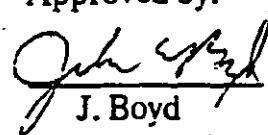


M 2149.01

H. Nejad/ct

Approved by:


J. Boyd9/20/31
Date

**AMERICAN CYANAMID RESEARCH COMPANY
AGRICULTURAL RESEARCH DIVISION
CHEMICAL DEVELOPMENT
P. O. Box 400
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Recommended Method of Analysis

Herbicide (CL 263,222): Determination of CL 263,222 Residues in soil by HPLC

A. Principle

CL 263,222 is extracted from soil with 0.5 N sodium hydroxide. The pH is adjusted to about 1.7-2.0, the resulting precipitate is filtered and CL 263,222 is absorbed from the extract on a C18-OH solid phase extraction cartridge. The compound is eluted from the C18-OH column with 50% methanol into a SPE strong cationic exchange cartridge. After eluting the compound from the SCX column with pH 6.5 phosphate buffer, the compound is partitioned from acidic solution into methylene chloride. The methylene chloride is evaporated to dryness, residual methylene chloride is eliminated by re-evaporation with methanol, and the residue is dissolved in water containing CL 263,499 (used as a marker for injection validation) for the measurement by high performance liquid chromatography. The validated sensitivity of the method is 5 ppb.

B. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Liquid Chromatograph: LC pump capable of isocratic operation up to 2 mL/min. (Kratos Spectroflow 400).

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NOTE: This method supercedes M 2149 to add typical chromatograms.

2. Detector: UV photometer set at 254 nanometers (Kratos Model 783).
3. Column: Supelcosil LC-8-DB reverse phase C8,5-micron, 15 cm x 4.6 mm deactivated for bases (Supelco Cat. No. 5-8347M).
4. Guard Column: Fitted with an LC-8-DB cartridge (supelcoguard, Supelco Cat. No. 5-9563M).
5. Sample Injector: Equipped with a 200-mcL loop (Rheodyne Model 7125).
6. Bottles: Wide-mouth, high density polypropylene, 8-oz. cap. (Nalgene Cat No. 2105-0008).
7. Reciprocating Shaker: Equipped to hold 8-oz. bottles horizontally (A. H. Thomas Co., Cat. No. 8291-510).
8. Centrifuge: Equipped with a head for 8-oz. bottles (Beckman Model J2-21).
9. pH Meter: Orion Model 701A or equivalent.
10. Vacuum Filtration Apparatus: A 500-mL suction flask fitted with a 600-mL Buchner porcelain funnel by means of a rubber adapter.
11. Filter paper: Glass microfibre, Whatman 934-AH, 12.5 cm diameter (Cat. No. 1827-125).
12. Column Oven: Spark Holland, SpH99.
13. Evaporation Flasks: 200-mL capacity.
14. General Laboratory Glassware: Assorted beakers, graduated cylinders and volumetric pipettes.
15. Rotary Evaporator: Buchler Instruments equipped with a heated water bath at 35-40°C.
16. Solid Phase Extraction Processing station: Cat. No. AI 6000, Analytical International.
17. Solid Phase Extraction:
 - a. Bond-Elute C18-OH Cartridge (1,000 mg): Cat. No. 0700-0000, Varian. (Distributed by Jones Chromatography, Lakewood, CO).

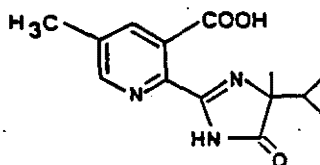
b. Bond-Elut Aromatic Sulfonic Acid Cartridge (SCX)(1000 mg): Cat. No. 1225-6011, Varian (Distributed by Jones Chromatography, Lakewood, CO).

