

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

**Reports:** ECM: MRID 45906202. 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 Soil by High Performance Liquid Chromatography with Mass Spectrometric Detection. Lab study ID: 14409 (Ricerca), 116-00 (Syngenta). Unpublished study performed by Ricerca, LLC, Concord, OH; submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Jun. 12, 2002.

ILV: MRID 45906205. Hargreaves, S. 2002. Independent Laboratory Validation of Syngenta Analytical Method 116-00 for the Determination of NOA-422601 (Avermectin B1a), NOA-421704 (Avermectin B1b), and NOA-427011 (8,9-Z Avermectin B1a) in Soil. Lab study ID: RJ3339B, 2236-02. Unpublished study performed by Syngenta, Bracknell, Berkshire, UK; submitted by Syngenta Crop Protection, Inc., Greensboro, NC. Dec. 12, 2002.

**Document No.:** MRIDs 45906202 & 45906205

**Guideline:** 850.6100

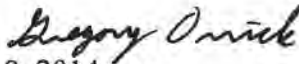
**Statements:** The method validations were conducted in compliance with FIFRA GLP or UK GLP standards. Signed and dated Data Confidentiality, GLP Compliance, Quality Assurance, and Report Approval (ECM report only) statements were provided for the ECM and ILV reports.

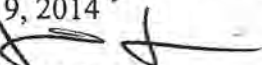
**Classification:** This analytical method is classified as **acceptable**. It was independently validated upon the second attempt, following closer adherence to the method procedure.

**PC Code:** 122804

**Primary Reviewer:** Gregory Orrick  
Environmental Scientist

**Secondary Reviewer:** James Lin  
Environmental Engineer

**Signature:**   
**Date:** Mar. 19, 2014

**Signature:**   
**Date:** Mar. 19, 2014

**Executive Summary**

This analytical method, 116-00, is designed for the quantitative determination of avermectin B1a, avermectin B1b, and 8,9-Z avermectin B1a in soil using LC-MS/MS (see Table 1). The method is quantitative for the analytes at the stated LOQ of 0.5 µg/kg. Whether the LOQ is below toxicological levels of concern is unknown in the absence of terrestrial plant toxicity studies. The independent laboratory was unsuccessful at validating the method at the first attempt. A second, more careful attempt achieved adequate recoveries for the analytes at the LOQ, but a single recovery for avermectin B1b was low at 10x the LOQ. The study sponsor excluded the single low recovery of avermectin B1b as an outlier. In this review, the low recovery is included because it reflects uncertainty with the repeatability of the method for avermectin B1b. However, it does not invalidate the reproducibility at the LOQ of avermectin B1b and the other analytes.

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

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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	45906202	45906205	X	Soil	6/12/02	Syngenta Crop Protection, Inc.	LC-MS/MS	0.5 µg/kg

## I. Principle of the Method

Fortified soil samples (10 g) are extracted twice with 70% (v/v) acetonitrile/water by shaking, centrifuging, and filtering. Extracts are combined and the organic extracts are removed via rotary evaporation. The remaining aqueous extract receives acetonitrile and ammonium hydroxide prior to passing through an SPE column that is rinsed with 25% acetonitrile/water and 1% ammonium hydroxide. Analytes are eluted with dichloromethane that is then removed with rotary evaporation. The remaining residue is dissolved in acetonitrile/water for analysis with LC-MS/MS. The method quantifies avermectin B1a, avermectin B1b, and 8,9-Z avermectin B1a in soil at the stated LOQ of 0.5 µg/kg.

## 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 with the exception that avermectin B1b had a single low recovery (37%) at 10x the LOQ.

