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October 17, 2001

MEMORANDUM

SUBJECT: Hydrogen Cyanamide Method Evaluation - Report No. ECM 0182W1
DP Barcode: D 262650

FROM: Aubry E. Dupuy, Jr., Branch Chief *Aubry E Dupuy, Jr.*
BEAD/Environmental Chemistry Laboratory

TO: Stephanie Sellers
Environmental Fate and Effects Division
Environmental Risk Branch II

THRU: Hardip Singh, Senior
Gatekeeper Team/IO
Environmental Fate and Effects Division

The EFED/Environmental Fate and Effects Division has requested an Environmental Chemistry Method Evaluation (ECME) on the determination of Hydrogen Cyanamide in seawater using the Wildlife International method, "Analytical Method Verification for the Determination of Hydrogen Cyanamide Residues in Saltwater".

The attached method evaluation report includes three parts:

Part I: Summary and Conclusions

In this section any problems encountered with the method and how they were handled are discussed. ECL's opinion of how well the method performed is also performed.

Part II: Analytical Results

In this section the individual results of each sample at each spiking level for each matrix are listed. The relative standard deviation (RSD) for each spiking level is also presented here.

Part II: Experimental Details

In this section any modification(s) that were made to this method, instrumental parameters, spiking levels, explanation of instrument



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calibration, representative sample and standard chromatograms and standard curves are listed and/or discussed.

If there are any questions regarding this report, please contact Christian Byrne at (228)-688-3213 or me at (228)-688-3212.

Attachments

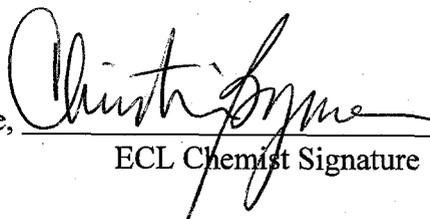
cc: Dr. Christian Byrne, QA Officer
BEAD/Environmental Chemistry Laboratory

Environmental Chemistry Method Validation Report Number ECM 0182W1

Dormex (50% Hydrogen Cyanamide) in Seawater

Environmental Chemistry Laboratory
Biological and Economic Analysis Division

October 11, 2001

Prepared by: Christian Byrne,  Date: 10/12/01
ECL Chemist Signature

Reviewed by: Elizabeth Flynt,  Date: 10/17/01
ECL QAC Signature

TABLE of CONTENTS

		Page
PART I	Summary and Conclusions	3
PART II	Analytical Results	4
PART III	Experimental Details	6
Appendix 1	Structure of Hydrogen Cyanamide	17
Appendix 2	Water Quality Characteristics for Seawater, Mississippi Sound	18

PART I Summary and Conclusions

We have completed the Environmental Chemistry Method Evaluation (ECME) for Hydrogen Cyanamide as Dormex (50% Hydrogen Cyanamide) in seawater. The method appears to be suitable for the detection of Hydrogen Cyanamide at levels at or greater than 0.010 mg a.i. (active ingredient)/L [0.010 ppm (parts-per-million); 10 ppb (parts-per billion)]. The method used for the ECME is entitled - **Analytical Method Verification for the Determination of Hydrogen Cyanamide Residues in Seawater** - MRID #448047-01. The performing laboratory was Wildlife International, Ltd., Easton, Maryland. There was no independent laboratory validation (ILV) received.

The analytical method involves the derivatization of the residues of Hydrogen Cyanamide at 80°C with a methanolic NBD-chloride solution followed by filtrations and analysis. The residues of Hydrogen Cyanamide are determined by high performance liquid chromatography (HPLC) using fluorescence spectrometric detection.

Wildlife International Ltd. submitted recovery data expressed as the concentration of 50% Hydrogen Cyanamide (Dormex); however, it then expressed the LOQ and LOD as concentrations of active ingredient, of which Dormex contained only 50.8%. For comparison purposes, all concentrations in this report were converted into active ingredient concentrations (mg a.i./L).

The limit of detection (LOD) for Hydrogen Cyanamide in surface water was 0.0112 mg a.i./L as calculated from the lowest standard concentration (0.100 mg a.i./L) and the signal-to-noise ratio by the registrant. ECL analyzed the LOD for Hydrogen Cyanamide in seawater at 0.010 mg a.i./L (calculated). The limit of quantitation (LOQ) was set at 0.100 mg a.i./L. The accuracy and precision results between ECL and the registrant at the various spiking concentrations used were comparable. The Wildlife International, Ltd. laboratory demonstrated average percent recoveries @ 2.04 mg a.i./L (20 x LOQ) and @ 0.204 mg a.i./L (2 x LOQ) of 103% and 104%, respectively. ECL analyses demonstrated average percent recoveries @ 1.01 mg a.i./L (10 x LOQ) and @ 0.101 mg a.i./L (LOQ) of 105 and 105, respectively. The Wildlife International, Ltd. laboratory demonstrated relative standard deviations (RSDs) @ 2.04 mg a.i./L (20 x LOQ) and @ 0.204 mg a.i./L (2 x LOQ) of 0.555% and 2.02%, respectively. The relative standard deviations (RSDs) for the ECL analyses @ 1.01 mg a.i./L (10 x LOQ) and @ 0.101 mg a.i./L (LOQ) were 0.935% and 0.717%, respectively. The LOD was detected @ 0.0101 mg a.i./L with a signal-to-noise ratio that exceeded 3:1 (sample:matrix blank).

ECL estimates that it takes approximately ten (10) working hours to extract and analyze one set of four (4) samples with appropriate blanks and standards.

PART II

Analytical Results

Method: SKW Trostberg AG, Project Number 248C-101,
"Analytical Method Verification for the Determination of Hydrogen Cyanamide in
Seawater."

