CYANAMID

Approved John E Boyd

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R. Tondreau/rt
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AMERICAN CYANAMID COMPANY
AGRICULTURAL RESEARCH DIVISION
CHEMICAL DEVELOPMENT
P. O. Box 400
Princeton, NJ 08540 USA

Recommended Method of Analysis

PURSUIT® Herbicide (AC 263,499): Determination of AC 263,499 Residues in Soil by HPLC

A. Principle

AC 263,499 is extracted from soil with 0.5N sodium hydroxide. The pH is adjusted to 2, the resulting precipitate is filtered and the compound is partitioned from the filtrate into methylene chloride. The methylene chloride is evaporated and further cleanup is achieved by passing an alkaline solution of the compound through a C-18 solid phase extraction column. The effluent from the C-18 column is acidified and the compound extracted from the acid solution using a strong cationic exchange SPE column. After eluting the compound from the column with pH 6.5 phosphate buffer, the compound is partitioned from acid solution into methylene chloride and concentrated for measurement by 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/minutes (Kratos Spectroflow 400).

2. Detector: UV photometer set at 254 nanometers (Kratos Model 783).

3. Column: Supelcosil LC-8-DB reverse phase C-8 5-micron 15 cm X 4.6 mm ID deactivated for bases (Supelco Cat. Number 5-8347).

4. Guard Column: Fitted with an LC-8-DB cartridge (Supelguard Brand, Supelco Cat. Number 5-9053).

5. Sample Injector: Equipped with a 500-mcL loop (Rheodyne Model 7125).

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6. Bottles: Wide-mouth, high density polyethylene, 16-ounce capacity (Nalgene Cat No. 2104).

7. Reciprocating Shaker: Equipped to hold 16-ounce bottles horizontally (A. H. Thomas Company, Number 8291-510).

8. Centrifuge: Equipped with a head for 500-mL bottles (Damon Model CU-5000, IEC Division, Needham, Massachusetts).

9. pH Meter: Orion Model 701A.

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: 9.0 cm (Whatman Number 40).

12. Separatory Funnels: 250- and 500-mL capacity.

13. Evaporation Flasks: 100- and 1,000-mL capacity.

14. General Laboratory Glassware: Assorted beakers, graduated cylinders and volumetric pipets.

15. Rotary Evaporator: Buchler Instruments equipped with a heated water bath at 35°C.

16. Solid Phase Extraction Processing Station: (Vac-Elut Brand, Analytichem International).

17. SPE Columns: Octadecyl (C-18), 500 mg in 3 mL tubes (J. T. Baker Chemical Company, Number 7020-3).

18. SPE Columns: Strong Cation Exchange (SCX) 500 mg in 3-mL tubes (Analytichem International, Number 617303).

19. SPE Adaptors: Luer to SPE Column (Analytichem International, Number 636001).

20. Disposable Syringes: 30-mL capacity, (Plastipak, Becton-Dickinson, Rutherford, New Jersey).

C. Reagents

1. Analytical Standard: AC 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, New Jersey.

2. Solvents: High purity, Burdick and Jackson brand (American Burdick and Jackson, Muskegon, Michigan).

a. Methylene Chloride b. Methyl Alcohol

3. Water, High Purity: Distilled or deionized.

4. Sodium Hydroxide: 50% solution, (J. T. Baker Chemical Company, Number 3727-1).

5. Hydrochloric Acid: Concentrated, Reagent Grade, (J. T. Baker Chemical Company, Number 9535-1).

6. Acetic Acid, Glacial: (J. T. Baker Chemical Company, Number 9507-1).

7. Potassium Phosphate, Dibasic: Powder, (J. T. Baker Chemical Company, Number 3252-1).

8. Extraction Solvent, 0.5N Sodium Hydroxide: Dilute 160 grams of 50% sodium hydroxide solution to 4 liters with high-purity water.

9. Hydrochloric Acid, 6N: Mix equal volumes of concentrated HCl and high-purity water.

10. Hydrochloric Acid 0.1N: Dilute 8.3 mL of concentrated HCL to one liter with high-purity water.

11. Sodium Hydroxide 0.1N: Dilute 8 grams of 50% sodium hydroxide to 1,000 mL with high-purity water.

12. Buffer Solution: Dissolve 50 grams of dibasic potassium phosphate in one liter of high-purity water and adjust to pH 6.5 ± 0.1 with 6N HCl.

13. HPLC Mobile Phase: Measure 400 mL of methanol into a 1,000-mL volumetric flask, add 40 mL of glacial acetic acid and dilute to the mark with high-purity water.

14. Filter Aid: (Celite 545 Johns-Manville Corporation).

D. Preparation of Standard Solutions

1. Stock Solution

Accurately weigh 100 mg ± 0.10 mg of AC 263,499 analytical standard into a 100-mL volumetric flask, dissolve the material in 5 mL of acetone and dilute to the mark with high-purity water. This solution contains 1,000 mcg of AC 263,499/ mL.

