INTRODUCTION

Tests were conducted to verify the performance of a method for the determination of Fenpyroximate TGAI in freshwater. In addition, the stability of the Fenpyroximate TGAI was determined under ambient conditions in glass, stainless steel and Teflon®-lined stainless steel test vessels. This study was conducted by Wildlife International, Ltd. and identified as Project Number 397C-101. 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). The method was verified by fortifying freshwater with the test substance and determining the recoveries. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 397C-101 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to verify the performance of methodology for analyses of Fenpyroximate TGAI in freshwater and to evaluate the stability of the test substance in different test vessels.

EXPERIMENTAL DESIGN

The stability of Fenpyroximate TGAI in freshwater was determined at approximately 20°C. Stability was determined in three types of vessels (glass, stainless steel and Teflon®-lined stainless steel) under ambient laboratory conditions. Stability was assessed by analyzing samples collected after several time intervals in each vessel type. Fenpyroximate TGAI was prepared at a nominal concentration of 10.0 µg a.i./L. Aliquots were transferred to either glass, stainless steel of Teflon®-lined stainless steel vessels. Sampling was performed following 1, 2, 3, 4, 6 and 24 hours. Duplicate samples were analyzed at each interval. For the validation, freshwater was fortified in replicates of five at three different concentrations and analyzed based on a method developed by Wildlife International, Ltd. Two matrix and two reagent blanks were analyzed concurrently with the verification set to evaluate potential analytical interferences. A calibration curve was prepared from external standards of Fenpyroximate TGAI to determine the test substance concentrations in samples.

MATERIALS AND METHODS

Test Substance

The test substance was received from Nichino America, Inc. on March 23, 2005 and was assigned Wildlife International, Ltd. identification number 7089 upon receipt. The test substance, described as a powder, was identified as: Fenpyroximate Technical; Lot/Batch Number: 911250; Chemical Abstract (CAS) Number: 134098-61-6. The test substance was stored under ambient conditions.

Analytical Standard

The analytical standard was received from Nihon Nohyaku Co., Ltd. on August 31, 2004 and was assigned Wildlife International, Ltd. identification number 6821 upon receipt. The analytical standard, described as a solid, was identified as: Fenpyroximate; Lot Number: 2AA0013E; Chemical Abstract (CAS) Number: 134098-61-6. The analytical standard had an expiration date of October 18, 2005 and a purity of 99.8% and was stored under refrigerated conditions. The certificate of analysis is presented in Appendix 2.

A second analytical standard was received from Chem Service on October 31, 2005 and was assigned Wildlife International, Ltd. identification number 7406A upon receipt. The analytical standard, described as a solid, was identified as: Fenpyroximate; Lot Number: 337-78B; Chemical Abstract (CAS) Number: 134098-61-6. The new standard had an expiration date of January 2009 and a purity of 99% and was stored under ambient conditions. The certificate of analysis is presented in Appendix 2.

Stocks/Standards Preparation

A stock solution of Fenpyroximate Technical (TGAI) were prepared by weighing 0.1000 grams of the test substance on an analytical balance. The test substance was transferred to a 100-mL class A volumetric flask, and brought to volume using acetone. The primary stock solution (1.00 mg/mL) was serially diluted in acetone to prepare 0.100, 0.0100 and 0.00100 mg/mL stock solutions. The 0.0100 mg/mL stock solution was used to fortify the quality control samples for the stability trial. The 0.00100 mg/mL stock solution was used to fortify the quality control samples for the freshwater verification.

For stability, a stock solution of Fenpyroximate analytical standard was prepared by weighing 0.05012 grams (weight corrected for purity) of the analytical standard on an analytical balance. The analytical standard was transferred to a 50.0-mL class A volumetric flask, and brought to volume using

acetone. The primary stock solution (1.00 mg a.i./mL) was serially diluted in acetone to prepare 0.100 and 0.0100 mg a.i./mL stock solutions. Calibration standards were prepared in acetone using the 0.0100 mg a.i./mL stock solution. The following shows the dilution scheme for the set of calibration standards:

Stock Concentration (mg a.i./mL)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (µg a.i./L)
0.0100	100	100	10.0
0.0100	250	100	25.0
0.0100	500	100	50.0
0.0100	750	100	75.0
0.0100	1000	100	100