18. SPE Adaptors: Analytichem International, Cat. No. 636001.
19. Stopcocks: One-Way Stopcocks (Applied Separations, Cat. No. 2406).
20. Disposable Svringes: Luer Slip Tip; 10-mL and 60-mL capacity (Scientific Products Cat. No. S9519-10S and S9519-60S).
21. Frit filter Reservoirs Disposable: 75-mL capacity (Analytichem International, Cat. No. 607520).
22. Analytical Balance: Sartorius Model R200D, readability 0.01/0.1 mg, weighing range 0-42 and 0-205 g.
23. Top-Loading Balance: Mettler Model PE 3000, readability 0.1 g, weighing range 0-3100 g.
24. Recorder: SP 4270 recording integrator, Spectra-Physics, Inc.
25. Pipettes: Pipettes capable of operation in range of 0-100 mcL and 100-1000 mcL (Rainnin edp Battery-Operated Motorized pipettes).
26. Karl Fisher Titrator (Aquatest 8): Model 128 (Photovolt Co., Indianapolis, IN).
27. Column Oven: HPLC column oven (SP8792, Spectra-Physics).

C. Reagents

1. Analytical Standards:

- a. CL 263,222 [nicotinic acid, 5-methyl-2-(4-isopropyl-4-methyl-oxo-2-imidazolin-2-yl)-], analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, Princeton, NJ.

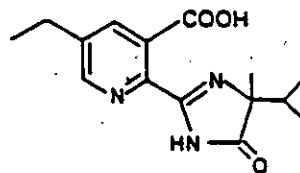


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- b. CL 263,499 [nicotinic acid, 5-ethyl-2-(4-isopropyl-4-methyl-oxo-2-imidazolin-2-yl)-], analytical grade, known purity, American Cyanamid Company, Agricultural Research Division, Princeton, NJ.



2. Solvents: High purity, HPLC grade (Burdick and Jackson; Baxter, Inc.).
- Methylene Chloride
 - Methanol
 - Acetonitrile
 - Hexane
3. Water: High purity, Deionized (Milli-Q water system)
4. Chemicals:
- Aldrich A. C. S. Reagent
Formic acid, 96% (Cat. No. 25,136-4)
 - "Baker Analyzed" Reagents, J. T. Baker Co.
Triethylamine (Cat. No. W635-07)
Hydrochloric Acid, concentrated (Cat. No. 9535-1)
Potassium Phosphate, dibasic (Cat. No. 3252-1)
Sodium Hydroxide, Pellets (Cat. No. 3722-1)
Filter Aid: (Celite 545 John-manville Corporation).
5. Solutions:
- Extraction solvent, 0.5N Sodium Hydroxide: Dissolve 80 g of sodium hydroxide pellets in 4 liter of deionized water.
 - 50 % Methanol in water: Mix equal volumes of methanol and high purity water and mix well.
 - Hydrochloric Acid, 6N: Measure equal volumes of concentrated HCl and high purity water.

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- d. Hydrochloric Acid, 0.1N: Dilute 8.3 mL of concentrated HCl to one liter with high purity water.
- e. pH 2.0 high purity Water: Adjust pH of high purity water to 2.0 with 6N HCl.
- f. Phosphate Buffer Solution pH 6.5: Dissolve 50 g of dibasic potassium phosphate in one liter of high purity water and adjust to pH 6.5 with 6N HCl.
- g. Phosphate Buffer Solution pH 3.5: Dissolve 5 g of dibasic potassium phosphate in one liter of high purity water and adjust to pH 3.5 with 6N hydrochloric acid.
- h. High Purity Water: Millipore, Milli-Q water or equivalent.
- i. Liquid Chromatographic Mobile Phase: (see 5, Notes on the Method)

Mix 250 mL acetonitrile, 10 mL of formic acid, and 740 mL high purity water. Filter through a Rainin Nylon-66 (0.45 μ m) filter or equivalent.
- j. High Marker Stock Solution (1000 mcg/mL)(see 14, Notes on the Method): Dissolve 50 mg CL 263,499 in 2.5 mL acetone in 50-mL volumetric flask and bring to volume.
- k. Intermediate Marker Stock Solution (100.0 mcg/mL): Transfer 10 mL aliquot of High Marker stock solution (1000 mcg/mL) to 100-mL volumetric flask bring it to volume with high purity water.
- l. Working Marker Solution (0.20* mcg/mL): From Intermediate Marker Stock Solution (100.0 mcg/mL), take 2 mL and dilute it to one liter with high purity water.

