1b	0.05 µg/L	10	76-111	93	11	12
	0.5 µg/L	10	84-110	96	6.8	7.1
	10 µg/L	10	91-110	98	6.9	7.0
8,9-Z Avermectin B1a	0.05 µg/L	10	88-107	100	5.3	5.3
	0.5 µg/L	10	93-120	104	9.4	9.1
	10 µg/L	10	101-121	110	8.4	7.6

Table 3. Independent Validation Method Recoveries for Analytes in Surface Water

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Avermectin B1a	0.05 µg/L	5	81-96	87	5.5	6.3
	0.5 µg/L	5	88-99	93	4.4	4.7
Avermectin B1b	0.05 µg/L	5	67-94	83	10	12
	0.5 µg/L	5	55-89	74	13	17
8,9-Z Avermectin B1a	0.05 µg/L	5	75-98	85	9.6	11
	0.5 µg/L	5	75-100	86	9.7	11

III. Method Characteristics

The LOD was calculated as 4x the baseline noise in a control sample. The LOQ was determined as the lowest fortification concentration with adequate accuracy (mean recoveries within 70-120%) and precision (RSDs ≤20%). The method was reproducible for all analytes at the stated LOQ of 0.05 µg/L. Recoveries for avermectin B1b initially included low values (≤60%) at both concentrations that were improved by reducing the HPLC injection volume to 10 µL, as stated in the method, to reduce matrix suppression. Also, the HPLC mobile phase gradient needed to be changed to prevent carry-over problems with the auto-sampler. Overall, however, the method was repeatable (*i.e.*, quantitative) with care taken.

Table 4. Method Characteristics

	Avermectin B1a	**Avermectin B1b**	**8,9-Z Avermectin B1a**
Limit of Quantitation (LOQ)	0.05 µg/L	0.05 µg/L	0.05 µg/L
Limit of Detection (LOD)	0.003 µg/L	0.003 µg/L	0.004 µg/L
Linearity (calibration curve r^2 and concentration range)	r^2 = 1.000 (0.25 – 10 µg/L)	r^2 = 0.9968 (0.25 – 10 µg/L)	r^2 = 0.9999 (0.25 – 10 µg/L)
Repeatable	Yes, with care	Yes, with care	Yes, with care
Reproducible	Yes	Yes	Yes
Specific	Yes	Yes	Yes

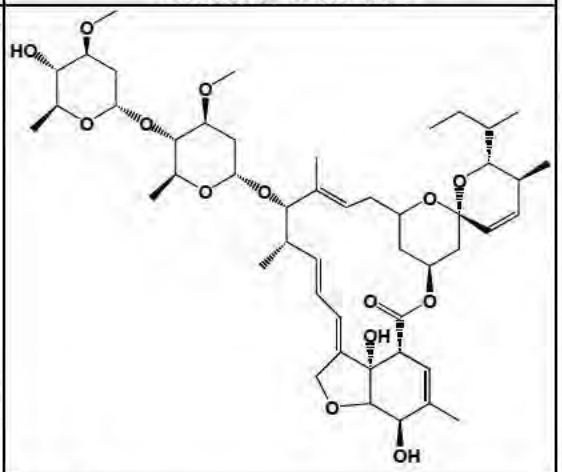
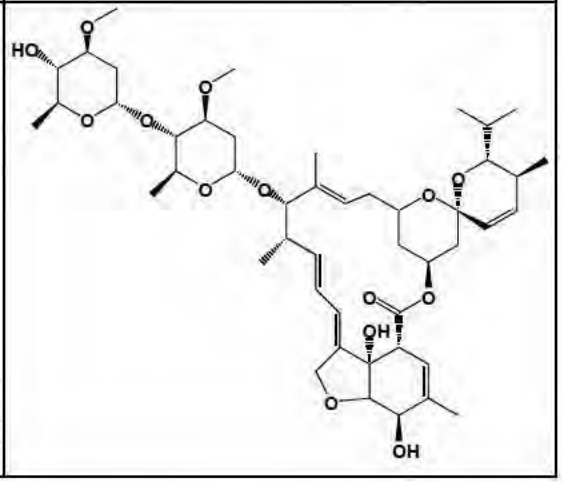
IV. Method Deficiencies and Reviewer's Comments

