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INTRODUCTION

Verification samples were fortified and analyzed to evaluate the performance of a method for the analysis of CAPTAN Technical in saltwater. The study was conducted by Wildlife International Ltd. and identified as Project Number 493C-101. The analyses of the samples were performed at Wildlife International Ltd. using gas chromatography (GC) with electron capture detection (ECD). Verification samples were prepared and analyzed between November 4, 1998 and November 5, 1998. All raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 493C-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 CAPTAN Technical in saltwater used at Wildlife International Ltd. for proposed environmental effects studies.

EXPERIMENTAL DESIGN

Saltwater was fortified at four different concentrations and analyzed, based on a method developed at Wildlife International, Ltd. Reagent and matrix blanks were analyzed concurrently to evaluate potential analytical interferences. Calibration curves were prepared from external standards of CAPTAN Technical to determine the test substance sample concentrations.

MATERIALS AND METHODS

Test Substance

The test substance was received from CAPTAN Task Force (Midwest Research Institute) on July 1, 1998 and was assigned Wildlife International Ltd. identification number 4525 upon receipt. The test substance was described as a white powder and was identified as: CAPTAN Technical; Lot Number: 55; Batch Number: 01; Purity: 99.8%; Expiration Date: June 3, 1999. The test substance was stored refrigerated. CAPTAN Technical was used to prepare calibration standards and matrix fortification samples.

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Reagents and Solvents

All solvents used in the method were of HPLC grade or equivalent. All reagents were of ACS reagent grade or higher quality.

Saltwater

The water used for testing was prepared at Wildlife International Ltd. from natural seawater collected at Indian River Inlet, Delaware. The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 μm , diluted to a salinity of approximately 20‰ with filtered well water, and pumped into a 37,800-L storage tank where it was aerated with spray nozzles. Prior to delivery to the test system, the 20‰ saltwater again was filtered (0.2 μm) to remove microorganisms and particles. Salinity and pH measurements taken during the four-week period immediately preceding the test are presented in Appendix I. The results of periodic analyses performed to measure the concentrations of selected contaminants in saltwater used by Wildlife International Ltd. are presented in Appendix II.

Analytical Method

The analytical method entailed an ethyl acetate extraction of the aqueous sample. Samples were prepared using 100 mL of acidified filtered saltwater (pH 5, acidified using phosphoric acid, reagent grade, 86.5%). The samples were fortified, as appropriate, with CAPTAN Technical stock solutions prepared in acetone (HPLC grade, 99.9+%). Five milliliters of ethyl acetate (HPLC grade, 99.9+%) was added to each sample. The samples were vortexed for approximately one minute. The ethyl acetate layer (upper) was decanted, diluted as necessary with ethyl acetate and placed in an autosampler vial for analysis. A method flow chart is provided in Figure 1.

Concentrations of CAPTAN Technical were determined using a Hewlett-Packard Model 5890 Gas Chromatograph equipped with an Electron Capture Detector (ECD). Chromatographic separations were achieved using a J & W DB-5 column (30 m x 0.53 mm, 1.50 μm film thickness). The instrument parameters are summarized in Table 1.

Calibration Curve and Limit of Quantitation (LOQ)

Calibration standards of CAPTAN Technical, ranging in concentration from 10.0 to 100 $\mu\text{g a.i./L}$, were analyzed with the verification sample set. Linear regression equations were generated using the peak area

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responses versus the respective concentrations of the calibration standards. The calibration curve is presented in Figure 2. The concentration of CAPTAN Technical in the samples was determined by substituting the peak area responses into the linear regression equation. Representative chromatograms of low and high calibration standards are presented in Figures 3 and 4, respectively.

The method limit of quantitation (LOQ) for the verification analyses was set at 0.00500 mg a.i./L, calculated as the product of the lowest calibration standard (10.0 μ g a.i./L) and the dilution factor of the matrix blank samples (0.500).

Reagent and Matrix Blank Samples

Three reagent blanks (ethyl acetate) and three matrix blanks (acidified filtered saltwater) were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during sample analysis (Table 2). Representative chromatograms of reagent and matrix blank samples are presented in Figures 5 and 6, respectively.

Method Verification Samples

During the method verification, acidified filtered saltwater was fortified in triplicate at 0.0100, 0.100, 1.00 and 10.0 mg a.i./L.

Example Calculations

The analytical result and percent recovery for sample number 493C-101-VMAS-36, nominal concentration of 10.0 mg a.i./L, was calculated as follows.

Initial Volume: 10 mL

Final Volume: 5.00 mL

Dilution Factor: 125

Peak Area: 66127

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$$\text{CAPTAN Technical } (\mu\text{g a.i./L}) \text{ at instrument} = \frac{\text{Peak area} - (\text{Y-intercept})}{\text{Slope}}$$

Slope: 705.370

Intercept: -1003.6

$$= (66127 + 1003.6) / 705.370 = 95.17 \mu\text{g a.i./L}$$

$$\text{CAPTAN Tech. (mg a.i./L) in final extract} = \frac{\text{CAPTAN Tech. } (\mu\text{g a.i./L}) \text{ at instrument} \times \text{Dilution factor}}{1000}$$

$$= (95.17 \mu\text{g a.i./L} \times 125) / 1000 = 11.9 \text{ mg a.i./L}$$

$$\text{Percent of Nominal Concentration} = \frac{\text{CAPTAN Tech. (mg a.i./L) in final extract}}{\text{CAPTAN Tech. (mg a.i./L) in initial sample}} \times 100$$

$$= (11.9 \text{ mg a.i./L} / 10.0 \text{ mg a.i./L}) \times 100$$

$$= 119\%$$

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Table 1

Typical GC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 5890 Gas Chromatograph (GC) Equipped with a Model G1030A Chemstation
DETECTOR:	Hewlett-Packard Electron Capture Detector (ECD)
ANALYTICAL COLUMN:	J&W DB-5 Column (30 m x 0.53 mm, 1.50 μ m film thickness)
INJECTOR TEMPERATURE:	250 °C
OVEN TEMPERATURE:	Initial temperature: 150 °C Initial hold time: 1.00 minute Ramp: 20 °C/minute Final temperature: 300 °C Final hold time: 2.00 minutes
DETECTOR TEMPERATURE:	300 °C
CARRIER GAS:	Helium, 4.0 mL/minute
TOTAL FLOW:	40 mL/min (at split vent)
MAKE-UP GAS:	Argon/Methane, to 42 mL/minute
INJECTION VOLUME:	1 μ L
CAPTAN TECHNICAL PEAK RETENTION TIME:	~ 9.2 minutes

**METHOD OUTLINE FOR THE ANALYSIS OF
CAPTAN TECHNICAL IN SALTWATER**

Prepare calibration standards in ethyl acetate using
volumetric flasks and gas-tight syringes.

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Prepare 10 mL samples using acidified (pH5) filtered saltwater.
Fortify recovery samples with the appropriate CAPTAN Technical stock solution(s).
Unfortified acidified (pH5) filtered saltwater will serve as the matrix blank.

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Add 5 mL of ethyl acetate to each 10 mL sample.
Vortex each sample for approximately one minute.

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Decant the ethyl acetate layer (upper).
Dilute the samples, as necessary, with ethyl acetate.

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Transfer samples and standards to autosampler vials for analysis by GC/ECD.

Figure 1. Analytical method flow chart for the analysis of CAPTAN Technical in acidified (pH 5) filtered saltwater.