TABLE 1. Recovery of Hydrogen Cyanamide from Seawater
Hydrogen Cyanamide - **LOD (0.010 mg a.i./L)** ⁽¹⁾

<u>Sample #</u>	<u>Added, mg/L (mg a.i./L)</u>	<u>Detected (mg a.i./L)</u>
LOQ-1	0.000	0.000
LOQ-2	0.000	0.000
LOD-3	0.010	Detected
LOD-4	0.010	Detected
LOD-5	0.010	Detected
LOD-6	0.010	Detected

Recovery of Hydrogen Cyanamide from Seawater
Hydrogen Cyanamide - **LOQ (0.100 mg a.i./L)**

<u>Sample #</u>	<u>Added, mg/L (mg a.i./L)</u>	<u>Detected (mg a.i./L)</u>	<u>Recovery (%)</u>
LOQ-1	0.000	0.000	
LOQ-2	0.000	0.000	
LOQ-3	0.101	0.107	106
LOQ-4	0.101	0.106	105
LOQ-5	0.101	0.105	104
LOQ-6	0.101	0.107	106
		Mean	105
		⁽²⁾ SD	0.754
		⁽²⁾ RSD	0.717%

TABLE 1. (continued)

Recovery of Hydrogen Cyanamide from Seawater
Hydrogen Cyanamide - 10 x LOQ (1.00 mg a.i./L)

<u>Sample #</u>	<u>Added, mg/L (mg a.i./L)</u>	<u>Detected (mg a.i./L)</u>	<u>Recovery (%)</u>
10 x LOQ-1	0.00	0.00	
10 x-LOQ-2	0.00	0.00	
10 x-LOQ-3	1.01	1.05	104
10 x-LOQ-4	1.01	1.07	106
10 x LOQ-5	1.01	1.07	106
10 x LOQ-6	1.01	1.07	106
		Mean	105
		⁽²⁾ SD	0.985
		⁽²⁾ RSD	0.935%

NOTES:

⁽¹⁾ The detection of the samples spiked at this levels demonstrated peak heights that produced signal-to-noise ratios when compared to the peak heights of the matrix seawater blank of greater than 3:1.

⁽²⁾ SD - Standard Deviation; RSD - Relative Standard Deviation

PART III
Experimental Details

General Description of Method:

Extraction and Filtration

A 2 ml volume of seawater or calibration standard was measured into a 15 ml graduated centrifuge tube. The seawater had been fortified with the appropriate quantity of spiking solution of Dormex (50 %Hydrogen Cyanamide). One milliliter of 0.37 M potassium tetraborate solution was added and vortexed. Two milliliters of methanolic 0.025 M NBD-Cl solution was added and vortexed. This mixture was heated to 80°C for 30 minutes. The solution was allowed to cool to room temperature, when one milliliter of 1.2 M hydrochloric acid solution was added and vortexed. The final solution was filtered through a PFTE Acrodisc 0.45 µm filter into HPLC vials for analysis. The residues of Hydrogen Cyanamide were determined by high performance liquid chromatography (HPLC) with a fluorescence spectrometric detector.

Minor Change:

The calibration standards were prepared in HPLC water instead of the estuarine seawater.

Special Precautions to be Taken:

None

Source of Analytical Reference Standards:

The standards were received from two sources:

EPA National Pesticide Standard Repository, Fort Meade, Maryland
Analytical Standard
Hydrogen Cyanamide, 1059-46, Lot 802 102
100.7% Purity, Expiration Date -11/28/02,
100 mg, Chemical Structure (Appendix 1).

SKW Trostberg Chemicals, Inc., Marietta, Georgia
Pesticide: Dormex (50% Hydrogen Cyanamide in water), Lot 031101
50.2% Purity, Expiration Date 11/04/01
25 ml

Sample Matrix:

The seawater was collected by the staff of the USEPA Environmental Chemistry Laboratory at a shoreline along the Mississippi Sound in Mississippi.. Water quality characteristics are listed in Appendix 2.

Instrumentation for Quantitation:

HPLC Chromatograph: Waters Model 2690
Model 747 Fluorescence Detector
Column: Zorbax Phenyl, 5 µm, 4.6 mm x 250 mm, Lot #

Instrument for Confirmation: None applicable

Instrument Parameters:

Mobile Phase: Programmed
Solvent A: 0.01 M Potassium phosphate, monobasic @ pH 3.6
Solvent B, Methanol

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

Column Temperature: 45°C
Flow Rate: 1 ml/min
Excitation Wavelength: 470 nm
Excitation Wavelength: 530 nm
Injector Volume: 50 µl

Sample Calculation:

Calibration standards were prepared at 0.01, 0.05, 0.10, 0.25, 0.50, 0.75 and 1.0 µg a.i./ml:

A standard curve was prepared by plotting the concentration of Hydrogen Cyanamide (µg a.i./ml) (x-axis) versus peak area of the calibration standards (fluorescence area (fa))/ µg a.i./ml (y-axis). A linear regression equation was determined:

$$y \text{ (area of the sample)} = m \text{ [slope]} * x \text{ (concentration of the sample)} + b \text{ [x-intercept]}$$

$$\text{Concentration of the sample (µg a.i./ml)} = (\text{Area of the sample} - b) / (m)$$

$$\text{Hydrogen Cyanamide Concentration (µg a.i./ml)} = \text{mg a.i./L (ppm a.i.)}$$

$$\text{Recovery} = \frac{\text{Concentration Detected}}{\text{Concentration Added}} \times 100\%$$