For the purity check, calibration standards were prepared in 50% methanol : 50% HPLC-grade bottled water using the 1.00 mg a.i./mL stock solution. The following shows the dilution scheme for the set of calibration standards:

Stock Concentration (mg a.i./mL)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (mg a.i./L)
1.00	30.0	10.0	3.00
1.00	50.0	10.0	5.00
1.00	100	10.0	10.0
1.00	200	10.0	20.0
1.00	300	10.0	30.0

For the validation, a stock solution of Fenpyroximate analytical standard was prepared by weighing 0.05051 grams (weight corrected for purity) of the analytical standard on an analytical balance. The analytical standard was transferred to a 50.0-mL class A volumetric flask, and brought to volume using acetone. The primary stock solution (1.00 mg a.i./mL) was serially diluted in acetone to prepare 0.100 and 0.0100 mg a.i./mL stock solutions. Calibration standards were prepared in acetone using the 0.0100 mg a.i./mL stock solution. The following shows the dilution scheme for the set of calibration standards:

Stock Concentration (mg a.i./mL)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (µg a.i./L)
0.0100	100	100	10.0
0.0100	250	100	25.0
0.0100	500	100	50.0
0.0100	750	100	75.0
0.0100	1000	100	100

Reagents and Solvents

All solvents used in this study were HPLC grade or equivalent.

Test System

The freshwater used to prepare the stability determinations and method verification studies was obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The well water was characterized as moderately-hard water. The means and ranges of specific conductance, hardness, alkalinity and pH measurements of the well water during the four-week period immediately preceding the test are presented in Appendix 4.

The well water 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 use, the water again was filtered to 0.45 µm in order to remove microorganisms and fine particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International, Ltd. are presented in Appendix 4.

Analytical Methods

Stability

The method used for the analysis of Fenpyroximate in freshwater for the stability trial was based upon methodology developed by Wildlife International, Ltd. The analytical method consisted of adding 20 mL of dichloromethane (DCM) to each sample and allowing the organic and aqueous layers to separate by shaking for approximately one minute. The lower layer (DCM) was drained into a roundbottom flask. To the aqueous fraction, 20 mL of DCM was added and shaken as above. Each extract was combined in its respective roundbottom flask. Each sample was then rotary evaporated using a waterbath and samples were evaporated to dryness under nitrogen. The requisite volume of acetone was added to each roundbottom flask and swirled to dissolve all residues. An aliquot of each extract was transferred to an autosampler vial and samples were submitted for analysis. Concentrations of Fenpyroximate in the samples were determined by gas chromatography using an Agilent Model 6890 Gas Chromatograph (GC) with an Agilent Model 5973 Mass Selective Detector operated in the SIM mode. Chromatographic separations were achieved using an Agilent 19091S-433 HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness). Typical instrumental parameters are presented in Table 1 and a method flowchart is provided in Figure 1.

Freshwater Validation

The method used for the analysis of Fenpyroximate in freshwater for the validation was based upon methodology developed by Wildlife International, Ltd. The analytical method consisted of adding 100 mL of dichloromethane (DCM) to each sample and allowing the organic and aqueous layers to separate by shaking for approximately one minute. The lower layer (DCM) was drained into a roundbottom flask. To the aqueous fraction, 100 mL of DCM was added and shaken as above. Each extract was combined in its respective roundbottom flask. Each sample was then rotary evaporated using a waterbath. Silica columns were conditioned with two column volumes of 50% DCM : 50% hexane. The columns were drained to the top of the silica bed and samples were loaded onto respective column. Roundbottom flasks were rinsed with 3 X ~2 mL of 50% DCM : 50% hexane and the rinses were added to the column. Full vacuum was applied for 30 minutes to dry the columns. The samples were eluted with one column volume of 30% acetone : 70% hexane into graduated centrifuge tubes and evaporated to dryness under nitrogen. The requisite volume of 70% acetonitrile : 30% HPLC-grade water was added and vortexed to dissolve all residues. An aliquot of each extract was transferred to an autosampler vial and samples were submitted for analysis. Concentrations of Fenpyroximate in the samples were determined by gas chromatography using an Agilent Series 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector. Chromatographic separations were achieved using a YMC PACK ODS AM column (150 m x 4.6 mm, 3 µm particle size). Typical instrumental parameters are presented in Table 2 and a method flowchart is provided in Figure 2.