2. Intermediate Solution

Pipet 10 mL of the stock solution into a 100-mL volumetric flask and dilute to the mark with high-purity water. This solution contains 100 mcg of AC 263,499/mL and is designated the Intermediate Standard Solution.

3. HPLC Working Standards

Pipet a 1-mL aliquot of the Intermediate Standard Solution into a 100-mL volumetric flask and dilute to the mark with high-purity water. This solution contains 1.00 mcg of AC 263,499/mL.

Pipet a 1-mL aliquot of the Intermediate Standard Solution into a 200-mL volumetric flask and dilute to the mark with high-purity water. This solution contains 0.50 mcg of AC 263,499/mL.

Pipet a 10-mL aliquot of the 0.50 mcg/mL solution into a 100-ml volumetric flask and dilute to the mark with high-purity water. This solution contains 0.05 mcg of AC 263,499/mL.

4. Fortification Solutions

For purposes of fortification of soil samples for recovery studies, use standard solutions in water such that a 1-mL aliquot of solution added to 50 grams of soil will yield the desired fortification level. For example, 1 mL of the 0.50 mcg/mL HPLC Working Standard added to 50 grams of soil will yield a 10 ppb fortification level.

E. Liquid Chromatographic Conditions

Set up the instrument for isocratic operation at a flow rate of 1.5 to 2.0 mL/minute. Set the UV detector at 254 nanometers. Instrument sensitivity should be set so that a 500-mcL injection of the 0.05 mcg/mL standard gives a chromatographic peak height of about 30 ± 10 millimeters or the equivalent in integrator units. The retention time for AC 263,499 is about 4 to 7 minutes using these operating conditions.

F. Linearity Check

Check linearity of chromatograph response by obtaining chromatograms of the three Working Standards, recording response as peak height (see Note M.5). Plot responses versus concentration. Significant departure from a straight line plot indicates faulty standard preparation or instrument malfunction which must be corrected before analyzing samples.

G. Recovery Studies

The ability of the analyst to perform the procedures must be demonstrated on fortified control soil prior to analysis of unknown samples. Pipet a 1-mL aliquot of fortification standard onto a 50-gram sample of control soil and mix the sample thoroughly. Let the sample stand for at least 15 minutes before proceeding with the analysis described in Section J.

H. Sample Preparation

1. Allow frozen soil samples to thaw completely in an airtight container just before analyzing.

2. Mix the thawed soil thoroughly and remove large stones and vegetation to obtain a homogeneous sample.

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I. Moisture Determination

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

J. Sample Analysis (See Note M.1.)

1. Extraction

a. Accurately weigh 50 ± 0.1 g of soil into a 16-ounce wide-mouth polyethylene bottle (see Note M.2.).

b. Measure exactly, in a 250-mL graduated cylinder, 200 mL of 0.5N sodium hydroxide and pour the solvent into the bottle containing the sample. Stopper the bottle and shake the contents for one hour on a reciprocating shaker.

c. Centrifuge the sample at 2,500 ± 100 rpm for 10 minutes.

d. Decant exactly 150-mL of the supernatant extract and transfer the solution to a 400-mL beaker.

e. Adjust the sample extract to pH 1.7 to 2.0 with 6N hydrochloric acid.

f. Add 10 ± 1 gram of Celite 545 to the beaker, stir the contents and filter the mixture by suction through a Whatman Number 40 filter paper.

g. Wash the beaker and filter cake with two 25-mL portions of 0.1N hydrochloric acid.

2. Partitioning (See Note M.3.)

a. Transfer the filtrate to a 500-mL separatory funnel. Add 200 mL of methylene chloride, stopper the funnel and shake the contents vigorously for 30 seconds. Let the layers separate for about 5 minutes; with most soils, the lower layer will not clear but will consist of an emulsion.

b. Draw off the lower layer into a 250-mL separatory funnel, add 5 to 10 mL of methanol, stopper and invert the funnel to mix the contents. Allow the phases to separate for about 5 minutes and draw off the clear bottom layer into a 1,000-mL evaporation flask. Do not draw off the interfacial emulsion into the flask but return it along with the upper layer to the original 500-mL separatory funnel.

c. Repeat the extraction of the aqueous phase with two additional 100-mL portions of methylene chloride. Follow the procedure described in Steps J.2.a and J. 2.b, including the addition of 5 to 10 mL of methanol each time to break the emulsion.

d. Evaporate the methylene chloride sample solution down to about 10 mL on a rotary evaporator and transfer the sample to a 100-mL evaporation flask, using 10 to 20 mL of methylene chloride to effect complete transfer.

e. Evaporate the solution to dryness.