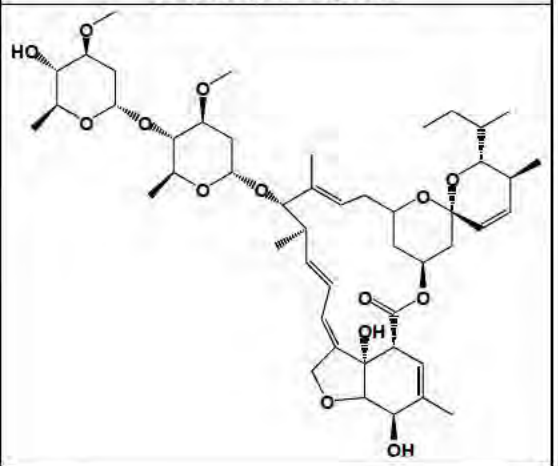
The independent laboratory was successful at validating the method at the first attempt. However, recoveries for avermectin B1b initially included low values (≤60%) at both concentrations that were improved by reducing the HPLC injection volume to 10 µL, as stated in the method, to reduce matrix suppression. Also, the HPLC mobile phase gradient needed to be changed to prevent carry-over problems with the auto-sampler. These changes do not appear to reflect deficiencies with the analytical method.

The LOQ (0.05 µg/L) is greater than toxicological levels of concern for estuarine/marine invertebrates at acute (0.01 µg/L) and chronic (0.00035 µg/L) exposure durations.

Attachment 1: Chemical Names and Structures

Table 1. Abamectin (Avermectin B1a plus B1b) and Its Environmental Transformation Products. [A]

Code Name/ Synonym	Chemical Name	Chemical Structure
Avermectin B_{1a} **NOA 422601** **MK 936**	**IUPAC:** (10E,14E,16E)-(1R,4S,5′S,6S,6′R,8R,12S,13S,20R,21R,24S)-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 **CAS:** 5-O-demethyl-avermectin A1a **CAS No.:** 65195-55-3 **Formula:** $C_{48}H_{72}O_{14}$ **MW:** 873.1 g/mol **SMILES:** CC[C@H](C)[C@@H]1[C@H](C=C[C@@]2(O1)C[C@@H]3C[C@H](O2)C/C=C(/[C@H]([C@H](/C=C/C=C/4\CO[C@H]5[C@@]4([C@@H](C=C([C@H]5O)C)C(=O)O3)O)C)O[C@H]6C[C@@H]([C@H]([C@@H](O6)C)O[C@H]7C[C@@H]([C@H]([C@@H](O7)C)O)OC)OC)\C)C	
Avermectin B_{1b} **NOA 421704**	**IUPAC:** (10E,14E,16E)-(1R,4S,5′S,6S,6′R,8R,12S,13S,20R,21R,24S)-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 **CAS:** 5-O-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-avermectin A1a **CAS No.:** 65195-56-4 **Formula:** $C_{47}H_{70}O_{14}$ **MW:** 859.1 g/mol **SMILES:** C[C@@H]\1[C@@H](/C(=C/C[C@H]2O[C@]3(O[C@@H]([C@H](C=C3)C)[C@@H](C)C)C[C@H](C2)OC(=O)[C@H]4[C@@]5(/C(=C/C=C1)/CO[C@@H]5[C@@H](C(=C4)C)O)O)/C)O[C@@H]6O[C@H]([C@@H]([C@H](C6)OC)O[C@@H]7O[C@H]([C@@H]([C@H](C7)OC)O)C)C	

Code Name/ Synonym	Chemical Name	Chemical Structure
8,9-Z Avermectin B_{1a} **NOA 427011**	**CAS:** 5-O-demethyl-, (8Z)-(9Cl)-avermectin A1a **CAS No.:** 113665-89-7 **Formula:** $C_{48}H_{72}O_{14}$ **MW:** 873.1 g/mol **SMILES:** CC[C@H](C)[C@@H]1[C@H](C=C[C@@]2(O1)C[C@@H]3C[C@H](O2)C/C=C(/[C@H]([C@H](\C=C/C=C/4\CO[C@H]5[C@@]4([C@@H](C=C([C@H]5O)C)C(=O)O3)O)C)O[C@H]6C[C@@H]([C@H]([C@@H](O6)C)O[C@H]7C[C@@H]([C@H]([C@@H](O7)C)O)OC)OC)\C)C	

[A] MW means "molecular weight".