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 $Na_2CO_3/NaHCO_3$ 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
Centrifuge 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, 5μm, 250 mm x 4.6 mm I.D.

High Performance Liquid Chromatographic Conditions

Mobile Phase A: 10 mM Na_2HPO_4 / 10 mM NaH_2PO_4 (aqueous)
Mobile Phase B: 55% CH_3CN : 45% 10 mM Na_2HPO_4 / 10 mM NaH_2PO_4
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 μL
Excitation wavelength: 360 nm
Emission wavelength: 495 nm
Sensitivity: 1 Volt output
Filter: 0.5 second
Autosampler runtime: 20 minutes
Attenuation (integrator): 2^4
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 ($NaH_2CO_2H_2O$) 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 (µL)	Final volume of dilution (mL)	Final concentration of dilution (µg/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 µg/L.
2. Inject 50µL of the 5.00 µg/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 (µg/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:

Analytical Result (µg/g) = A X D.F.

where:

Analytical Result = concentration of cyanamide (µg/g)

A = concentration (µg/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 µg/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.