

2.0 INTRODUCTION

Verification samples were fortified and analyzed to evaluate the performance of a method for the analysis of NOA446510 in filtered saltwater. The study was conducted by Wildlife International, Ltd. and identified as Project Number 528C-123. The analyses of the samples were performed at Wildlife International, Ltd. using High Performance Liquid Chromatography (HPLC) with variable wavelength detection. Verification samples were prepared and analyzed on November 15, 2004. All raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 528C-123 in archives located on the Wildlife International, Ltd. site.

3.0 PURPOSE

The purpose of this study was to verify the performance of a method for analysis of NOA446510 in saltwater to be used at Wildlife International, Ltd. for proposed environmental effects studies.

4.0 EXPERIMENTAL DESIGN

The study was performed based on procedures in *Residues: Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414 (1)*. Filtered saltwater was fortified at three different concentrations and analyzed using a method developed by Wildlife International, Ltd. Reagent and matrix blanks were analyzed concurrently with the matrix fortification sets to evaluate potential analytical interferences. A calibration curve was prepared from external standards of NOA446510 to determine the test substance concentrations in the samples.

5.0 MATERIALS AND METHODS

5.1 Test and Reference Substances

The test substance was received from Syngenta on October 28, 2004 and was assigned Wildlife International, Ltd. Identification Number 6912 upon receipt. The test substance, a solid, was identified as: NOA446510 Technical; Batch Number SEZ 3 BP004; FL041251; CAS Number 374726-62-2. The test substance had a reported purity of 96.1% and an

expiration date of October 31, 2005. The test substance was stored under ambient conditions. The Certificate of Analysis is presented in Appendix 4.

The analytical standard was received from Syngenta on April 13, 2004 and was assigned Wildlife International, Ltd. Identification Number 6644 upon receipt. The analytical standard, a beige powder, was identified as: NOA446510; Batch Number AMS 1131/2; CAS Number 374726-62-2. The analytical standard had a reported purity of 99.0% and an expiration date of June 2005. The analytical standard was stored under ambient conditions. The Certificate of Analysis is presented in Appendix 4.

5.2 Stocks/Standards Preparation

A stock solution of NOA446510 technical was prepared by accurately weighing 0.1043 g (corrected for purity) of the test substance on an analytical balance. The test substance was transferred to a 100-mL volumetric flask, and brought to volume using acetonitrile. The primary stock solution contained 1.00 mg a.i./mL of NOA446510 technical and was diluted using acetonitrile to prepare 0.100 and 0.0100 mg a.i./mL stock solutions. The 1.00, 0.100 and 0.0100 mg a.i./mL stock solutions were used to fortify the method verification samples.

A stock solution of NOA-446510 was prepared by accurately weighing 0.02526 g (corrected for purity) of the analytical standard on an analytical balance. The analytical standard was transferred to a 50.0-mL volumetric flask, and brought to volume using acetonitrile. The primary stock solution contained 0.500 mg a.i./mL of NOA-407854 analytical standard and was serially diluted using acetonitrile to prepare 0.100, 0.0100, 0.00100 mg a.i./mL stock solutions. The 0.0100 mg a.i./mL stock solution was used to prepare the calibration standards in filtered saltwater. The following shows the dilution scheme for the set of calibration standards:

Stock Concentration <u>mg a.i./mL</u>	Aliquot (<u>μL each</u>)	Final Volume (<u>mL</u>)	Standard Concentration (<u>mg a.i./L</u>)
0.0100	50.0	10.0	0.0500
0.0100	150	10.0	0.150
0.0100	250	10.0	0.250
0.0100	350	10.0	0.350
0.0100	500	10.0	0.500

5.3 Reagents and Solvents

All solvents used were HPLC grade or equivalent. All reagents were ACS reagent grade or higher quality.

5.4 Test System

The water used for testing was natural seawater collected at Indian River Inlet, Delaware, and diluted to a salinity of approximately 20‰ with well water. Mean salinity and pH measurements taken during the four-week period immediately preceding the test are presented in [Appendix 1](#).

The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 μm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. Prior to delivery to the end user system, the water again was filtered (0.45 μm) to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in saltwater used by Wildlife International, Ltd. are presented in [Appendix 2](#).

5.5 Analytical Method

The method used for the analysis of the method verification samples was developed by Wildlife International, Ltd. The analytical method consisted of preparing standards and QC samples in filtered saltwater, transferring to autosampler vials and analyzing by HPLC/UV analysis.

