

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Cyanimide

MRID #: 436468-01

Matrix: Soil

Analysis: HPLC/UV

This method is provided to you by the Environmental Protection Agency's (EPA) Environmental Chemistry Laboratory (ECL). This method *is not* an EPA method but one which was submitted to EPA by the pesticide manufacturer to support product registration. EPA recognizes that the methods may be of some utility to state, tribal, and local authorities, but makes no claim of validity by posting these methods. Although the Agency reviews *all* Environmental Chemistry Methods submitted in support of pesticide registration, the ECL evaluates only about 30% of the currently available methods. Most methods perform satisfactorily but some, particularly the older methods, have deficiencies. Moreover, the print quality of the methods varies considerably because the methods originate from different sources. Therefore, the methods offered represent the best available copies.

If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

1950

CONFIDENTIAL - SECURITY INFORMATION

CONFIDENTIAL - SECURITY INFORMATION

CONFIDENTIAL - SECURITY INFORMATION

CONFIDENTIAL - SECURITY INFORMATION

CONFIDENTIAL - SECURITY INFORMATION

CONFIDENTIAL - SECURITY INFORMATION

CONFIDENTIAL - SECURITY INFORMATION

CONFIDENTIAL - SECURITY INFORMATION

The following information is being furnished to you for your information only. It is not to be disseminated outside your agency. This information is being furnished to you for your information only. It is not to be disseminated outside your agency. This information is being furnished to you for your information only. It is not to be disseminated outside your agency.

CONFIDENTIAL - SECURITY INFORMATION
1. If you are to 000-254-111 is a copy of the information you may wish to use
CONFIDENTIAL - SECURITY INFORMATION

TITLE: A High Performance Liquid Chromatographic Method for Measurement of Cyanamide Residues in Soil

AUTHOR: Timothy Zee Kendall

DATE: June 17, 1993

ABSTRACT: A high performance liquid chromatographic (HPLC) method is described for the analysis of cyanamide in soil. The method employs an acetone extraction of the soil followed by centrifugation, evaporation of a portion of the sample and derivatization with dansyl chloride. The derivatized sample extracts are chromatographed using gradient elution and quantified with fluorescence detection. Recoveries of cyanamide from Oregon soil fortified in the range of 0.0100-0.100 mg/kg resulted in a mean recovery of 92.1% (S.D. 16.7) with a recovery range of 65.7%-118%. Control values were all <0.00500 mg/kg. The LOD in the Oregon soil matrix is 0.00500 mg/kg with the LOQ of 0.010 mg/kg. Recoveries of cyanamide from Washington soil fortified in the range of 0.0250-0.100 mg/kg resulted in a mean recovery of 73.0% (S.D. 10.4) with a recovery range of 62.8%-102%. Recoveries from Washington samples fortified at the 0.0100 mg/kg level resulted in artificially high recoveries due to matrix contribution. Control values indicate that the Washington matrix elevates the LOD to approximately 0.0100 mg/kg with a LOQ at 0.0250 mg/kg.

INTRODUCTION

The method for determining cyanamide residue in soil is presented. The methodology employed is novel and achieves detection limits lower than previously reported. The method was validated with soil collected from sites in Oregon and Washington (soil characterization in Tables 1 and 3). The method is flexible enough to allow for the variable matrix associated with soil.

PRINCIPLE AND APPLICATION

Cyanamide is extracted and concentrated from soil by acetone partition; the soil-solvent mixture is shaken on a shaker table and then centrifuged. Following evaporation of a portion of the extract, the residues are dissolved in an acetone solution of dansyl chloride and a pH 9.0 $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer solution. The solution is then heated at 50 °C for one hour. An aliquot of this solution is analyzed by gradient elution HPLC using fluorescence detection. Quantification of cyanamide is effected by linear regression analysis of the peak heights for samples and reference standards.