(* see 12, Notes on the Method).

D. Preparation Of Standard Solution

1. Preparation of Fortification/HPLC Standard Solutions without Marker
 - a. Stock Fortification/HPLC Standard Solutions without marker
(Prepare monthly, store in amber bottles in refrigerator)

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1) High Stock Fortification/HPLC Standard Solution without marker (1000 mcg/mL)

Accurately weigh 50 mg \pm 0.05 mg of CL 263,222 analytical standard into a 50-mL volumetric flask, dissolve the material in 2.5 mL of acetone and dilute to the mark with high purity water. This solution contains 1000 mcg CL 263,222/mL.

2) Intermediate Stock Fortification/HPLC Standard Solution without marker (100.0 mcg/mL)

Measure a 10-mL of the High Stock Fortification/HPLC Standard Solution without marker (1000 mcg/mL) into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 100.0 mcg CL 263,222/mL.

3) Low Stock Fortification/HPLC Standard Solution without marker (10.00 mcg/mL)

Measure a 10-mL of the Intermediate Stock Fortification/HPLC Standard Solution without marker (100.0 mcg/mL) into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 10.00 mcg CL 263,222/mL.

b. Working Fortification/HPLC Standard Solutions without marker
(Prepare monthly, store in amber bottles in refrigerator)

1) (1.00 mcg/mL)

Pipet a 1-mL aliquot of the Intermediate Stock Fortification/HPLC Standard Solution without marker (100.0 mcg/mL) into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 1.00 mcg CL 263,222/mL.

2) (0.50 mcg/mL)

Measure a 5-mL aliquot of the 10.00 mcg/mL Low Stock Fortification/HPLC Standard Solution without marker into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 0.50 mcg CL 263,222/mL.

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3) (0.25 mcg/mL)

Measure a 25-mL aliquot of the 1.00 mcg/mL Working Fortification/HPLC Standard solution without marker into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 0.25 mcg CL 263,222/mL.

4) (0.08 mcg/mL)

Pipet a 0.8-mL aliquot of the 10.00 mcg/mL Low Stock Fortification/HPLC Standard solution without marker into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 0.08 mcg CL 263,222/mL.

5) (0.05 mcg/mL)

Pipet a 5-mL aliquot of the 1.00 mcg/mL Working Fortification/HPLC Standard solution without marker into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 0.05 mcg CL 263,222/mL.

6) (0.02 mcg/mL)

Pipet a 2-mL aliquot of the 1.00 mcg/mL Working Fortification/HPLC Standard solution without marker into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 0.02 mcg CL 263,222/mL.

7) (0.01 mcg/mL)

Pipet a 1-mL aliquot of the 1.00 mcg/mL Working Fortification/HPLC Standard solution without marker into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 0.01 mcg CL 263,222/mL.

c. Fortification Guidance

For purposes of fortification of soil samples for recovery studies, use Fortification/HPLC standard solutions without marker such that a 0.1-2.0 mL of solution added to 25 grams soil will yield the desired fortification level. For example, 0.5 mL of the 0.25 mcg/mL HPLC Working solution added to 25 g soil yield a 5 ppb fortification level.

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2. Preparation of HPLC Standard Solutions with Marker Standard.

a. Stock Solutions

(Prepare monthly, store in amber bottles in refrigerator)

1) High Stock HPLC Standard Solution with marker (1000 mcg/mL)

Accurately weigh $50 \text{ mg} \pm 0.05 \text{ mg}$ of AC 263,222 analytical standard into a 50-mL volumetric flask, dissolve the material in 2.5 mL of acetone and dilute to the mark with Working Marker Standard Solution (0.20 mcg/mL). This solution contains 1000 mcg CL 263,222/mL and 0.20 mcg/mL CL 263,499.

