

INTRODUCTION

Verification samples were fortified and analyzed to evaluate the performance of a method developed by Wildlife International, Ltd. for the analysis of Tau-Fluvalinate in freshwater and saltwater. This study was conducted by Wildlife International, Ltd. and identified as Project Number 636C-101. The analyses of the samples were performed at Wildlife International, Ltd. using Gas Chromatography with Electron Capture Detection (GC/ECD). Samples for the definitive test were prepared and analyzed between February 1 and February 3, 2007. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 636C-101 in archives located on the Wildlife International, Ltd. site.

PURPOSE

The purpose of this study was to verify the performance of methodology for the analysis of Tau-Fluvalinate in freshwater and saltwater to be used by Wildlife International, Ltd. to perform environmental effects studies.

EXPERIMENTAL DESIGN

Freshwater and saltwater were fortified at four different concentrations and analyzed based on methodology developed by Wildlife International, Ltd. Three reagent blank and three matrix blank samples were analyzed with each fortification set to evaluate potential analytical method interferences. Calibration curves were generated from analyses of standard solutions of Tau-Fluvalinate analyzed with each series of method verification samples.

MATERIALS AND METHODS

This study was conducted according to the protocol "Analytical Method Verification for the Determination of Tau-Fluvalinate in Freshwater and Saltwater" (Appendix 1).

Test Substance

The test substance was received from Wellmark International on August 9, 2006 and was assigned Wildlife International, Ltd. identification number 7681 upon receipt. The test substance, described as a viscous liquid, was identified on the label as: tau-Fluvalinate Technical; Lot #: 060623962; CAS #: 102851-06-9. The test substance had a reported purity of $92.52 \pm 0.30\%$ and an expiration date of July 31, 2007. The test substance was stored refrigerated. A Certificate of Analysis for the test substance is presented in Appendix 2.

Analytical Standard

The analytical standard was received from Wellmark International on August 9, 2006 and was assigned Wildlife International, Ltd. identification number 7682 upon receipt. The analytical standard, described as a viscous liquid, was identified on the label as: tau-Fluvalinate Analytical Standard; Lot #: 209-140-02; ID # ARS06-12-MIP1. The standard had a reported purity of $95.6 \pm 0.8\%$ and an expiration date of February 9, 2008. The analytical standard was stored refrigerated. A Certificate of Analysis for the analytical standard is presented in Appendix 3.

Reagents and Solvents

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

Test Systems**Freshwater**

The freshwater used to prepare the freshwater method verification samples 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 organic and inorganic constituents in well water used by Wildlife International, Ltd. are presented in Appendix 5.

Saltwater

The saltwater used to prepare the saltwater method verification samples 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 6.

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 organic and inorganic constituents in saltwater used by Wildlife International, Ltd. are presented in Appendix 7.

Analytical Method

The methods used for the analyses of Tau-Fluvalinate in freshwater and saltwater were developed by Wildlife International, Ltd. Freshwater and saltwater verification samples were fortified with a stock solution of Tau-Fluvalinate test substance in acetone and extracted with dichloromethane. The extracts were evaporated to dryness and reconstituted with acetonitrile. The samples were analyzed by gas chromatography with electron capture detection (GC/ECD).

Concentrations of Tau-Fluvalinate technical in the samples were determined using an Agilent Model 5890 Gas Chromatograph (GC) equipped with an Electron Capture Detector (ECD). Chromatographic separations were achieved using a ZB-5 column (0.53 mm x 30 m, 1.5 μm film thickness). Instrumental parameters for the analysis of Tau-Fluvalinate are summarized in Table 1 and a method flowchart is provided in Figure 1.

Stocks/Standards Preparation

A stock solution of Tau-Fluvalinate was prepared by weighing 0.01080 grams (weight corrected for purity) 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 (0.100 mg a.i./mL) was diluted in acetone to prepare 0.0100, 0.00100 and 0.000100 mg a.i./mL stock solutions. The 0.00100 mg a.i./mL stock solution was used to prepare the freshwater and saltwater verification samples.