ANALYTICAL METHOD

Reagents

Cyanamide, Lot No. RD-467-034, 99% active ingredient, supplied by CFPI
Acetone, Burdick and Jackson "or equivalent", HPLC grade
Acetonitrile, Burdick and Jackson "or equivalent", HPLC grade
Dansyl chloride, Aldrich Chemical, 98% a.i.
Sodium carbonate, Fisher Scientific, AR grade
Sodium bicarbonate monohydrate, Aldrich Chemical, 99.7% a.i.
Sodium phosphate monobasic, Mallinckrodt, 99.3% a.i.
Sodium phosphate dibasic, Mallinckrodt, AR grade
Dichlorodimethylsilane, EM Science
E-Pure water, Barnstead "or equivalent", ASTM type II

Equipment

Balance, Ohaus Galaxy 160 "or equivalent", four-place analytical balance
Flasks, volumetric, assorted sizes
Shaker table, LabLine "or equivalent"
Centrifuge, Beckman GPR (or equivalent), 0-5000 rpm
Centrifuga bottles, 200 mL, Pyrex
Heating block, Thermolyne 17600 dry bath
Graduated centrifuge tubes, Pyrex, 15 mL capacity
Pipets, volumetric, assorted sizes
Serum bottles, Wheaton, assorted sizes, with Teflon-lined lids and metal crimp caps

Syringes, Hamilton, assorted sizes

Apparatus

High Performance Liquid Chromatographic System

Liquid chromatograph solvent pumps (2), Waters Model 6000A, Waters Model 590, in series

Solvent programmer, Waters Model 680

Autosampler, Waters Model 710B Intelligent Sample Processor

Detector, Waters Model 470 programmable fluorescence detector

Integrator, Hewlett-Packard Model 3396 A

Chromatographic Column, Metachem Inertsil ODS-2, 5 μ m, 250 mm x 4.6 mm I.D.

High Performance Liquid Chromatographic Conditions

Mobile Phase A: 10 mM Na₂HPO₄ / 10 mM NaH₂PO₄ (aqueous)

Mobile Phase B: 55% CH₃CN : 45% 10 mM Na₂HPO₄ / 10 mM NaH₂PO₄

Gradient program:

Time (min)	Flow (ml/min)	%A	%B	Curve
—	1.20	70	30	—
7.00	1.20	40	60	6
12.0	1.50	0	100	1
13.0	1.20	70	30	1

Injection volume: 50 μ L

Excitation wavelength: 367 nm

Emission wavelength: 495 nm

Sensitivity: 1 Volt output

Filter: 0.5 second

Autosampler runtime: 20 minutes

Attenuation (integrator): 2⁴

Threshold (integrator): 4

Peak width (integrator): 0.10

Attenuation (detector): 32

Gain (detector): 10

Detailed Procedure

I. Preparation of Stock, Buffer and Standard Solutions

A. Cyanamide Stock (Solutions):

1. Weigh 100 milligrams (a.i.) of cyanamide on an analytical balance.

2. Transfer the cyanamide to a 100-mL volumetric flask and dissolve to the mark with acetone.
3. In order to prepare appropriate stocks for fortification, make serial dilutions (in acetone) as follows:

Concentration of stock (mg/L)	Volume of stock used (mL)	Final volume of dilution (mL)	Final concentration of dilution (mg/L)
1000	10.0	100	100
100	10.0	100	10.0
10.0	10.0	100	1.00
1.00	10.0	100	0.100
0.100	10.0	100	0.0100

4. Transfer each stock solution to its properly labeled 100-mL amber serum vial and seal with a Teflon-lined crimp cap.
5. Store all stock solutions in a refrigerator maintained at 4°C.

B. Sodium carbonate/sodium bicarbonate buffer:

1. Prepare 0.2 M sodium carbonate by weighing 2.12 grams of anhydrous sodium carbonate (Na_2CO_3) into a 100 mL volumetric flask and dissolving with E-Pure[®] water.
2. Prepare 0.2M sodium bicarbonate monohydrate by weighing 1.68 grams of sodium bicarbonate ($\text{NaH}_2\text{CO}_2\text{H}_2\text{O}$) into a 100 mL volumetric flask and dissolving with E-Pure[®] water.
3. Prepare the carbonate/bicarbonate buffer by volumetrically combining 4.0 mL of 0.2M sodium carbonate with 46 mL of 0.2M sodium bicarbonate and diluting the solution to 200 mL with E-Pure[®] water.
4. Store sodium carbonate and sodium bicarbonate stocks and prepared buffer at 4°C.