2) Intermediate Stock HPLC Standard Solution with marker (100.0 mcg/mL)

Measure a 10-mL of the high stock HPLC Standard solution with marker (1000 mcg/mL) into a 100-mL volumetric flask and dilute to the mark with Working Marker Standard Solution (0.20 mcg/mL). This solution contains 100.0 mcg CL 263,222/mL and 0.20 mcg/mL CL 263,499.

3) Low Stock HPLC Standard Solution with marker (10.00 mcg/mL)

Measure a 10-mL of the Intermediate Stock HPLC Standard Solution with marker (100.0 mcg/mL) into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 10.00 mcg CL 263,222/mL and 0.20 mcg/mL CL 263,499.

b. HPLC Working Standard Solution with marker (Interspersed Standard) (0.08 mcg/mL)

(Prepare monthly, store in amber bottles in refrigerator)

Pipet a 0.8- mL aliquot of the 10.00 mcg/mL Low Stock Fortification/HPLC Standard solution with marker into a 100-mL volumetric flask and dilute to the mark with high purity water. This solution contains 0.08 mcg CL 263,222/mL and 0.20 mcg/mL CL 263,499.

c. General Guidance

Use HPLC Working Standard with marker to monitor drifting of the chromatographic response as well as to document the proper injection. Use 0.01, 0.02, 0.05, 0.08, and 0.25 mcg/mL Fortification/HPLC Working

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Standards without marker for the linear regression equation. Use 0.25, 0.50, 1.00, and 10 mcg/mL Fortification/HPLC Standards without marker for fortification.

E. Liquid Chromatographic Conditions (see 5. Notes on the Method)

Set up the instrument for isocratic operation at a flow rate of 1.0 mL/min. Set the UV detector at 254 nanometers. A column oven operated at about 35°C can be helpful in improving peak shape and resolution from interfering peaks and in minimizing variation in retention time. Instrument sensitivity should be set so that a 200- μ L injection of the 0.08 mcg/mL standard gives a chromatographic peak height of about 50 \pm 10 millimeters or the equivalent in integrator units. The retention time for CL 263,222 is about 4.5-5.5 minutes and that of CL 263,499 is about 7-8 minutes using the indicated mobile phase (250 mL Acetonitrile, 10 mL Formic Acid, and 740 mL Water) and the operating conditions.

F. Sample Preparation

1. Allow frozen soil samples to thaw completely in airtight containers just before analyzing.
2. Mix the thawed soil thoroughly and remove large stones and vegetation to obtain a homogeneous sample.

G. Recovery studies

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. Two fortified samples should also be processed with each daily set of samples analyzed, one of which must be at the validated sensitivity limit.

1. Weigh a 25 g subsample of control soil into a centrifuge bottle (8-oz. capacity).
2. Add to the soil dropwise an accurately measured volume of standard fortification solution.
3. Mix the sample well before adding the extraction solvent.
4. Continue with the extraction and cleanup steps as described in the method.

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H. Moisture Determination

Determine the moisture content of the soil to be analyzed by Karl Fisher titration or other validated methodology.

