Study Number 02-S502

Report Title Abamectin

Independent Laboratory Validation of Syngenta Analytical Method 116-00 for the Determination of NOA-422601 (Abamectin B1a), NOA-421704 (Abamectin B1b) and NOA-427011 (8,9-Z Abamectin B1a) in Soil.

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Summary

The analytical procedure documented in Syngenta study report 116-00 (Appendix 7) was independently validated by laboratory personnel totally unfamiliar with the method and using equipment (supplies and instrumentation) not previously used by the method developer.

Untreated soil was fortified with NOA-422601, NOA-421704 and NOA-427011 at 0.0005 mg kg^{-1} and at 0.005 mg kg^{-1} in quintuplet and analysed using the supplied analytical procedure.

The recovery of NOA-422601, NOA-421704 and NOA-427011 was determined for each sample and the results used to determine the validity of the method.

Validation of the method was unsuccessful at the first attempt for all analytes.

After consultation with the sponsor, experiments were carried out to determine the cause of the failure. The cause of failure was not established. In a second validation attempt, measures were taken to eliminate contamination and particular care with both the rotary evaporation step and the solid phase extraction (SPE) step was exercised.

After consultation with the sponsor, the samples were reanalysed using a lower injection volume (10 µL instead of 20 µL) to see if the low recovery was due to unusual matrix suppression. Reanalysis of the samples with the lower injection volume made no significant difference to any of the recoveries:

The sponsor reviewed the two data sets and accepted the data using the 10 µL injection volume,

The reagent blank and control samples analysed as part of this study showed no detectable residues of any of the analytes.

1 Introduction

The aim of this study was to achieve an independent laboratory validation of the analytical method described in Syngenta study report 116-00 (Appendix 7) for the determination of NOA-422601, NOA-421704 and NOA-427011 in soil. Specifically to:

a) Prove that soil procedural recoveries fortified with NOA-422601, NOA-421704 and NOA-427011 in soil can be taken through the analytical method, and a mean recovery of 70% - 120% of the fortified amount can be achieved with an overall relative standard deviation (RSD) of ≤ 20%.

b) Demonstrate that the relationship between sample concentration and detector response is linear over the working range of the method.

The validation was carried out by personnel totally unfamiliar with the method and using equipment (supplies and instrumentation) not previously used by the method developer, thus meeting the requirements as permitted in OPPTS 850.7100 and EPA guidelines PR Notice 96-1.

2 Materials

2.1 Test and Reference Substances

The following analytical standards were utilised in this study.

Figure 1

Compound : NOA-422601 (Abamectin B1a)

CAS Number : 65195-55-3

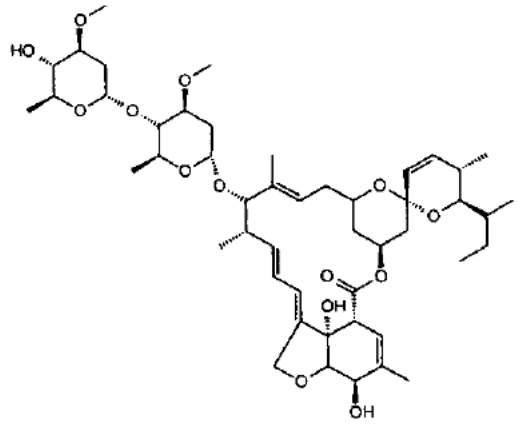


Figure 2

Compound : NOA-421704 (Abamectin B1b)

