INTRODUCTION

Verification samples were fortified and analyzed to evaluate the performance of a method for the analysis of aqueous hydrogen cyanamide (50% w/w) in filtered saltwater solutions. The study was conducted by Wildlife International Ltd. and identified as Project Number 248C-101. The analyses of the filtered saltwater samples were performed at Wildlife International Ltd. using high performance liquid chromatography (HPLC) with fluorescence detection. Fortification samples were prepared and analyzed between May 4, 1998 and May 7, 1998. All original raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 248C-101 in archives located on the Wildlife International Ltd. site.

PURPOSE

The purpose of this study was to verify a method for measuring the test substance residues in saltwater used by Wildlife International Ltd. in support of environmental fate and/or effects studies.

EXPERIMENTAL DESIGN

Filtered saltwater was fortified at four different concentrations and analyzed based on a method supplied by the Sponsor. Reagent and matrix blanks were analyzed to evaluate possible analytical interferences. A calibration curve was prepared from standard solutions to determine the test substance sample concentrations.

MATERIALS AND METHODS

Test Substance

The test substance was received from SKW Trostberg AG on March 27, 1998 and was assigned Wildlife International Ltd. identification number 4416 upon receipt. The test substance was described as a liquid and was identified as: Aqueous Hydrogen Cyanamide (50% w/w); CYANAMID L 500; CH. NR.: 807601 with an expiration date of March, 1999. The test substance had a reported purity of 50.8%. The test substance was stored under refrigerated conditions.

Analytical Standard

The analytical standard was received from SKW Trostberg AG on March 27, 1998 and was assigned Wildlife International Ltd. identification number 4417 upon receipt. The analytical standard was described as a clear semisolid and was identified as: Cyanamid F1000: Lot No.: 031002. The analytical standard had a reported purity of 100.3% and was stored under refrigerated conditions.

Reagents and Solvents

Solvents/Reagents	Grade	Purity	Supplier
Potassium tetraborate tetrahydrate	ACS	99%	Aldrich Chem. Co.
4-chloro-7-nitrobenzofuran (NBD-Cl)	ACS	98%	Aldrich Chem. Co.
Hydrochloric acid	ACS	5.0 N	Lab Chem
Potassium dihydrogenphosphate	ACS	99.99%	Aldrich Chem. Co.
o-Phosphoric acid	ACS	85%	Mallinckrodt
Methanol	HPLC	99.9+%	Burdick & Jackson
NanoPure® water	eq. to ASTM Type II		Barnstead

Dilution Water

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 ppt water 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 used for the processing and analysis of aqueous hydrogen cyanamide (50% w/w) in filtered saltwater was developed at Wildlife International, Ltd. Samples, diluted as necessary,

and calibration standards were derivatized in the following manner. Two milliliters of each standard and test solution were transferred to 15-mL graduated centrifuge tubes to which 1.00 mL of (0.37M) potassium tetraborate solution was added. The solution was mixed by vortex, then 2.00 mL of methanolic (0.025M) NBD-CL solution was added and the contents of the tube were vortexed again. Solutions were then heated in a heating block at 80°C for 30 minutes. One milliliter of 1.2M HCl was added to each solution and mixed by vortex. Solutions were then filtered through a PTFE Acrodisc 0.45 μm filter into vials for HPLC analysis.

Concentrations of aqueous hydrogen cyanamide (50% w/w) in the calibration standards and aqueous samples were determined by high performance liquid chromatography using a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with a fluorescence detector operated at an excitation wavelength of 470 nm and an emission wavelength of 530 nm. Chromatographic separations were achieved using a Zorbax Phenyl analytical column (250 mm x 4.6 mm I.D., 5 μm particle size). The instrument parameters are summarized in Table 1. A method flow chart is provided in Figure 1.

Calibration Curve, Limit of Detection and Limit of Quantitation

Calibration standards, ranging in concentration from 0.100 to 1.00 mg a.i./L, were analyzed with the verification sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A representative calibration curve is presented in Figure 2. The concentration of aqueous hydrogen cyanamide (50% w/w) 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 shown in Figures 3 and 4, respectively.

The method limit of detection (LOD) for the method verification analyses was defined as a peak reflecting three times the signal-to-background ratio. The background peak height of a matrix blank was 3.95536. The peak height of a low (0.100 mg a.i./L) calibration standard was 106.18207 (signal). The signal-to-background ratio was 26.84. Calculated as three times the standard concentration divided by the signal-to-background ratio, the LOD was 11.2 μg a.i./L.

The method limit of quantitation (LOQ) for the method verification analyses was set at 0.100 mg a.i./L as determined by the product of the lowest standard (0.100 mg a.i./L) and the dilution factor of the matrix blanks (1.00). This LOQ was equivalent to 0.197 mg/L as product.