I. Sample Analysis(see 1 to 4, Notes on the Method)

1. Extraction

- a. Weigh 25 ± 0.1 g of soil into 8-ounce wide-mouth polypropylene bottle.
- b. Using a 250-mL graduated cylinder, measure 100 mL of 0.5 N sodium hydroxide and pour the solvent into the bottle containing the sample. Stopper the bottle and shake the contents for about one hour on a reciprocating shaker.
- c. Centrifuge the sample at about 3000 rpm for about 15 minutes.
- d. Decant exactly 80 mL of the supernatant extract into a 100-mL graduated cylinder and transfer the solution to a 400-mL beaker (Rinse the graduated cylinder with 0.1N HCl).
- e. Adjust the sample pH to pH 1.7-2.0 (preferably 1.7) with 6N HCl (Rinse the pH meter probe with pH 2.0 water).
- f. Add 5 ± 0.5 g of Celite 545 to the beaker, stir the contents and filter the mixture by suction through a Whatman No.934-AH, 12.5 cm glass fiber filter paper into a 250-mL separatory funnel.
- g. Wash the beaker and filter cake with two 25-mL portions of pH 2.0 water.

2. Solid Phase Extraction

- a. Prepare a Bond-Elute C18-OH cartridge using an Analytichem International VAC-Elute processing station by washing the cartridge with 5 mL methanol and 5 mL of water.
- b. Connect a 75-mL fritted reservoir onto the top of C18-OH cartridge using an adapter and stopcock. Pull the sample solution using vacuum through C18-OH cartridge (1 drop/sec). Discard the effluent.

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- c. Wash reservoir and C18-OH cartridge with two 5-mL portion of 0.1 N HCl solution. Use vacuum and discard the wash.
 - d. Prepare a Bond-Elute aromatic sulfonic acid by washing the cartridge with 5 mL of hexane, 5 mL methanol, and 5 mL high purity water.
 - e. Connect the C18-OH cartridge onto the top of the SCX column using an adapter.
 - f. Connect a 60-mL disposable syringe barrel onto the top of the tandem cartridge system, using an adapter and stopcock. Add 40 mL of 50% methanol in water into the syringe and pull the solution through the tandem cartridge (1 drop/sec). Discard the effluent.
 - g. Remove the top part (60-mL syringe and C18-OH cartridge) and wash the SCX cartridge with 5 mL of methanol, followed by 5 mL of high purity water and 5 mL phosphate buffer pH 3.5. Discard the wash.
 - h. Using an adapter attach a clean 10-mL syringe to the top of SCX cartridge (use syringe with Luer Slip Tip to ensure proper fit) and add 10 mL of pH 6.5 phosphate buffer. With the syringe plunger, force the solution through the packing (1 drop/sec), collecting the eluate in a 250-mL separatory funnel containing 50 mL of 0.1 N HCl.
 - i. Add 25 mL of methylene chloride to the separatory funnel, stopper, and shake vigorously for 1 min.
 - j. Let the layers separate and draw off the bottom layer into a 200-mL evaporation flask. Repeat the extraction with two 25-mL portion of methylene chloride. Evaporate the combined methylene chloride extract to dryness. Re-evaporate with 20 mL methanol.
 - k. Dissolve the residue in exactly 5 mL of Working Marker Standard Solution (0.20 mcg of CL263,499/mL water).
 - l. Inject about 0.5 mL into HPLC equipped with 200-mL loop (see 13, Notes on the Method).
- J. Liquid Chromatography(see 5 to 8, Notes on the Method)

Ascertain \ln/\ln (power curve) regression equation of the five working Fortification/HPLC standard without marker (0.01, 0.02, 0.05, 0.10, 0.25 mcg/mL) as described in the method (see K., Calculations). Inject a 200-mL aliquot of each sample solution containing 0.20 mcg/mL CL 263,499 then the HPLC Working

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standard (Interspersed Standard). Evaluate the ratio of response of the marker in the sample and standard solution (Peak HT. of the marker in the sample/Peak HT of the marker in the standard). Samples for which this ratio differs by more than 10 % must be reinjected. Repeat the sequence of four sample injections followed by a standard until all the samples have been injected. Evaluate Drift Index {Drift Index = [(Marker Response in Interspersed Standard)(100)/(Marker average Response in the Interspersed Standard)]} and Deviated Drift Index (DDI) (DDI = |Drift Index - 100|). If DDI is more than 10% in the groups surrounded by Interspersed Standard, repeat the injection of the five Working Fortification/HPLC standards and ascertain ln/ln regression equation for those groups of samples. Similarly, sample groups surrounded by standard injections that indicate Retention Time drift of greater than 10 % should be reinjected. The response difference between the same concentration of the Working Standards with or without marker should not exceed 5%.