CAS Number : 65195-56-4

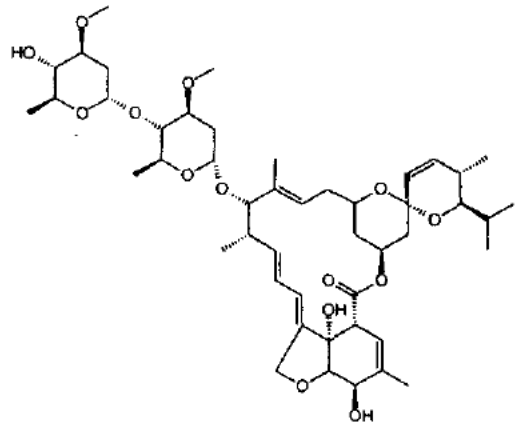
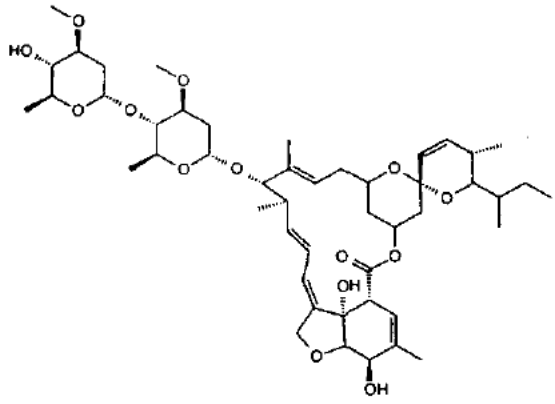


Figure 3

Compound : NOA-427011 (8,9-Z Abamectin B1a)

CAS Number : 113665-89-7



NOA-422601 Analytical Standard Reference, batch reference MRH-I-35-2. Purity 99.5%. Reanalysis date November 2002.
NOA-421704 Analytical Standard Reference, batch reference SCR-I-47. Purity 99.1%. Reanalysis date June 2004.
NOA-427011 Analytical Standard Reference, batch reference TEW-IX-5-1. Purit 98.8%. Reanalysis date December 2002.

a) The analytical standards were obtained from Syngenta Crop Protection, Analytical Development and Product Chemistry GS2131, Münchwilen AG, Breitenloh 5, CH-4333 Münchwilen and Syngenta Crop Protection Inc. PO Box 18300, Greensboro NC 27419, USA. These standards are stored in the Environmental Sciences standard store at <-18°C and were used within their expiry date.

2.2 Test System

The control sample utilised in this study is described in Tables 2 and 3.

Table 2. : Control Samples

Matrix Type	Sample Number	Trial Reference	Source
Soil- sandy clay loam	1	J6426/1	Spalding, Lincolnshire, UK

Table 3. : Soil Physicochemical Properties (Ref. 1)

Soil Depth (cm)	pH [1]	pH [2]	CEC[3] (meq/100 g)	O.M.[4] (%)	WHC[5] @ 1/3 bar (%)	WHC[5] @ 15 bar (%)	Sand (%)	Silt (%)	Clay (%)	USDA Class
0-15	7.5	6.8	9.2	2.3	28.2	12.7	66	14	20	Sandy clay loam

[1] pH measured in water
[2] pH measured in $CaCl_2$
[3] CEC = cation exchange capacity
[4] O.M. = organic matter
[5] WHC = water-holding capacity

3 Methods

3.1 Preparation and Stability of Analytical Standard Solutions

Individual stock solutions of NOA-422601, NOA-421704 and NOA-427011 were prepared in acetonitrile, according to the procedures described in the analytical method (Appendix 7). These were subsequently used to prepare working standard solutions in acetonitrile or mixed working standard solutions in acetonitrile:water (50:50 v/v).

NOA-422601, NOA-421704 and NOA-427011 standards in acetonitrile or acetonitrile:water (50:50 v/v) are assumed to be stable when stored in amber bottles at ≤ 7°C for up to 4 months after preparation.

3.2 Fortification Levels

Recovery of the analyte through the analytical procedure was assessed by fortifying aliquots of soil with NOA-422601, NOA-421704 and NOA-427011. Five replicate recoveries were carried out at the LOQ (0.0005 mg kg^{-1}) and five replicate recoveries were carried out at ten times the LOQ (0.005 mg kg^{-1}). Fortification levels are summarised in Table 4.

Table 4 : Fortification Levels

Fortification Level (mg kg^{-1})	Number of Replicates
Control	2
0.0005	5
0.005	5

3.3 Analytical Procedures

3.3.1 Sample Analysis

Samples were analysed according to procedures described in detail in Syngenta study report 116-00 (Appendix 7). The method was followed as written, with the following modifications:

- The instrument used for analysis was a PE Sciex API 3000 LC-MS-MS and minor modifications were made to the instrument parameters in order to maximize sensitivity. (see Appendix 1 for full details of analytical instrumentation).

- The chromatographic conditions listed in Table 1 of method 116-00 were modified to eliminate carry-over problems with the analyte NOA-427011 observed when using the gradient program specified in method 116-00. An isocratic mobile phase of methanol:water (88:12, v/v) was used and the run time was reduced to 6 minutes.