Concentrations of NOA446510 in the samples were determined using high performance liquid chromatography (HPLC) with variable wavelength detection. The instrument, an Agilent Model 1100 High Performance Liquid Chromatograph was equipped with an Agilent Model 1100 Variable Wavelength Detector operated at 220 nm. Chromatographic separations were achieved using a YMC-PACK ODS AM column (150 mm x 4.6 mm, 3 µm particle size). Instrumental parameters for the analysis of NOA446510 are summarized in [Table 1](#) and a method flowchart is provided in [Figure 1](#).

5.6 Calibration Curves and Limit of Quantitation (LOQ)

Calibration standards of NOA446510, ranging in concentration from 0.0500 to 0.500 mg a.i./L, were analyzed with the sample set. A linear regression equation was generated using the peak area responses versus the respective concentrations of the calibration standards. The calibration curve is presented in [Figure 2](#). The concentration of NOA446510 in the samples was determined by substituting the peak area response of the samples into the linear regression equation. Representative chromatograms of low- and high-level calibration standards are presented in [Figures 3](#) and [4](#), respectively.

The limit of quantitation (LOQ) for the method verification analysis in filtered saltwater was set at 0.0500 mg a.i./L based upon the product of the concentration of the lowest calibration standard (0.0500 mg a.i./L) and the dilution factor of the matrix blank samples (1.00).

5.7 Reagent and Matrix Blanks

Concurrent with the series of matrix fortification samples, two reagent blank samples (containing HPLC-grade bottled water) and two matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses ([Table 2](#)). A representative chromatogram of a reagent blank is presented in [Figure 5](#). A representative chromatogram of a matrix blank for filtered saltwater is presented in [Figure 6](#).

5.8 Saltwater Method Verification Samples

Filtered saltwater was fortified at 0.100, 1.00 and 10.0 mg a.i./L using stock solutions of NOA446510 prepared in acetonitrile. Samples fortified at 0.100, 1.00 and 10.0 mg a.i./L containing NOA446510 yielded mean recoveries of 101, 88.6 and 94.1%, respectively (Table 2). A representative chromatogram of method verification sample is presented in Figure 7.

5.9 Example Calculations

The analytical result and percent recovery for sample number 528C-123-VMAS-3, with a nominal concentration of 0.100 mg a.i./L in filtered saltwater was calculated using the following equations:

$$\text{Concentration of NOA446510 in sample (mg a.i./L)} = \frac{\text{peak area} - (\text{y-intercept})}{\text{slope}} \times \text{dilution factor}$$

$$\text{Percent of nominal concentration} = \frac{\text{Measured concentration of sample (mg a.i./L)}}{\text{Nominal concentration of sample (mg a.i./L)}} \times 100$$

Peak Area = 24.28618

Y-Intercept = -2.2439

Slope = 262.60

Dilution Factor = 1.00

$$\text{Concentration of NOA446510 in sample (mg a.i./L)} = \frac{24.28618 + 2.2439}{262.60} \times 1.00$$

$$\text{Concentration of NOA446510 in sample (mg a.i./L)} = 0.101$$

$$\text{Percent of nominal concentration} = \frac{0.101 \text{ mg a.i./L}}{0.100 \text{ mg a.i./L}} \times 100$$

$$\text{Percent of nominal concentration} = 101\%$$

Table 1 Typical HPLC Operational Parameters
for the Analysis of NOA446510 in Filtered Saltwater

INSTRUMENT:	Agilent Model 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Model 1100 Variable Wavelength Detector			
ANALYTICAL COLUMN:	YMC-PACK ODS AM (150 x 4.6 mm, 3- μ m particle size)			
STOP TIME:	15 minutes			
FLOW RATE:	1.000 mL/min			
SOLVENT A:	0.1% H ₃ PO ₄			
SOLVENT B:	CH ₃ CN			
GRADIENT ELUTION PROFILE:	Time (min)	%A	%B	Flow (mL/min)
	0.01	90.0	10.0	1.000
	1.00	90.0	10.0	1.000
	9.00	5.0	95.0	1.000
	10.00	5.0	95.0	1.000
	10.10	90.0	10.0	1.000
	15.00	90.0	10.0	1.000
OVEN TEMPERATURE:	40°C			
INJECTION VOLUME:	100 μ L			
NOA446510 RETENTION TIME:	Approximately 9.6 minutes			
WAVELENGTH:	220 nm			

Figure 1 Analytical method flow chart
for the analysis of NOA446510 in filtered saltwater.