C. Cyanamide Standards:

1. In order to prepare representative standards fortify 15 mL *silanized* centrifuge tubes (as specified below). To each centrifuge tube is then volumetrically added 1.0 mL of a 1.0 mg/mL acetone solution of dansyl chloride and 1.0 mL of a pH 9.0 Na_2HCO_3 / NaH_2CO_3 aqueous buffer solution. Each tube is capped, vortexed for approximately 30 seconds and then heated in a dry heating block at 50°C for one hour. The solutions are removed from the bath, allowed to cool to room temperature and volumetrically diluted to 10.0 mL with 50% acetone : 50% pH 9 sodium carbonate/sodium bicarbonate buffer. Fortify standards as follows:

Concentration of stock (mg/L)	Volume of stock used (μ L)	Final volume of dilution (mL)	Final concentration of dilution (μ g/L)
1.00	500	10.0	50.0
1.00	350	10.0	35.0
1.00	250	10.0	25.0
1.00	100	10.0	10.0
1.00	50	10.0	5.00

2. Prepare fresh standards daily.

II. Control Sample Fortification

A. Processing and Dry-Weight Determination of Soil Samples:

1. Ensure that all glassware is fully silanized with dichlorodimethylsilane prior to sample processing.
2. Rinse all glassware with reagent grade acetone.
3. Remove the appropriate sets of soil cores from the freezer. Document (in the cyanamide logbook) the time of and person responsible for the removal of cores from the freezer.
4. Determine the upper end of each soil core.
5. Using a PVC tube cutter, measure, mark and remove the top 15 cm of each core and combine the appropriate cores in a mixing bowl. Allow the soil sections to thaw.
6. Homogenize until a uniform mixture is obtained (ca 10-20 minutes).
7. Remove a 50-gram portion of the mixed soil into a tared, prelabeled, 200 mL Pyrex centrifuge bottle. Document this analytical weight.
8. Remove a second soil sample (approximately 10-15 grams) and determine its soil (dry-weight) content.

B. Quality Control Sample Fortification

1. For preparation of quality control or method validation samples, fortify each preweighed soil sample (contained in a centrifuge bottle) with cyanamide by volumetric addition of the prepared stock solutions.

III. Extraction and Derivatization

1. To each soil sample add 100 mL of acetone. Secure the centrifuge bottles to a rotary shaker table. Set the table at 250 rpm and allow samples to shake for approximately 30 minutes.
2. Transfer the bottles to a centrifuge and centrifuge at 1500 rpm for 10 minutes.
3. Volumetrically remove 2.0 mL of the acetone extract from each sample, transfer to a 15 mL centrifuge tube and evaporate to dryness (at room temperature) under a gentle stream of nitrogen.
4. To each sample, volumetrically add 1.0 mL of a 1.0 mg/mL solution of dansyl chloride (in acetone) and 1.0 mL of a pH 9.0 Na_2HCO_3 / NaH_2CO_3 aqueous buffer solution. Vortex the sample briefly in order to ensure complete mixing.
5. Place the samples in a preheated dry block and heat at 50 °C for one hour.
6. Remove the samples from the block and allow to cool to room temperature.
7. Proceed to Section IV, High Performance Liquid Chromatography.

IV. High Performance Liquid Chromatography

A. Method: High performance liquid chromatographic conditions for the analysis of derivatized cyanamide standards and samples have been determined. Close adherence to these parameters is necessary in order to obtain adequate sensitivity and resolution.