Sample Calculation for Q-3:

$$\begin{aligned} \text{Extract Concentration } (\mu\text{g a.i./ml}) &= (1250440 \text{ fau-140582fa}) / (10360291 \text{ fa}/\mu\text{g a.i./ml}) \\ &= 0.107 \mu\text{g a.i./ml} \end{aligned}$$

$$\begin{aligned} \text{Concentration (Hydrogen Cyanamide) as} \\ \text{active ingredient } (\mu\text{g a.i./ml}) &= 0.107 \mu\text{g a.i./ml} \times (\text{mg}/1000 \mu\text{g}) \times (1000 \text{ ml/L}) \\ &= 0.107 \text{ mg a.i./L} \end{aligned}$$

$$\text{Recovery} = \frac{0.107 \text{ mg a.i./L}}{0.101 \text{ mg a.i./L}} \times 100\% = 106\%$$

Chromatograms and Calibration Curves:

A. Calibration Standards Hydrogen Cyanamide for Determination of the Recovery of Hydrogen Cyanamide from Seawater @ 0.033 ppm a.i. & 0.100 ppm a.i.

Calibration Standard-1 (LOQ)	0.01 mg a.i./L
Calibration Standard-2 (LOQ)	0.05 mg a.i./L
Calibration Standard-3 (LOQ)	0.10 mg a.i./L
Calibration Standard-4 (LOQ)	0.25 mg a.i./L
Calibration Standard-5 (LOQ)	0.50 mg a.i./L

Linear Regression Graph - Hydrogen Cyanamide (LOQ)

B. Hydrogen Cyanamide Fortification, Seawater @ 0.033 mg a.i./L , 0.100 mg a.i./L, & 1.00 ppm mg a.i./L

LOQ-1	Method Blank (LOQ & LOD)
LOQ-2	Seawater Matrix Blank (LOQ & LOD)
LOD-3	Seawater Fortified @ 0.010 mg a.i./L (Replicate #1)
LOQ-3	Seawater Fortified @ 0.101 mg a.i./L (Replicate #1)
10 x LOQ-3	Seawater Fortified @ 1.01 mg a.i./L (Replicate #1)

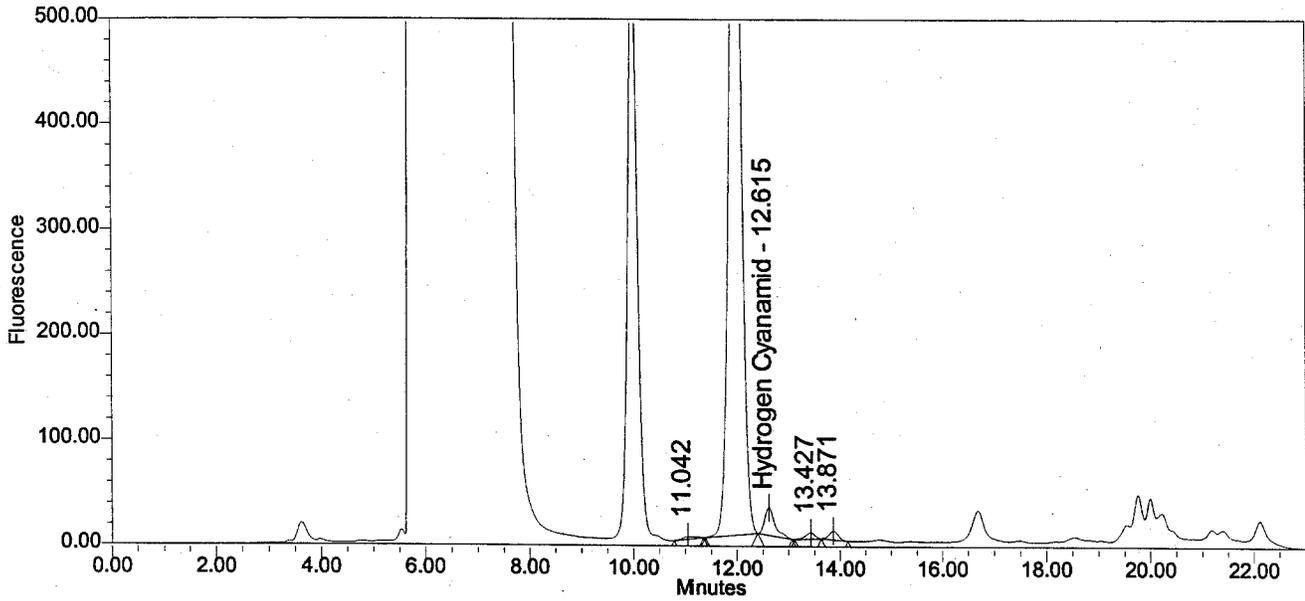
Notes:

(1) Abbreviations -

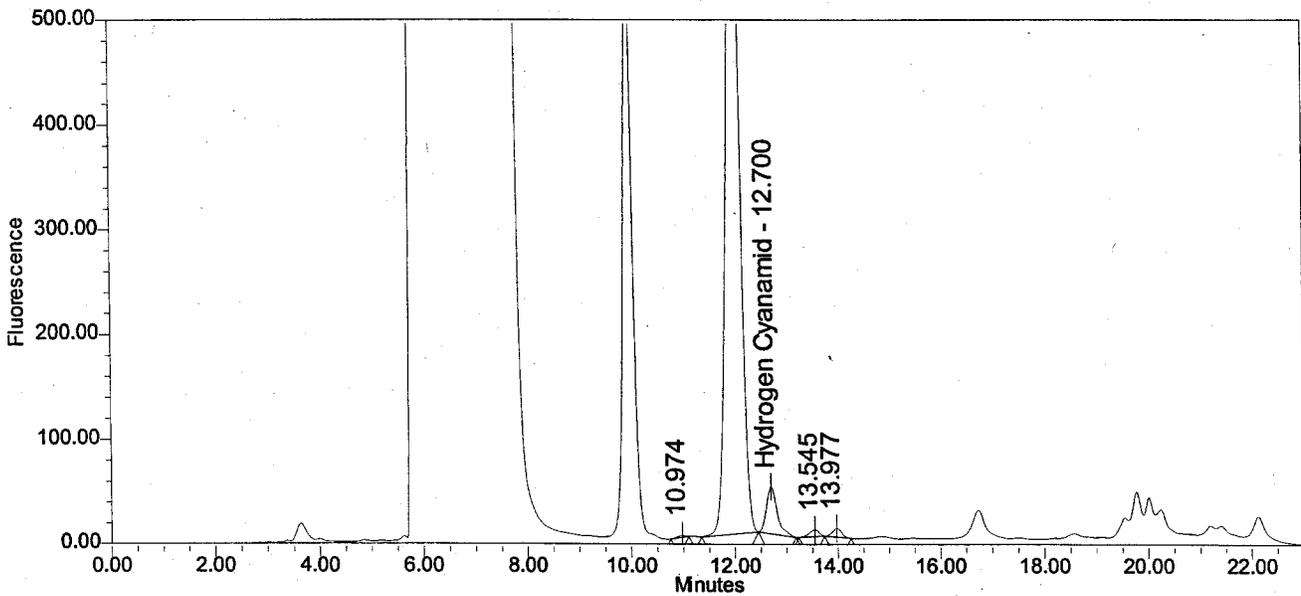
LOQ-1: Limit of Quantitation & Detection Extraction Series	-	Solvent Blank
LOQ-2: Limit of Quantitation & Detection Extraction Series	-	Matrix Blank

LOD-3: Limit of Quantitation & Detection Extraction Series -	Replicate #3 - @ 0.010 mg a.i./L (Replicate #1)
LOQ-3: Limit of Quantitation & Detection Extraction Series -	Replicate #3 - @ 0.101 mg a.i./L (Replicate #1)
10 x LOQ-3: Limit of 10 x Quantitation Extraction Series -	Replicate #3 - @ 1.01 mg a.i./L (Replicate #1)

Chromatograms:

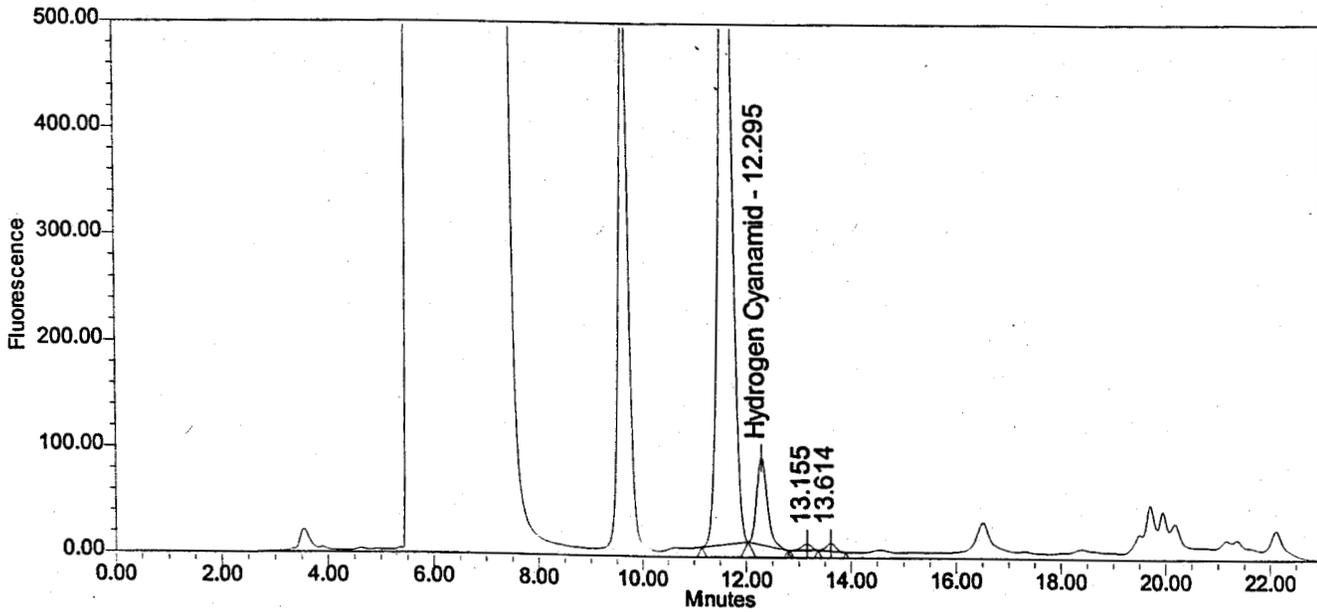


Calibration Standard-1 @ 0.01 mg a.i./L (Replicate #1) for LOQ, LOD Peak Area =360480