Calibration Curves

Calibration standards of Fenpyroximate, ranging in concentration from 10.0 to 100 µg a.i./L, were prepared and analyzed with the appropriate sample set. For the purity check, calibration standards ranged from 3.00 to 30.0 mg a.i./L. Five calibration standards were analyzed with each set of samples. The standards were injected at the beginning and end of each run, and one standard was injected, at a minimum, after every five samples. Regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A representative calibration curve for the stability trial is presented in Figure 3. A representative calibration curve for the validation is presented in Figure 10. The concentration of Fenpyroximate in the samples was determined by substituting the peak area responses of the samples into the applicable regression equation. Representative chromatograms of low and high-level calibration standards are presented in Figures 3 and 4, respectively.

Matrix Fortification Samples

For the stability trial, samples of freshwater water were fortified at 10.0 µg a.i./L at hours 0, 3, 6 and 24 using a stock solution of Fenpyroximate TGAI in acetone. The samples were analyzed concurrently with the stability samples. The measured concentrations for the matrix fortification samples ranged from 101 to 150% of fortified concentrations (Table 6). A chromatogram of a representative matrix fortification sample is presented in Figure 6.

Method Verification Samples

Freshwater was fortified at 0.180, 1.00 and 3.00 µg/L using stock solutions containing Fenpyroximate TGAI in acetone. Samples fortified at 0.180, 1.00 and 3.00 µg/L yielded mean recoveries of 119, 107 and 99.7% of nominal concentrations, respectively (Table 8). A representative chromatogram of a freshwater matrix fortification is presented in Figure 13.

Limit of Quantitation

The limit of quantitation (LOQ) for the method verification analyses in freshwater was set at 0.122 µg/L based upon the product of the concentration of the lowest calibration standard (10.0 µg a.i./L) and the dilution factor of the matrix blank samples (0.0120), corrected for purity (98.8%).

Reagent and Matrix Blanks

Two reagent blanks and two matrix blanks were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses (Table 8). Representative chromatograms of a reagent blank and matrix blank are presented in Figures 11 and 12, respectively.

Determination of Stability

The stability of Fenpyroximate in freshwater was determined as follows. Freshwater samples were prepared in glass, stainless steel and Teflon®-lined stainless steel test vessels at a nominal concentration of 10.0 µg/L. The test vessels were placed in a diluter at approximately 20°C for the duration of the study. Freshwater samples fortified with Fenpyroximate were volumetrically collected in duplicate and processed according to Figure 1.

Purity Check

The purity of Fenpyroximate was determined by preparation of triplicate solutions which were analyzed against external standards to ensure test material integrity and verify percent active ingredient (Table 7).

Example Calculations

The analytical result and percent recovery for sample number 397C-101-2 with a nominal concentration of 10.0 µg/L, were calculated using the following equations:

$$\text{Concentration of Fenpyroximate in sample (µg/L)} = \frac{\text{peak area - (Y-intercept)}}{\text{slope}} \text{ X dilution factor}$$

$$\text{Percent of nominal concentration} = \frac{\text{measured concentration in sample (µg/L)}}{\text{nominal concentration in sample (µg/L)}} \text{ X } 100$$

Peak area = 1067.00
Y-intercept = 283.41190
Slope = 14.8041
Dilution Factor = 0.200

$$\text{Concentration of Fenpyroximate in sample (µg/L)} = \frac{1067.00 - 283.41190}{14.8041} \text{ X } 0.200$$

Concentration of Fenpyroximate in sample (µg/L) = 10.6 µg/L

$$\text{Percent of nominal concentration} = \frac{10.6 \text{ µg/L}}{10.0 \text{ µg/L}} \text{ X } 100$$

Percent of nominal concentration = 106%

Table 1

Typical Gas Chromatograph (GC) Operational Parameters

INSTRUMENT:	Agilent Model 6890 Gas Chromatograph (GC)
DETECTOR:	Agilent Model 5973 Mass Selective Detector operated in SIM mode
ANALYTICAL COLUMN:	Agilent 19091S-433 HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness)
INJECTOR TEMPERATURE:	250°C
OVEN:	Initial temperature: 80°C Initial hold time: 1.00 minute Ramp: 15°C/minute Final temperature: 280°C Final hold time: 0.00 minute
DETECTOR TEMPERATURE:	300°C
INJECTION VOLUME:	2.0 µL
CARRIER GAS:	Helium
HEAD PRESSURE:	~9.32 psi
APPROXIMATE FENPYROXIMATE RETENTION TIME:	10 minutes