3. Solid Phase Extraction

a. Condition a C-18 solid phase extraction column by washing with 3 mL of methanol followed by 3 mL of water. Prepare an aromatic sulfonic acid (SCX) column by washing with 3 mL of water.

b. By means of a Luer-to-SPE adaptor, attach a 30-mL syringe barrel to the top of each column for use as reservoirs.

c. With no vacuum applied, place the SCX unit on the SPE processing unit and add 10 mL of 0.1N hydrochloric acid to the reservoir.

d. Dissolve the sample in 5 mL of 0.1N sodium hydroxide and pour this solution into the reservoir of the C-18 column. Using the syringe plunger, force the sample solution through the C-18 column at a rate of 4 to 5 drops/second and collect the eluate in the SCX reservoir containing the hydrochloric acid. Wash the sample flask with two 5-mL portions of high-purity water pass these washes through the C-18 column and into the SCX reservoir.

e. Apply vacuum to the SPE processing station, alloving the effluent to go to waste. After the solution has passed completely through the SCX column, remove the reservoir and wash the column with 3 mL of methanol followed by 2 mL of high-purity water. Remove the column from the processing station.

f. Place a clean 30-mL syringe barrel on to the SCX column and add 10 mL of pH 6.5 phosphate buffer. With the syringe plunger, force the solution through the packing, collecting the eluate in a separatory funnel containing 50 mL of 0.1N hydrochloric acid. Add 5 mL of methanol to the reservoir and force this solvent through the packing, collecting the eluate in the separatory funnel.

g. Add 25 mL of methylene chloride to the separatory funnel, stopper, and shake the contents vigorously for 30 seconds. Let the layers separate and drav off the bottom layer into a 100-mL evaporation flask. Repeat the extraction with an additional 25-mL portion of methylene chloride. Evaporate the combined methylene chloride layers to dryness and dissolve the residue in exactly 5.0 mL (see Note M.9.) of high-purity water.

K. Liquid Chromatography (See Notes M.4. to M.6.)

Just before the analysis of samples, inject the three Working Standard Solutions and ascertain linearity of response. Inject duplicate 500-mcL aliquots each of two sample solutions (four injections), then one of the standard solutions to check for drift in chromatographic response. Repeat the sequence of four sample injections followed by a standard until all the samples have been injected; use a different standard for each of these checks. Samples for which the duplicate injections differ by more than 10% should be reinjected. Similarly, sample groups surrounding standard injections that indicate response drift of greater than 10% should be reinjected. Average the responses obtained for each sample and calculate the AC 263,499 content of the original sample according to the following section.

L. Calculations

Calculate the AC 263,499 content of the original sample, uncorrected for sample moisture, using Equation 1 and that Standard Solution which most nearly approximates sample response. Calculate the AC 263,499 content on a dry weight basis using Equation 2.

$$(1)\ \text{ppb AC 263,499}_{\text{(uncorrected)}} = \frac{R(SAM) \times C \times V1 \times V3 \times V5 \times D.F. \times 1{,}000}{R(STD) \times W \times V2 \times V4}$$

Where: R(SAM) = Sample response (millimeters or integrator units).

R(STD) = Standard response (millimeters or integrator units).

V1 = Volume of extraction solvent (mL).

V2 = Aliquot of extraction solvent used for analysis (mL).

V3 = Volume of sample solution used for HPLC (mL).

V4 = Volume of sample solution injected (mcL).

V5 = Volume of standard solution injected (mcL).

C(STD) = Concentration of AC 263,499 in working standard (mcg/mL).

W = Sample weight (grams).

D.F. = Dilution Factor (see Note M.8.).

$$(2)\ \text{ppb}_{\text{(dry weight basis)}} = \text{Uncorreted ppb} \times \frac{100}{(100 - \%\ \text{moisture})}$$

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M. Notes on the Method

1. Process soil samples from extraction to completion of elution without interruption. Sample solutions for HPLC are stable for at least one week if stored at room temperature in tightly stoppered containers.

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 the methylene chloride partitioning step (Section J.2.), transferring the bottom layer to another separatory funnel and addition of methanol is a means of breaking up emulsions which result with most soils. Follow the procedure as described whether emulsions form or not.

4. For the HPLC mobile phase, the methanol:water ratio may be varied from 30:70 to 40:60 in order to obtain optimum chromatography with regards to peak shape and resolution from sample background peaks. The acetic acid content should be held at 4%. The addition of 0.1% triethylamine to the mobile phase can sometimes be helpful in resolving the peak of interest from background peaks.

5. 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. If peak shape does not improve after changing a guard column, prepare a fresh batch of mobile phase.

6. Chromatographic peak heights may be measured either using an electronic integrator or a millimeter rule. When using the latter, record response as peak height in millimeters times attenuation.

7. If the chromatographic response exceeds that of the most concentrated calibration standard, 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 calculations as described in Note M.8.

8. Dilution Factor (D.F.) = 1 unless sample response exceeds the range covered by the Working Standard Solutions and it is necessary to dilute the sample solution further with water by a factor of D times to obtain a response within the range of the standards.

9. Some soil matrices suppress HPLC response of the compound when the specified final volume of 5.0 mL is used. If recovery values for a particular soil average below 75%, increase the final volume to exactly 10.0 mL for that soil type. If preferred and the available equipment is adequately sensitive, it is acceptable to use the 10.0 mL final volume for all soil types.

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