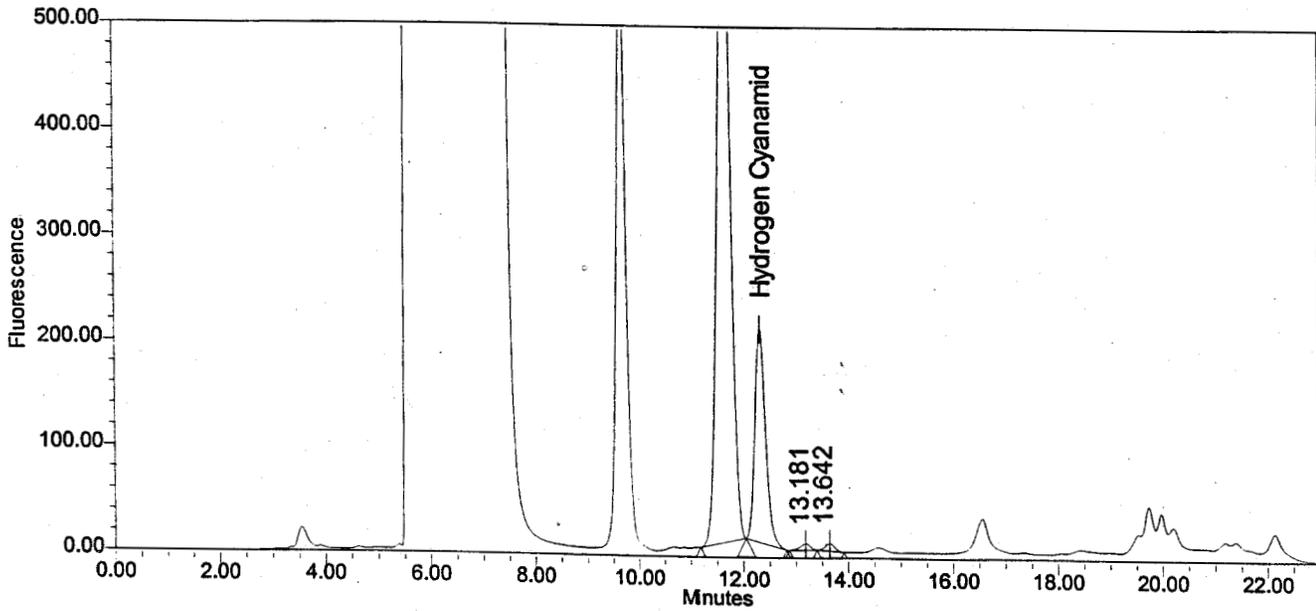


Calibration Standard-2 @ 0.05 mg a.i./L (Replicate #01) for LOQ, LOD Peak Area =645430

Chromatograms:

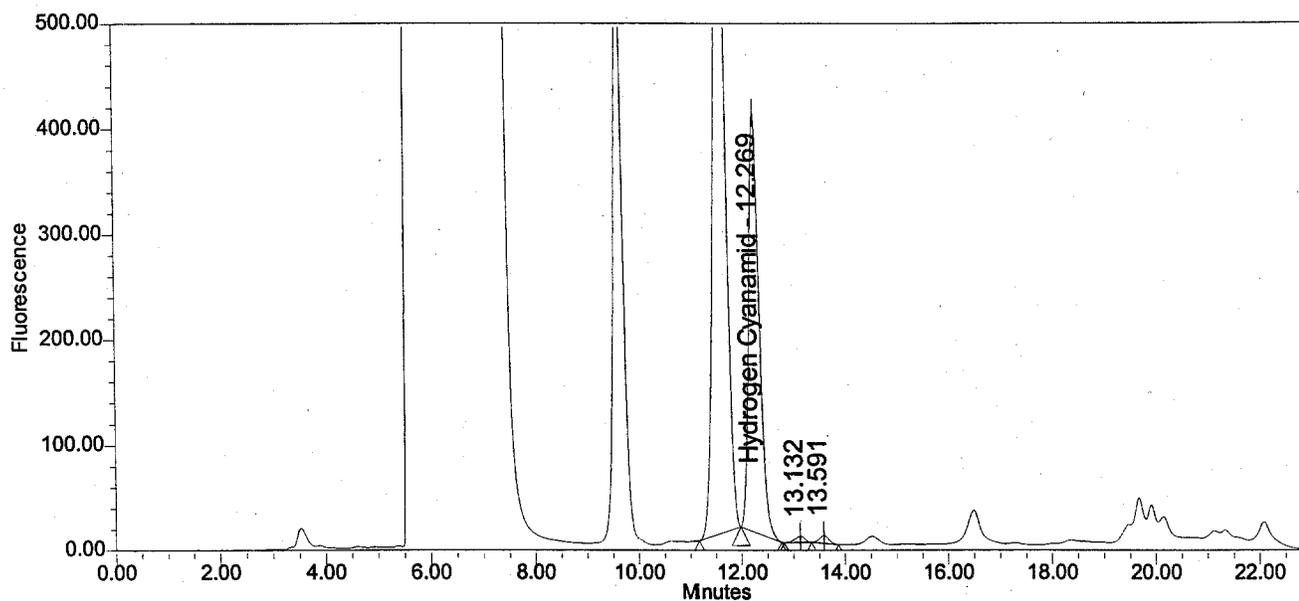


Calibration Standard-3 @ 0.10 mg a.i./L (Replicate #01) for LOQ, LOD Peak Area =1147410