Reagent and Matrix Blanks

Along with the series of fortification samples analyzed for the method verification, three reagent (prepared in NanoPure® water) and three matrix (prepared in filtered saltwater) blanks were analyzed to determine possible interferences. No interferences were observed at or above the LOQ (0.197 mg/L) during the sample analyses (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, filtered saltwater matrix was fortified in triplicate at 0.402, 4.02, 40.2 and 402 mg/L using stock solutions containing aqueous hydrogen cyanamide (50% w/w) in NanoPure® water. Samples fortified at 0.402, 4.02, 40.2 and 402 mg/L yielded mean recoveries of 104, 103, 102 and 105%, respectively (Table 2). Representative chromatograms of low and high-level matrix fortifications are presented in Figures 7 and 8, respectively.

Example Calculations

Sample number 248C-101-VMAS-12, nominal concentration of 402 mg/L in filtered saltwater.

Initial Volume: 0.200 mL
Final Volume: 50.0 mL
Dilution Factor: 250
Peak Area: 11089.5
Purity: 50.8%

Calibration curve equation.
Slope: 12994.30
Intercept: -104.89114

$$\text{Aq. HC}^1 \text{ (mg a.i./L) at instrument} = \frac{\text{Peak area - (Y-intercept)}}{\text{Slope}}$$

$$= (11089.5 + 104.89114) / 12994.30 = 0.8615 \text{ mg a.i./L}$$

$$\text{Aq. HC}^1 \text{ (mg/L) in sample} = \frac{\text{Aq. HC}^1 \text{ (mg a.i./L) at instrument x Final volume}}{\text{(Initial volume x Purity)}}$$

$$= (0.8615 \text{ mg a.i./L} \times 50.0 \text{ mL}) / (0.200 \text{ mL} \times 0.508) = 424 \text{ mg/L}$$

$$\text{Percent of Nominal Concentration} = \frac{\text{Aq. HC}^1 \text{ (mg/L) in sample}}{\text{Aq. HC}^1 \text{ (mg/L) fortified}} \times 100$$

$$= (424 \text{ mg/L} / 402 \text{ mg/L}) \times 100 = 105\%$$

[1] Aq. HC = Aqueous hydrogen cyanamide (50% w/w)

Table 1

Typical HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1090 High Performance Liquid Chromatograph with a Jasco FP-920 Fluorescence Detector.
ANALYTICAL COLUMN:	Zorbax Phenyl Column (250 x 4.6 mm, 5 μm particle size)
STOP TIME:	20 minutes
FLOW RATE:	1.00 mL/min
OVEN TEMPERATURE:	45°C
MOBILE PHASE:	Solvent A: 0.01M Potassium dihydrogenphosphate at pH 3.6 Solvent B: Methanol

Time	Solvent A	Solvent B
0.00	70%	30%
2.00	70%	30%
13.00	50%	50%
13.01	40%	60%
16.00	40%	60%
16.01	70%	30%
20.00	70%	30%

INJECTION VOLUME:	50 μL
AQUEOUS HYDROGEN CYANAMIDE(50% w/w) RETENTION TIME:	Approximately 12.0 to 12.4 minutes
EXCITATION WAVELENGTH:	470 nm
EMISSION WAVELENGTH:	530 nm
GAIN:	x 100

METHOD OUTLINE FOR THE PROCESSING OF AQUEOUS HYDROGEN CYANAMIDE (50% w/w) IN FILTERED SALTWATER

Prepare calibration standards in filtered saltwater using volumetric flasks and gas-tight syringes.

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Prepare recovery samples in filtered saltwater using volumetric flasks and gas-tight syringes. The reagent blank will be unfortified NanoPure® water; the matrix blank will be unfortified filtered saltwater. Dilute samples as necessary with filtered saltwater.

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DERIVATIZATION STEPS (Samples and Standards)

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Add 1.0 mL of 0.37M potassium tetraborate solution to a 15-mL centrifuge tube.

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Add 2.0 mL of the standard solution or test solution to the 15-mL centrifuge tube. Vortex/mix.

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Add 2.0 mL of methanolic 0.025M NBD-CL solution to each 15-mL centrifuge tube and vortex/mix.

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Heat the centrifuge tubes using a heating block at 80°C for 30 minutes.

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Add 1.0 mL of 1.2 M HCl solution. Vortex/mix.

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Filter the solutions through an Acrodisc PTFE 0.45 μm filter into HPLC vials.

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Analyze using HPLC equipped with fluorescence detection.

Figure 1. Analytical method flow chart for the analysis of aqueous hydrogen cyanamide (50% w/w) in filtered saltwater.