Table 2

Typical HPLC Operational Parameters

INSTRUMENT:	Agilent Series 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector
ANALYTICAL COLUMN:	YMC-Pack ODS AM (150 mm x 4.6 mm, 3-µm particle size)
STOP TIME:	15 minutes
FLOW RATE:	1.000 mL/min
SOLVENT A:	70% (0.1% H_3PO_4)
SOLVENT B:	30% (CH_3CN)

GRADIENT ELUTION PROFILE:

Time (min)	%A	%B	Flow (mL/min)
0.01	70.0	30.0	1.000
1.00	70.0	30.0	1.000
7.00	2.0	98.0	1.000
10.00	2.0	98.0	1.000
10.10	70.0	30.0	1.000
15.00	70.0	30.0	1.000

OVEN TEMPERATURE:	40°C
INJECTION VOLUME:	100 µL
FENPYROXIMATE RETENTION TIME:	Approximately 9.4 minutes
WAVELENGTH:	220 nm

METHOD OUTLINE FOR THE ANALYSIS OF FENPYROXIMATE IN FRESHWATER FOR THE STABILITY TRIAL

Rinse separatory funnels and roundbottom flasks with dichloromethane.

↓

Transfer the requisite volume of freshwater to the separatory funnels. Fortify recovery samples with the appropriate stock solution(s).

↓

Using a graduated cylinder, add 20 mL of dichloromethane to each sample. Stopper and shake each sample (with venting) for approximately one minute. Allow the organic and aqueous layers to separate. Drain the dichloromethane (lower) layer into a roundbottom flask.

↓

Add 20 mL of dichloromethane to the separatory funnel containing the aqueous fraction. Shake and partition as described above and combine each extract in its respective roundbottom flask.

↓

Rotary evaporate each sample to approximately 0.50 – 1.0 mL using waterbath maintained at ~40°C. Do not rotary evaporate to dryness.

↓

Evaporate the samples to dryness under a gentle stream of nitrogen.

↓

Volumetrically add the requisite volume of acetone to each roundbottom flask and swirl well in order to dissolve all residues.

↓

Transfer an aliquot of each extract to an autosampler vial. Submit samples of analysis.

Figure 1. Analytical method flowchart for the analysis of Fenpyroximate for the stability trial.

METHOD OUTLINE FOR THE ANALYSIS OF FENPYROXIMATE IN FRESHWATER FOR THE VALIDATION

Rinse separatory funnels and roundbottom flasks with dichloromethane.

↓

Transfer the requisite volume of freshwater to the separatory funnels. Fortify recovery samples with the appropriate stock solution(s). Unfortified freshwater will serve as the matrix blank.

↓

Using a graduated cylinder, add 100 mL of dichloromethane to each sample. Stopper and shake each sample (with venting) for approximately one minute. Allow the organic and aqueous layers to separate. Drain the dichloromethane (lower) layer into a roundbottom flask.

↓

Add 100 mL of dichloromethane to the separatory funnel containing the aqueous fraction. Shake and partition as described above and combine each extract in its respective roundbottom flask.

↓

Rotary evaporate each sample to approximately 1-2 mL using waterbath maintained at ~40°C. Do not rotary evaporate to dryness.

↓

Condition silica columns with two column volumes of 50% DCM : 50% hexane. Drain columns to top of silica bed (do not allow to go to dryness). Load samples onto respective column. Rinse roundbottom flasks with 3 X ~ 2 mL of 50% DCM : 50% hexane and add to column.

↓

Drain to dryness under slight vacuum. Apply full vacuum for 30 minutes to dry column.

↓

Elute samples with one column volume of 30% acetone : 70% hexane into 15 mL graduated centrifuge tubes.

↓

Evaporate to dryness under nitrogen.

↓

Using the graduations on the centrifuge tubes, add the requisite volume of 70% acetonitrile : 30% HPLC-grade water and vortex in order to dissolve all residues.

↓

Transfer an aliquot of each extract to an autosampler vial. Submit samples of analysis.

Figure 2. Analytical method flowchart for the analysis of Fenpyroximate for the validation.