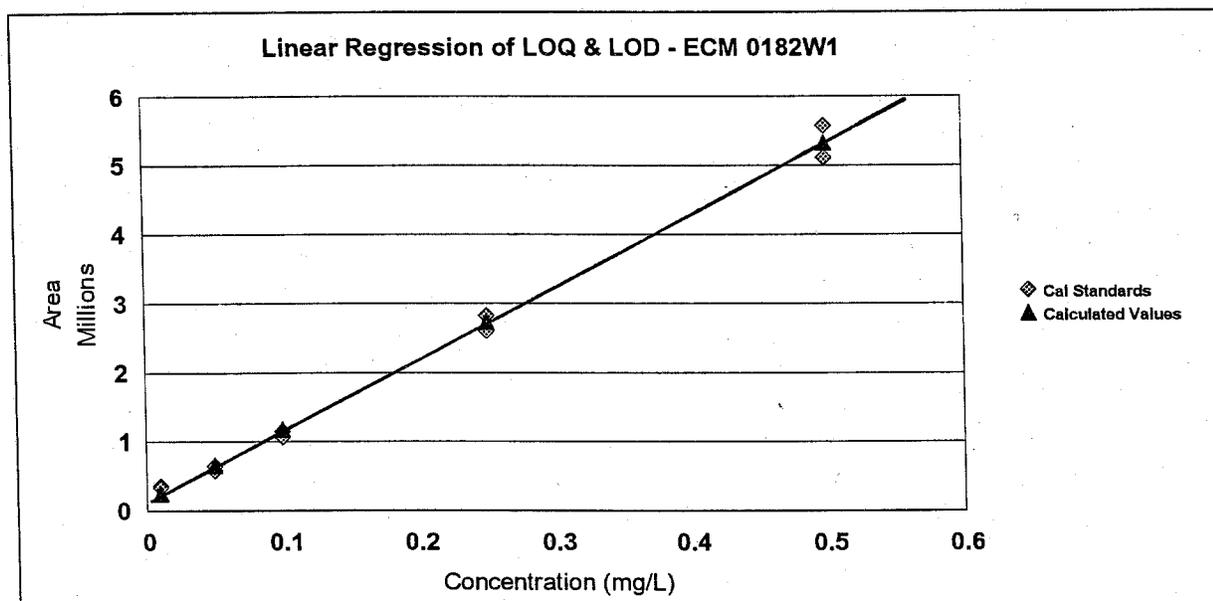


Calibration Standard-4 @ 0.25 mg a.i./L (Replicate #01) for LOQ, LOD Peak Area =2824830

Chromatograms:



Calibration Standard-5 @ 0.50 mg a.i./L (Replicate #01) for LOQ, LOD Peak Area =5580570

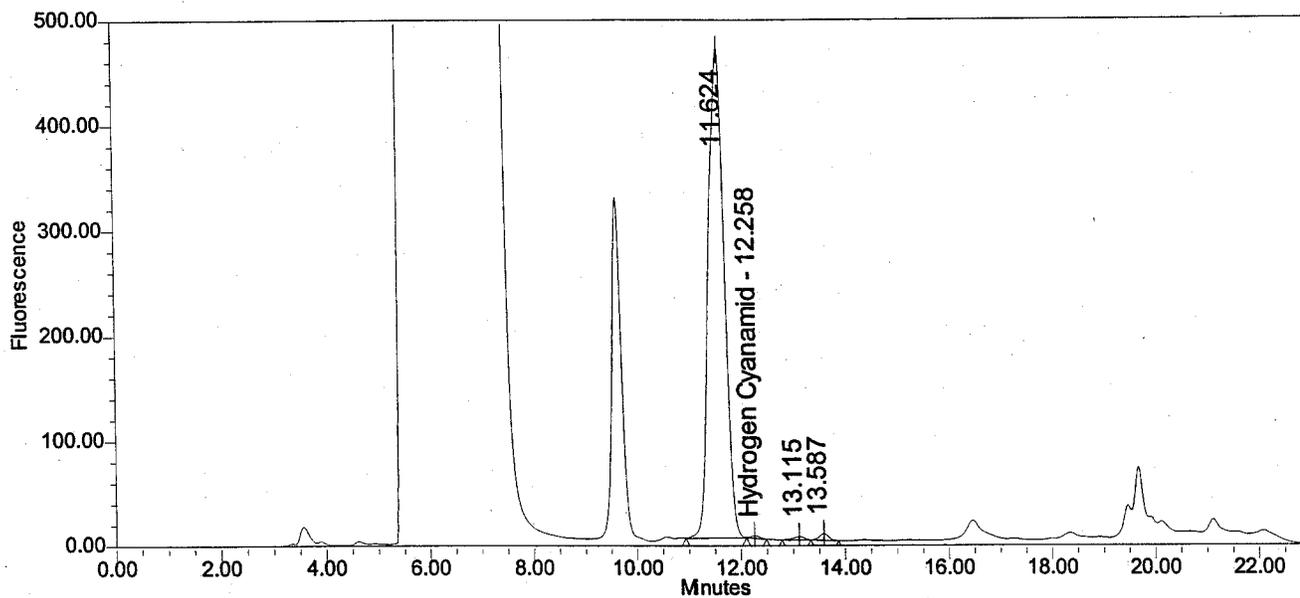


Linear Regression Graph for Hydrogen Cyanamid

Concentration (mg a.i./L) = [Sample Peak Area - 140582 fau] / (10360291 fau/mg a.i./L)