- A 20 µL injection volume was used to enable measurement of the 0.0001 µg mL^{-1} concentration mixed standard in the linearity check, but the 10 µ volume was sufficient to accurately measure residues of NOA-422601, NOA421704 and NOA-427011 in soil at the LOQ of 0.0005 mg kg^{-1}.

The percentage recovery obtained for each sample was calculated and these results were used to assess the relative standard deviation of the analytical method.

3.3.2 Detector Linearity

Standard solutions of NOA-422601, NOA-421704 and NOA-427011 at concentration levels from 0.0001 μg mL^{-1} to 0.01 μg mL^{-1} (equivalent to 2 to 200 pg of analyte injected on to the column using a 20 μL injection volume) were analysed in triplicate by LC-MS-MS, using the conditions as specified in this report. The mean detector response was plotted against standard concentration and the correlation coefficient determined using Microsoft Excel (Figures 10-12).

3.3.3 Time Required for Analysis

Based on experience gained during this study, it is estimated that a batch of up to 13 samples, including control and recovery samples, could be taken through the procedure within a working day, assuming an nine hour working period. Final determination by LC-MS-MS was carried out overnight (see Table 11).

3.3.4 Statistical Analysis

The 95% confidence intervals for the true average recoveries at each spiking level were calculated using Microsoft Excel (Tables 6 and 7).

6 Recommendations

The following comments on method 116-00 indicate where minor improvements or clarification may be useful.

- Table 1 : HPLC System and Operating Conditions.

  Due to carry-over problems with the autosampler used for this study (CTC HTS PAL), it was necessary to modify the mobile phase from a gradient to an isocratic mobile phase of methanol/water (88:12, v/v). It is recommended that the isocratic mobile phase be used when using this type of autosampler.

Instrument Description

Pump	:	Agilent 1100 series quaternary pump model number G1311A
Degasser	:	Agilent 1100 series model number G1322A
Column Oven	:	Agilent 1100 series model number G1316A fitted with column switching valve
Detector	:	PE Sciex API 3000 triple quadrupole mass spectrometer
Autosampler	:	CTC HTS PAL

Chromatography Conditions

Column	:	Phenyl hexyl 150 mm × 2.0 mm, 5 µm particle size
Mobile phase	:	Methanol:water v/v (88:12)
Column flow rate	:	0.3 mL min^{-1}
Injection volume	:	10 µL
Stop Time	:	6 minutes
Column oven temperature	:	40°C

Mass Spectrometer Conditions – NOA-422601 and NOA-427011

Interface	:	TurboIonSpray
Polarity	:	Positive
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 8 (arbitrary units)
Temperature (TEM)	:	400°C
Ionspray voltage	:	5500 V
Collision gas setting (CAD)	:	Nitrogen set at 8 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	895.5
Q3 mass	:	751.5
Dwell time	:	300 ms

Resolution Q1	:	Low
Resolution Q3	:	Unit
Declustering potential (DP)	:	195 V
Focusing potential (FP)	:	350 V
Entrance potential (EP)	:	10 V
Collision energy (CE)	:	59 V
Collision cell exit potential (CXP)	:	26 V
Electron multiplier setting (CEM)	:	2600 V

Mass Spectrometer Conditions – NOA-421704

Interface	:	TurboIonSpray
Polarity	:	Positive
Nebuliser gas (NEB)	:	Nitrogen set at 12 (arbitrary units)
Curtain gas (CUR)	:	Nitrogen set at 8 (arbitrary units)
Temperature (TEM)	:	400°C
Ionspray voltage	:	5500 V
Collision gas setting (CAD)	:	Nitrogen set at 8 (arbitrary units)
Scan type	:	MRM
Q1 mass	:	881.5
Q3 mass	:	737.5
Dwell time	:	300 ms
Resolution Q1	:	Low
Resolution Q3	:	Unit
Declustering potential (DP)	:	195 V
Focusing potential (FP)	:	350 V
Entrance potential (EP)	:	10 V
Collision energy (CE)	:	59 V
Collision cell exit potential (CXP)	:	26 V
Electron multiplier setting (CEM)	:	2600 V