B. Analysis:

1. Prepare standard solutions containing dansyl cyanamide. Standard solution concentrations used for the recovery study were 5.00, 0.0100, 25.0, 35.0 and 50.0 $\mu\text{g/L}$.
2. Inject 50 μL of the 5.00 $\mu\text{g/L}$ standard solution. Identify the dansyl cyanamide peak by its retention time and document the peak height. Adjust the attenuation so that the peak signal results in at least a ten percent deflection from the baseline.
3. Inject 50 μL of each of the standards, document the peak heights, and determine the coefficient of determination for the line. The coefficient of determination should be greater than or equal to 0.985.
4. Inject 50 μL of several samples. In general, a full standard set should be analyzed for every 10-12 samples.
5. Inject a full complement of standards and document peak heights.
6. Repeat steps 3-5 until all samples have been injected.
7. Construct a standard curve for dansyl cyanamide by plotting peak height observed versus the concentration ($\mu\text{g/L}$) of the standard injected.
8. The standard linear regression analysis for dansyl cyanamide is used to determine the concentration in each sample.
9. In order to determine the analytical result for each sample, the following equation is

used:

$$\text{Analytical Result } (\mu\text{g/g}) = A \times \text{D.F.}$$

where:

Analytical Result = concentration of cyanamide ($\mu\text{g/g}$)

A = concentration ($\mu\text{g/L}$) of extract from the regression analysis

D.F. = dilution factor, ratio of the final extract volume (L) to the initial sample mass (g). NOTE: If the initial sample mass represents a wet weight, the analytical result is expressed as $\mu\text{g/kg}$ wet weight. To convert the analytical result to a dry weight basis, multiply by the wet-to-dry weight ratio determined in step II.A.8.

RESULTS AND DISCUSSION

Soil samples were fortified with cyanamide. Each sample was extracted with acetone, shaken, centrifuged, evaporated to dryness and derivatized with dansyl chloride. Cyanamide residues were analyzed by HPLC using fluorescence detection.

The method validation/recovery study was run on soil collected from Oregon and Washington and in duplicate on separate days in order to evaluate the ruggedness and relative precision of the adopted method. Linear regression analyses for the response of cyanamide standards (as the dansyl derivative) are shown in Figure 31. Accuracy and precision of the recovery from Oregon and Washington soil is presented in Figure 32.

Recoveries from Oregon soil fortified with cyanamide at 0.100 mg/kg (wet weight) ranged from 65.7% to 107% with an average recovery of 81.6% (S.D. 15.6); recoveries at 0.0500 mg/kg ranged from 68.7% to 105% with an average recovery of 88.1% (S.D. 17.8); recoveries at 0.0250 mg/kg ranged from 73.5% to 114% with an average recovery of 95.5% (S.D. 14.2); recoveries at 0.0100 mg/kg ranged from 77.2% to 118% with an average recovery of 103% (S.D. 14.3). The LOD for cyanamide was 0.00500 mg/kg, calculated as the predicted analytical result at one half of the LOQ (0.0100 mg/kg). The LOQ was determined as a function of the wet weight of the sample. A summary of recovery data obtained from Oregon soil is presented in Table 32. Representative chromatograms of Oregon soil extracts containing cyanamide at the 0.100, 0.0500, 0.0250, 0.0100 mg/kg and control levels are shown in Figures 33-37, respectively.

Recoveries from Washington soil fortified with cyanamide at 0.100 mg/kg (wet weight) ranged from 62.8% to 72.4% with an average recovery of 65.6 (S.D. 3.63); recoveries at 0.0500 mg/kg ranged from 71.7% to 77.3% with an average recovery of 74.3% (S.D. 2.25); recoveries at 0.0250 mg/kg ranged from 63.0% to 102% with an average recovery of 79.3% (S.D. 15.2); recoveries at 0.0100 mg/kg ranged from 123% to 154%

with an average recovery of 137% (S.D. 14.9). As observed in the control samples, a significant matrix interference resulted in artificially elevated recoveries at the 0.0100 mg/kg level. The LOD for cyanamide was compromised due to the matrix contribution of the Washington soil. The LOD for this soil should be considered to approximate 0.0100 mg/kg; the LOQ should be 0.0250 mg/kg. The LOQ was determined as a function of the wet weight of the sample. A summary of recovery data obtained from Washington soil is presented in Table 33. Representative chromatograms of Washington soil extracts containing cyanamide at the 0.100, 0.0500, 0.0250, 0.0100 mg/kg and control levels are shown in Figures 38-42, respectively.

SUMMARY

A procedure for the measurement of cyanamide in soil has been developed. The analytical procedure entails the extraction of cyanamide from soil, its derivatization, separation from artifacts by HPLC using gradient elution and its quantification by fluorescence detection.