AMERICAN CYANAMID COMPANY
AGRICULTURAL PRODUCTS RESEARCH DIVISION
HUMAN AND ENVIRONMENTAL SAFETY
P.O. BOX 400
PRINCETON, NEW JERSEY 08543-0400

Recommended Method of Analysis - M 2455.01

CL 354,825: HPLC Method for the Determination of Residues of CL 354,825 in Soil.

A. Principle

Residue of CL 354,825, a soil metabolite of CL 299,263, is extracted from soil with 0.5 N NaOH. The extract is initially cleaned-up by acidic precipitation and Celite filtration. The extract is then fractionated by C18 solid phase extraction (SPE). Final clean-up of CL 354,825 is achieved by SCX-SPE and methylene chloride partitioning. The resulting sample is analyzed by HPLC on a C8 reverse-phase column with detection by UV absorbance at 335 nm. Results are calculated by comparison of the peak response of the peak of interest with an external CL 354,825 standard. The validated sensitivity (LOQ, limit of quantitation) of this method is 5 ppb.

B. Reagents

This is a list of suggested reagents. Items from other suppliers that have been shown to be functionally equivalent may be substituted.

1. Analytical Standards: Analytical grade of known purity. American Cyanamid Company, Agricultural Products Research Division, P.O. Box 400, Princeton, New Jersey, 08543-0400.

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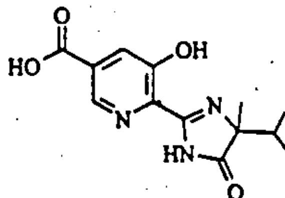
NOTE: This method supersedes M2455 and is procedurally similar to M2455 except for the minor procedural adjustments which are summarized on page 17.

a. CL 354,825:

Nicotinic acid, 5-hydroxy-6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-



2. Solvents:

B & J Brand High Purity Solvent, Baxter, Burdick and Jackson, Muskegon, MI.

a. Methanol, Catalog # 230-4
b. Acetonitrile UV, Catalog # 015-4
c. Methylene Chloride, Catalog # 300-4
d. Hexane UV, Catalog # 216-4

3. Chemicals: (ACS or "Analyzed" Grade)

Baker "Analyzed" Reagents, J.T. Baker, Inc.

a. Sodium hydroxide, J.T. Baker # 3722-01
b. Hydrochloric acid, concentrated, J.T. Baker # 9535-01
c. Potassium phosphate, monobasic, J.T. Baker # 3246-01
d. Potassium phosphate, dibasic, J.T. Baker # 3252-01
e. Potassium chloride, J.T. Baker # 3040-01

Other Suppliers

f. Formic acid, 98%, Fluka Chemika #06440.
g. Celite 545, acid washed filter aid, Johns-Manville Corp.

4. Water, Purified: Milli-Q UV Plus water system fitted with ion exchange, carbon and Organex-Q cartridges, Millipore, Bedford, MA.

5. HPLC Mobile Phase: 32% Acetonitrile/66% water/2% formic acid. Mix 320 mL acetonitrile, 660 mL purified water and 20 mL formic acid. Filter the mixture through a 0.45 micron microporous HPLC solvent filter (Gelman FP-Vericel).

6. Reagent Solutions:

All reagent solutions should be stored at room temperature in borosilicate glass reagent bottles unless noted otherwise. Larger or smaller amounts of these solutions can be made by adjusting the volumes and amounts proportionately.

a. 10 N NaOH. Carefully add and dissolve 400 grams of solid sodium hydroxide in about 600 mL of purified water using a magnetic stirrer. Caution, this solution becomes very hot. Let the solution cool and dilute to 1 liter with purified water. Store this solution in a high-density polyethylene (HDPE) bottle.

b. 0.5 N NaOH. Dilute 50 mL of 10 N NaOH to 1 liter with purified water. Store this solution in a high-density polyethylene (HDPE) bottle.

c. 6 N HCl. Slowly add 100 mL concentrated hydrochloric acid to 100 mL purified water that is being stirred in a glass beaker on a magnetic stirrer. Caution, this solution will become hot. Let cool and dilute to 200 mL.

d. 0.024 N HCl. Mix 2.0 mL concentrated HCl with 998 mL purified water.

e. 5% Methanol/0.024 N HCl. Mix 50 mL methanol with 950 mL 0.024 N HCL.

f. 1 M Potassium phosphate monobasic. Dissolve 27.2 g of potassium phosphate monobasic in about 150 mL purified water and dilute to 200 mL.

g. 1 M Potassium phosphate dibasic. Dissolve 34.8 g of potassium phosphate dibasic in about 150 mL purified water and dilute to 200 mL.

h. 100 mM Potassium phosphate monobasic. Dilute 50 mL 1 M potassium phosphate monobasic to 500 mL with purified water.

i. 100 mM Potassium phosphate dibasic. Dilute 50 mL 1 M potassium phosphate dibasic to 500 mL with purified water.

j. 100 mM Potassium phosphate, pH 5.0. Place 200 mL of 100 mM potassium phosphate monobasic in a beaker with magnetic stirring and titrate to pH 5.0 with 100 mM potassium phosphate dibasic using a pH meter.

k. 1% (v/v) Formic acid in water. Mix 5 mL formic acid (98%) with 495 mL purified water.

l. 10% (v/v) Methanol/Water. Mix 100 mL methanol with 900 mL purified water.

m. 20% (v/v) Methanol/Water. Mix 200 mL methanol with 800 mL purified water.

n. 0.5% (v/v) Formic acid in methylene chloride. Mix 1 mL of formic acid (98%) with 200 mL methylene chloride.

o. Saturated potassium chloride in methanol. Mix about 20 g of potassium chloride with 400 mL of methanol in a glass reagent bottle, cap and shake by repeated inversion for several minutes. Leave the mixture in the capped bottle for at least 2 hours and shake occasionally. There should be excess undissolved salt in the bottle. The solution should be removed without disturbing the salt. As the solution is used, additional methanol and/or potassium chloride can be added and saturated by shaking.