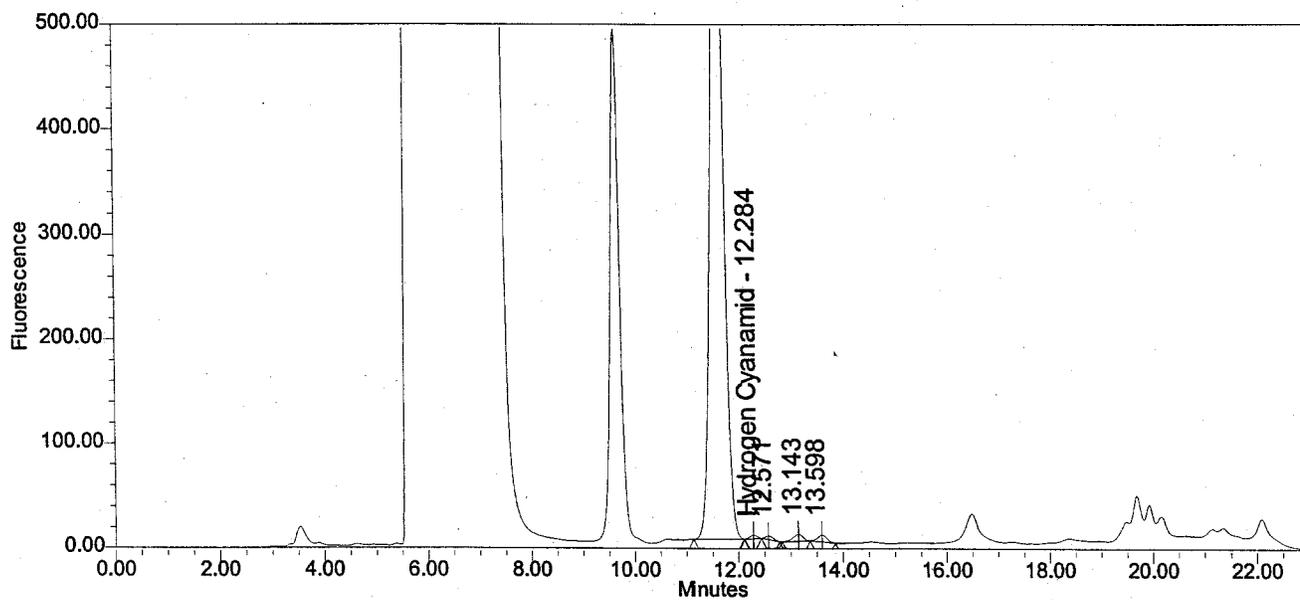
Observations = 10 R² = 0.9948

Chromatograms:



LOQ-1 (Method Blank) @ LOQ, LOD

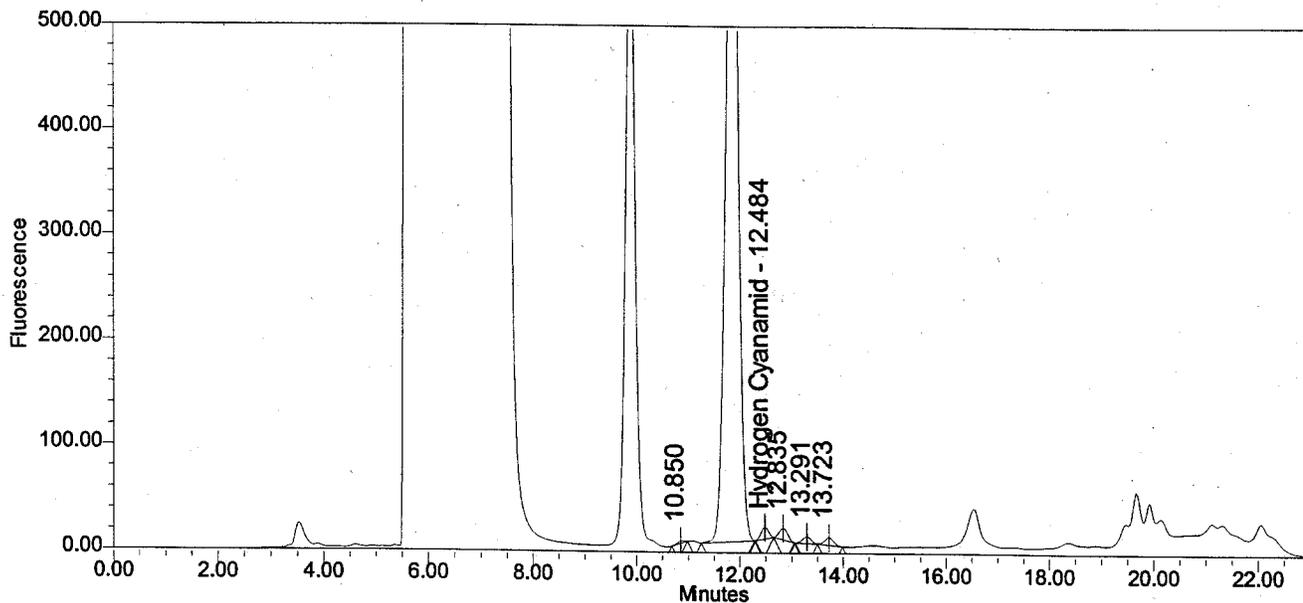
Peak Area = 28500



LOQ-2 (Matrix Seawater Blank) @ LOQ, LOD

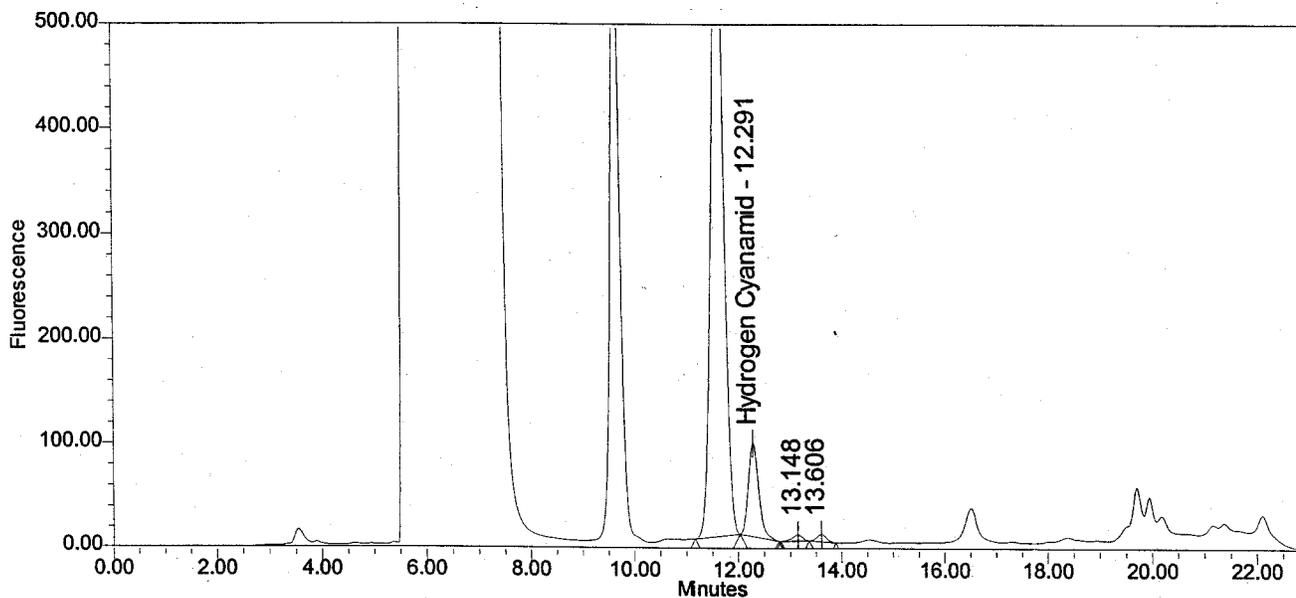
Peak Area = 32060

Chromatograms:



LOD-3 (Seawater Fortified @ 0.010 mg a.i./L - Replicate #1)

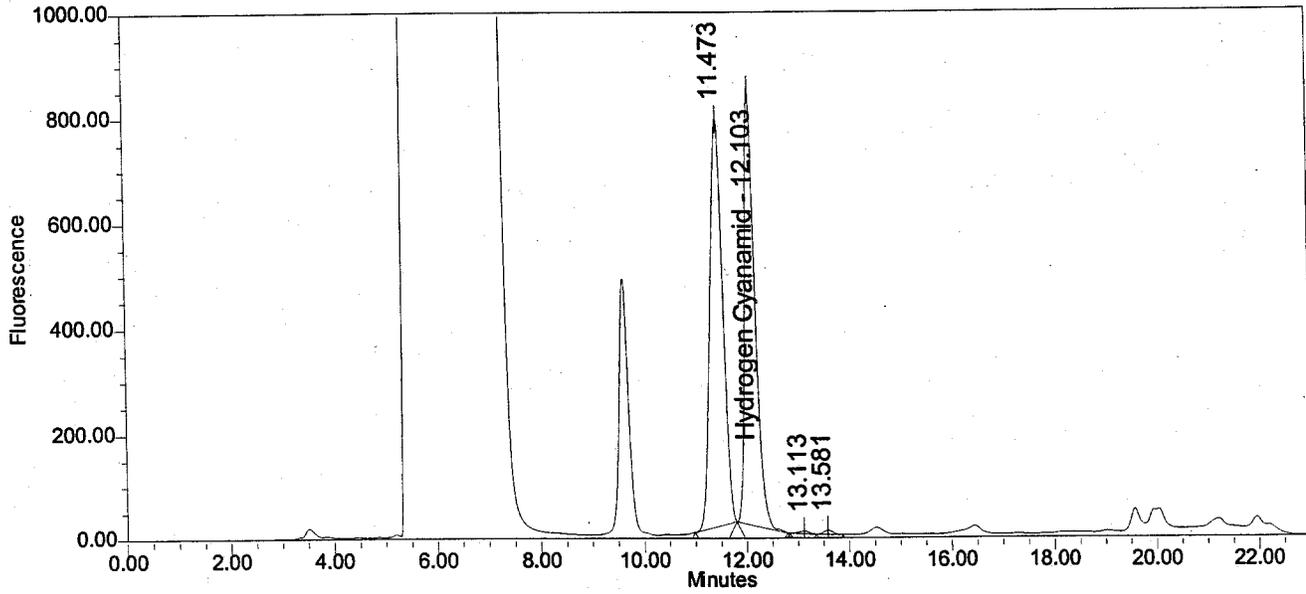
Peak Area = 112840



LOQ-3 (Seawater Fortified @ 0.101 mg a.i./L- Replicate #1)

Peak Area = 1250440

Chromatograms:

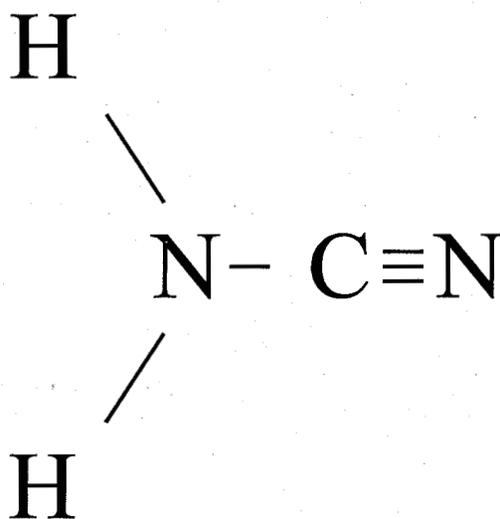


10 x LOQ-3 (Seawater Fortified @ 1.01 mg a.i./L- Replicate #1)

Peak Area = 11605869

Appendix 1

Chemical Structure of Hydrogen Cyanamide



Appendix 2

Water Quality Parameters of Seawater Collected
at a Shoreline on the Mississippi Sound at Henderson Point, Mississippi

Conducted by the USEPA/ECL

Parameter:	Technique	
pH	Electrode	6.65
Salinity	Electrode	16 ‰