K. Calculations(see 9 to 11, Notes on the Method)

1. Determine the natural logarithms (ln) of the concentration (ln C) and the response (ln R) for each of the Working Fortification/HPLC standard solutions in the calibration set.
2. Calculate the slope and intercept of the equation by linear regression of ln R versus ln C (equation 1)

$$(EQ. 1) \ln R = RI \ln C + \ln UR$$

where:

ln R = ln Response in Integrator Units

ln C = ln Concentration of the Working Fortification/HPLC standard solutions mcg/mL

RI = Response Index (Slope of ln/ln regression curve)

ln UR = ln Unit response (Intercept of the ln/ln regression curve).

3. Calculate the response expected at each standard concentration from the regression equation using Equation 2.

$$(EQ.2) R = e [RI \ln C + \ln UR]$$

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4. Compare the observed response with the expected response at each concentration; the two values should agree to within 10%.
5. Calculate CL 263,222 concentration in each of the unknown samples as follows:
 - a. Calculate the CL 263,222 concentration (ppm) in each of the processed sample extracts using Equation 3:
 - b. Calculate the concentration of CL 263,222 in the original sample as follows:

(EQ.3)

$$C = e^{[RI \ln C + \ln UR]}$$

(EQ.4)

$$\text{ppb} = \frac{(C) \times (V1) \times (V3) \times (V5) \times (DF) \times 1000 \text{ (uncorrected)}}{(W) \times (V2) \times (V4)}$$

Where:

C = Concentration (mcg/mL) of the CL 263,222 in the processed sample

W = Weight of sample taken for analysis in grams (25 g).

V1 = Volume in mL of extracting solvent (100 mL)

V2 = Volume in mL of extract taken for analysis (80 mL)

V3 = Volume in mL of final solution used for HPLC analysis (5 mL).

V4 = Volume in mL of sample solution injected (200 mL)

V5 = Volume in mL of standard solution injected (200 mL)

D.F. = Dilution Factor (see 9, Notes on the Method)

$$6. \quad \text{ppb (dry weight basis)} = \text{Uncorrected ppb} \times \frac{100}{(100 - \% \text{ moisture})}$$

Figure 1 demonstrates typical chromatograms of 0 (control soil), 5, 20, 80 and 200 ppb fortified control soil.

L. Notes on the Method

1. Process soil samples from extraction to completion of elution without interruption. Inject the extracted samples to the HPLC in the same day or a day after. If the samples are injected the next day, they must be stored in a refrigerator.

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2. When analyzing samples in pairs, use bottles of approximate equal weight so that balancing of the bottles will not be required in the centrifuging step.
3. In solid phase extraction do not let any of the SPE cartridges get dried.
4. In the methylene-chloride partitioning step (solid phase extraction step J.), allow about 10 minutes for the layers to separate. If necessary, use a disposable 9-inch pasteur pipet to break up the emulsion.
5. For the HPLC mobile phase, the acetonitrile/water ratio may be varied from 10/90 to 30/70 in order to obtain optimum chromatography with regard to peak shape and resolution from sample background peaks. The formic acid content also can be varied from 1 to 1.5% according to chromatographic condition. The addition of 0.1-0.3% triethylamine to the mobile phase can sometimes be helpful in resolving the peak of interest from the background peaks, however sometimes it causes base-line instability, especially in conjunction with higher concentration of formic acid.
6. Poorly shaped HPLC peaks may be due to guard column or analytical column contamination. Guard columns should always be used and replaced as necessary. Poor peak shapes can also occur when using contaminated mobile phase.
7. Chromatographic response may be measured either using an electronic integrator or a millimeter ruler. When using the latter, record response as peak height in millimeters times attenuation.
8. Some soil matrixes suppress HPLC response of the compound when the specified final volume of 5.0 mL is used. If the recovery values obtained for a particular soil are low, increase the final volume to exactly 10 mL for that soil type.
9. Linearity and range are markedly improved by the ln/ln transformation (also known as the "power curve") described by Dorschel, et al., Analytical Chemistry (1989), 61: 951A-968A, particularly at the low end of the curve. The calculations described here are based on that transformation and are most conveniently carried out using a computer-based spreadsheet such as LOTUS 1-2-3. They may also be carried out on a scientific calculator (such as the HEWLETT PACKARD 27S) which is equipped to convert to and from LOG or LN functions and to do linear regressions of logarithmic functions. The following series of steps provide an example of how to calculate the above values with the Hewlett-Packard 27S Scientific hand-calculator.