A stock solution of Tau-Fluvalinate analytical standard was prepared by weighing 0.01045 grams (weight corrected for purity) of the standard on an analytical balance. The analytical standard was transferred to a 100-mL class A volumetric flask, and brought to volume using acetonitrile. The primary stock solution (0.100 mg a.i./mL) was diluted in acetonitrile to prepare 0.0100 and 0.00100 mg a.i./mL stock solutions. The 0.00100 mg a.i./mL stock solution was used to prepare the calibration standards in acetonitrile. The following shows the dilution scheme for a set of calibration standards:

Stock Concentration (mg a.i./mL)	Aliquot (μ L)	Final Volume (mL)	Standard Concentration (μ g a.i./L)
0.00100	50.0	10.0	5.00
0.00100	150	10.0	15.0
0.00100	250	10.0	25.0
0.00100	350	10.0	35.0
0.00100	500	10.0	50.0

Calibration Curves

Calibration standards of Tau-Fluvalinate for both freshwater and saltwater, ranging in concentration from 5.00 to 50.0 μ g a.i./L were analyzed with each respective sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The calibration curves are presented in Figures 2 and 8. The concentration of Tau-Fluvalinate in the samples was determined by substituting the peak area responses of the samples into the linear regression equation. Representative chromatograms of low and high-level calibration standards for freshwater and saltwater are presented in Figures 3, 4, 9 and 10, respectively.

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) for the freshwater and saltwater method verifications was calculated to be 0.0667 μ g a.i./L based upon the product of the concentration of the lowest calibration standard (5.00 μ g a.i./L) and the dilution factor of the matrix blank samples (0.0133).

Reagent and Matrix Blank Samples

Concurrent with each series of method verification samples, three reagent blank and three matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses for each matrix (Tables 2 and 3, respectively). Representative chromatograms of reagent blanks in freshwater and saltwater are presented in Figures 5 and 11, respectively. Representative chromatograms of matrix blanks for freshwater and saltwater are presented in Figures 6 and 12, respectively.

Example Calculations

The analytical result and percent recovery for freshwater verification sample number 636C-101-MAS-19, nominal concentration of 1.00 µg a.i./L, were calculated using the following equations:

$$\text{Tau-Fluvalinate } (\mu\text{g a.i./L}) \text{ in sample} = \frac{\text{Peak area} - (\text{Y-intercept})}{\text{Slope}} \times \text{Dilution factor}$$

Peak area = 47660.40000

Y-intercept = -3088.11

Slope = 1863.84

Dilution Factor = 0.0400

$$\text{Concentration of Tau-Fluvalinate } (\mu\text{g a.i./L}) \text{ in sample} = \frac{47660.40000 + 3088.11}{1863.84} \times 0.0400$$

$$\text{Concentration of Tau-Fluvalinate in sample } (\mu\text{g a.i./L}) = 1.09$$

$$\text{Percent of nominal concentration} = \frac{1.09 (\mu\text{g a.i./L})}{1.00 (\mu\text{g a.i./L})} \times 100$$

$$\text{Percent of nominal concentration} = 109\%$$

Table 1

Typical Gas Chromatographic (GC) Operational Parameters

INSTRUMENT:	Agilent Model 5890 Gas Chromatograph (GC)
DETECTOR:	Agilent Electron Capture Detector (ECD)
ANALYTICAL COLUMN:	ZB-5 Column (0.53 mm x 30 m, 1.5 µm film thickness)
INJECTOR TEMPERATURE:	250°C
OVEN:	Initial temperature: 180°C Initial hold time: 1.00 minute Ramp: 20.0°C/minute Final temperature: 320°C Final hold time: 6.00 minutes
DETECTOR TEMPERATURE:	300°C
INJECTION VOLUME:	3.0 µL, splitless
CARRIER GAS:	Helium
HEAD PRESSURE:	Approximately 4 psi
MAKE-UP GAS:	Argon / Methane 5%, @ 60 psi
APPROXIMATE TAU- FLUVALINATE PEAK RETENTION TIME:	13.5 minutes