**Table 2. Initial Validation Method Recoveries for Analytes in Soil**

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Avermectin B1a	0.5 µg/kg	5	78.3-88.2	82.9	4.4	5.3
	5 µg/kg	5	85.5-91.2	87.4	2.3	2.6
Avermectin B1b	0.5 µg/kg	5	75.5-93.2	83.7	7.0	8.4
	5 µg/kg	5	87.6-98.2	94.1	4.3	4.6
8,9-Z Avermectin B1a	0.5 µg/kg	5	71.7-81.0	78.1	3.8	4.9
	5 µg/kg	5	80.8-89.6	85.6	3.4	4.0

**Table 3. Independent Validation Method Recoveries for Analytes in Soil**

Analyte	Fortification Level (units)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Avermectin B1a	0.5 µg/kg	5	90-96	93	2.2	2.4
	5 µg/kg	5	76-97	90	8.3	9.2
Avermectin B1b	0.5 µg/kg	5	81-92	85	4.4	5.1
	5 µg/kg	5	37-91	77	23	30
8,9-Z Avermectin B1a	0.5 µg/kg	5	91-104	99	4.9	4.9
	5 µg/kg	5	93-98	96	1.9	2.0

### 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  $\leq 20\%$ ). The method was reproducible for all analytes at the stated LOQ of 0.5  $\mu\text{g}/\text{kg}$ . But recoveries for all three analytes were not quantitative at the initial validation attempt and upon the second attempt, recoveries for avermectin B1b included one low value (37%) out of five values at 5  $\mu\text{g}/\text{kg}$ . Therefore, the method was only repeatable or quantitative with extra care taken, with uncertainty for avermectin B1b at 5  $\mu\text{g}/\text{kg}$ .

**Table 4. Method Characteristics**

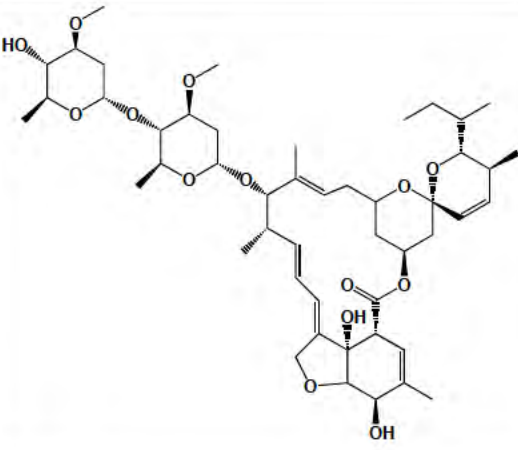
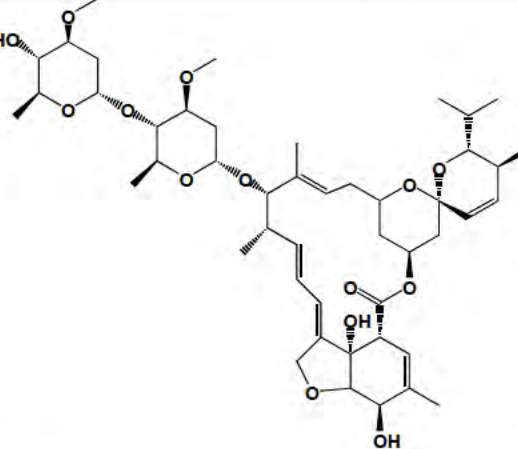
	<b>Avermectin B1a</b>	<b>Avermectin B1b</b>	<b>8,9-Z Avermectin B1a</b>
Limit of Quantitation (LOQ)	0.5 $\mu\text{g}/\text{kg}$	0.5 $\mu\text{g}/\text{kg}$	0.5 $\mu\text{g}/\text{kg}$
Limit of Detection (LOD)	0.1 $\mu\text{g}/\text{kg}$	0.2 $\mu\text{g}/\text{kg}$	0.07 $\mu\text{g}/\text{kg}$
Linearity (calibration curve $r^2$ and concentration range)	$r^2 = 0.9998$ (0.1 – 10 $\mu\text{g}/\text{L}$ )	$r^2 = 0.9998$ (0.1 – 10 $\mu\text{g}/\text{L}$ )	$r^2 = 1.000$ (0.1 – 10 $\mu\text{g}/\text{L}$ )
Repeatable	Yes, with care	Yes, with care	Yes, with care
Reproducible	Yes	Yes	Yes
Specific	Yes	Yes	Yes