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EXAMPLE**Procedure For Power-Curve Equation**

<u>Key Commands</u>	<u>Function</u>
CLR	(turn calculator on)
STAT	(enters into statistics mode)
GET	(options)
NEW	(accesses new list)
CLEAR DATA	(options current list)
YES	(clear current list)
0.01 INPUT	(enter the concentrations of the Working Fortification/HPLC standards)
0.02 INPUT	
0.05 INPUT	
0.08 INPUT	
0.25 INPUT	
EXIT	(exits list)
NAME	(choose a name for X axis)
CONC INPUT	(the X-variable was named CONC)
GET	
NEW	
293 INPUT	(enter the variable response values of the standards)(the listed values are examples here)
660 INPUT	
1644 INPUT	
2652 INPUT	
8270 INPUT	
EXIT	(exits list)
NAME	(choose a name for the Y axis)
RESP INPUT	(the Y-intercept was named RESP)
CALC	(allows for calculations to be performed)
MORE	(provides more calculation options)
FRCST	(displays a series of functions)
CONC	(selects CONC list as x-variables)
RESP	(selects RESP list as y-variables)
MORE	(options)
MODL	(options for type of regression)
PWR	(selects and performs power-curve as regression method)
M	(display slope = 1.03017)
B	(display y-intercept = 35391.3258)

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If the sample, for example has response 588 (Peak HT.), using equation 3 and 4 we do the following calculation;

$$c = e^{[(\ln 588 - \ln 35391.3258) / 1.03017]} = 0.01873$$

$$\text{ppb} = \frac{0.01873 \times 100 \times 5 \times 0.2 \times 1}{25 \times 80 \times 0.2} \times 1000 = 4.7$$

10. Dilution Factor (D) = 1 unless the chromatographic response exceeds that of the most concentrated calibration standard. If it is necessary to make an appropriate dilution of the sample with water to obtain a response within the range of the working standard solutions, include the dilution factor in the equation 4.
11. Other calibration methods such as bracketing standards may be used; in that situation, prepare a series of standard solutions with the same concentration as described in section D.1.b., with the exception that all containing the same amount of marker (0.2 mcg/mL). Inject a 200-mcL aliquot of the HPLC Working Standards (with the marker) which most nearly approximates samples response followed by 200-mcL aliquots of each of four sample solutions and the standard. Repeat this sequence until all the samples have been injected.
12. Working Marker Standard solution can be made in any concentration between 0.1-0.33 mcg/mL.
13. For higher sensitivity, a 500-mcL loop could be used. A problem may arise from an interfering peak which originates from methylene chloride and elutes very close to the peak of interest. If the presence of this peak is significant either with a 200 or 500-mcL loop, replacing a portion of the acetonitrile with methanol may resolve the problem.
14. Other markers such as CL 243,997 {[nicotinic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-], analytical grade, American Cyanamid Company, Agricultural Research Division, Princeton, NJ may be used if there is chromatography problem or interference with CL 263,499.

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