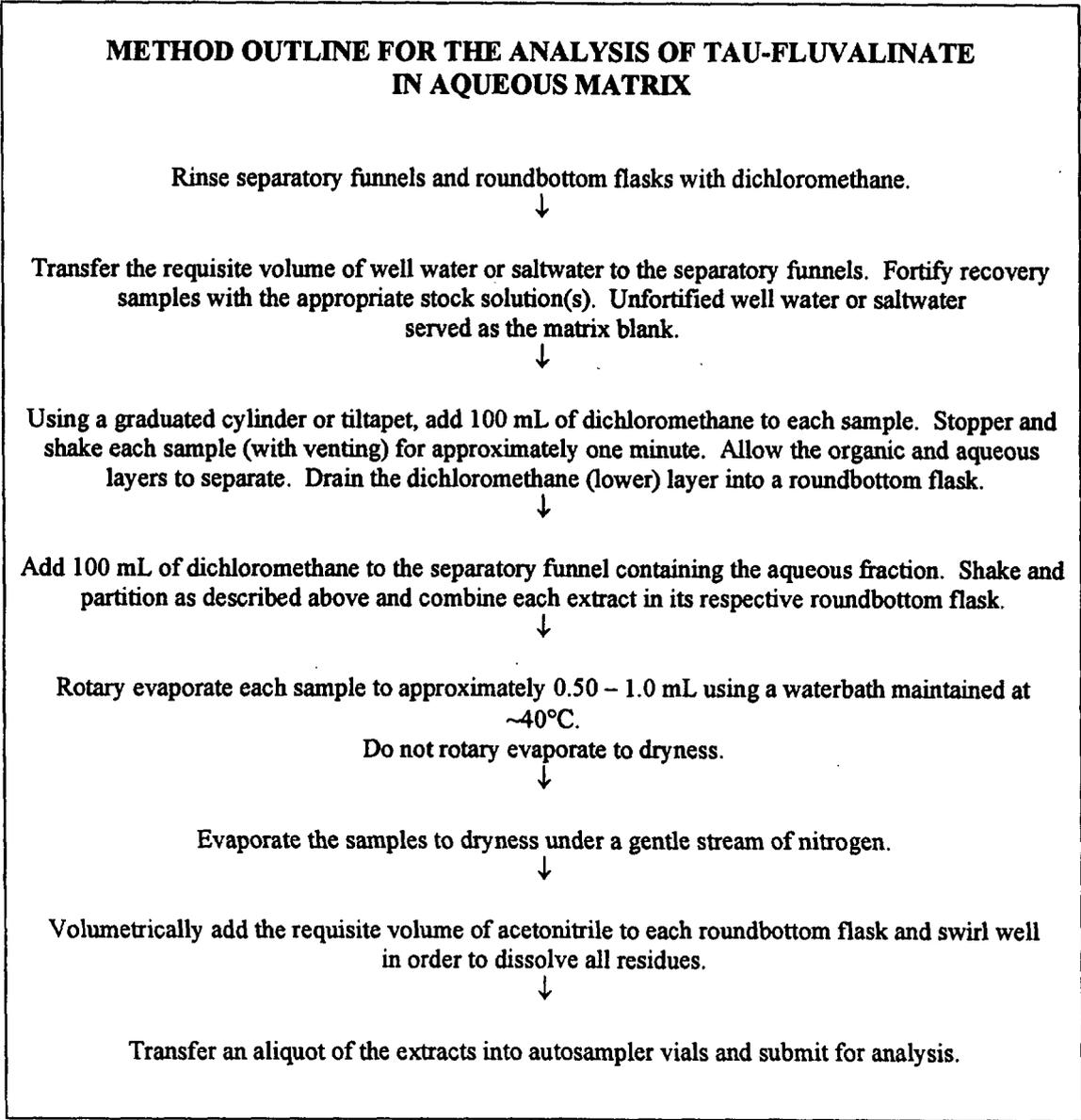


Figure 1. Analytical method flowchart for the analysis of Tau-Fluvalinate in freshwater and saltwater.