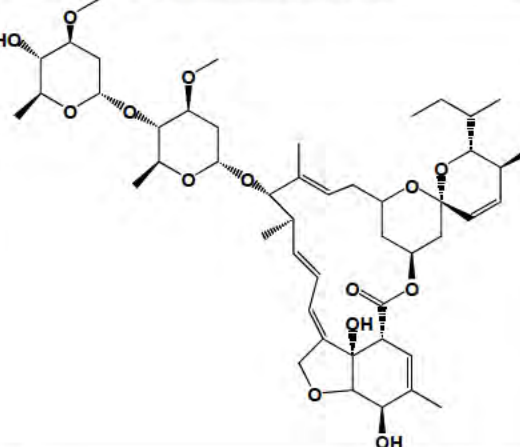
### IV. Method Deficiencies and Reviewer's Comments

The independent laboratory was unsuccessful at validating the method at the first attempt. A second, more careful attempt achieved adequate recoveries for the analytes at the LOQ, but a single recovery for avermectin B1b was low at 10x the LOQ. The second attempt involved greater efforts to avoid contamination, including using labware not previously used to analyze abamectin. Rotary evaporation was conducted slower to avoid bumping. Samples were sonicated and added to the SPE column slower, at  $\sim 1$  drop per second. And HPLC injection volumes were reduced to 10  $\mu\text{L}$ . These changes appear to reflect more careful laboratory conduct rather than deficiencies with the analytical method.

## Attachment 1: Chemical Names and Structures

Table 1. Abamectin (Avermectin B1a plus B1b) and Its Environmental Transformation Products. <sup>A</sup>

Code Name/ Synonym	Chemical Name	Chemical Structure
<b>Avermectin B<sub>1a</sub></b> <b>NOA 422601</b> <b>MK 936</b>	<p><b>IUPAC:</b> (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<sup>4,8</sup>.0<sup>20,24</sup>]-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-<math>\alpha</math>-L-arabino-hexopyranosyl)-3-O-methyl-<math>\alpha</math>-L-arabino-hexopyranoside</p> <p><b>CAS:</b> 5-O-demethyl-avermectin A1a</p> <p><b>CAS No.:</b> 65195-55-3</p> <p><b>Formula:</b> C<sub>48</sub>H<sub>72</sub>O<sub>14</sub>  <b>MW:</b> 873.1 g/mol  <b>SMILES:</b>  <chem>CC[C@H](C)[C@@H]1[C@H](C=C[C@@]2(O1)C[C@@]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</chem></p>	
<b>Avermectin B<sub>1b</sub></b> <b>NOA 421704</b>	<p><b>IUPAC:</b> (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<sup>4,8</sup>.0<sup>20,24</sup>]-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-<math>\alpha</math>-L-arabino-hexopyranosyl)-3-O-methyl-<math>\alpha</math>-L-arabino-hexopyranoside</p> <p><b>CAS:</b> 5-O-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)-avermectin A1a</p> <p><b>CAS No.:</b> 65195-56-4</p> <p><b>Formula:</b> C<sub>47</sub>H<sub>70</sub>O<sub>14</sub>  <b>MW:</b> 859.1 g/mol  <b>SMILES:</b>  <chem>C[C@@H]1[C@@H](/C=C/[C@H]2O[C@]3(O[C@@H]([C@H](C=C3)C)[C@@H](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)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</chem></p>	

Code Name/ Synonym	Chemical Name	Chemical Structure
<b>8,9-Z Avermectin B<sub>1a</sub></b> <b>NOA 427011</b>	CAS: 5-O-demethyl-, (8Z)-(9Cl)-avermectin A1a  <b>Formula:</b> C <sub>48</sub> H <sub>72</sub> O <sub>14</sub> <b>MW:</b> 873.1 g/mol <b>SMILES:</b> <chem>CC[C@@H](C)[C@@H]1[C@H](C=C[C@@]2(O1)C[C@@H]3C[C@H](O2)C=C/[C@@H]([C@H](C=C/C=C/4CO[C@H]5[C@@]4([C@@H](C=C([C@H]5O)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</chem>	

<sup>A</sup> MW means "molecular weight".

## Attachment 2: Statistics Spreadsheets



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