C. Apparatus and Supplies

This is a list of suggested apparatus and supplies. Items, from other suppliers, that have been shown to be functionally equivalent may be substituted.

1. Liquid Chromatograph: Hewlett Packard Series 1050 Modular HPLC System with Model 79852A Quaternary Pump with Column Oven Module, Model G1303A On-Line Degasser, Model 79853C Variable Wavelength Detector and Model 79855A Autosampler.

2. HPLC Column and Guard Column: Waters Symmetry C8, 4.6 mm I.D. x 150 mm length cartridge column with an integrated Symmetry C8 guard column, Waters, Milford MA.

3. Data System: Hewlett Packard HPLC 3D Chemstation Software Version G1307A Rev.A.02.02 on an HP Vectra 486/33 Computer Model 240-3.

4. Balance, Analytical: Mettler Model AT261, precision ± 0.05 mg.

5. Balance, Top-Loading: Sartorius Model L610D, precision ± 0.01 g.

6. Centrifuge: Sorvall RC-SC Plus centrifuge with GSA rotor for 250 mL bottles, Dupont . The centrifuge must be capable of applying approximately 5000 x G average to fully loaded 250-mL bottles.

7. Reciprocating Shaker: Eberbach Model 6010, VWR Scientific. This shaker produces a 1-1/2 inch throw and 280 oscillations/minute.

8. Rotary Evaporator: Büchi, Model RE 121 fitted with dry-ice finger condenser and Model 461 Water Bath. Brinkmann Instruments.

9. SPE Vacuum Manifold: IST, International Sorbent Technologies Ltd. VacMaster 10, Catalog Number 121-1016, fitted with PTFE Stopcock/Needles, Catalog Number 121-0001. Distributor, Jones Chromatography, Lakewood, CO. (303) 989-9200

10. Magnetic Stirrer. Thermolyne, Model Nuova II. 800 279-6294

11. Digital pH Meter. Orion digital ionalyzer, Model 501, fitted with a Beckman Futura Plus gel-filled combination electrode, Beckman part number 39846.

12. Laboratory Glassware: 1 liter, 250-mL and 100-mL graduated cylinders; 100-mL beakers; 100-mL volumetric flasks, 125-mL separatory funnels; 250-mL side-arm filtering flasks; 100-mL pear-shaped vacuum flasks with ground glass joints; 1-mL, 5-mL and 10-mL Class B or better pipets. All glassware should be made of borosilicate glass such as Corning Pyrex or Kimble Kimax glass. Rinse all glassware with methanol and air dry before use (see Method Note C.12, Section N).

13. Luer Stopcocks: Part number AI-121310-05, Varian Associates.

14. Silicone Tubing: 1/8 inch ID x 1/4 inch OD medical grade silicone tubing, Baxter.

15. HPLC Solvent Preparation and Delivery System: Ultraware, Kontes.

16. Microporous Disk Filters: FP-Vericel membrane filter, 47 mm, 0.45 micron, HPLC Certified, Gelman Sciences, Inc.

17. Autoinjector Vials: Crimp seal type vials (1-mL nominal), teflon/silicone/teflon type septa (11 mm) sealed with hand type crimper, Supelco, Inc.

18. Centrifuge Bottles. 250-mL polypropylene centrifuge bottles, Nalgene.

19. Plastic Syringe, Disposable: Luer-Lok, 10-mL capacity, Becton Dickinson.

20. Glass Syringe: Luer Metal Tip Syringe, 20-mL capacity, Popper and Sons, Inc. New Hyde Park, New York.

21. SPE Column Adapters: IST, International Sorbent Technologies Ltd. PTFE Adapters, Catalog Number 120-1100. Distributor, Jones Chromatography, Lakewood, CO.

22. Solid Phase Extraction Cartridges:

    a. C18. Waters Sep-Pak Plus Environmental C18 Cartridge, Catalog Number 23635.

    b. SCX. IST, International Sorbent Technology, Isolute SCX Column, 1g/6 mL, Catalog Number 530-0100-C, Distributor, Jones Chromatography, Lakewood, CO. Price 123.50

23. Reservoirs, Disposable: IST, International Sorbent Technology, 70-mL capacity, Catalog Number 120-1008F. Distributor, Jones Chromatography, Lakewood, CO.

24. HPLC Injection Syringe: Hamilton, Gastight Model 1000 Series, 100-mcL capacity.

25. Filter Funnel: Whatman disposable 934-AH glass fiber filter funnel, Catalog Number 1920-1827, Whatman International Ltd.

26. Magnetic Stir Bars: Teflon Coated, 7/8 inch.

D. Preparation of Standard Solutions

All of the standard solutions listed in this section should be stored in amber bottles at about 4°C and are stable for one month past the preparation date of the stock solution.

1. Stock Standard Solution:

   a. CL 354,825 Stock. Weigh accurately (to the nearest tenth of a mg) approximately 10 mg of CL 354,825 standard and place the known amount into a 100-mL volumetric flask. Add 50 mL of methanol and agitate by swirling to dissolve the solid. If the solid does not dissolve completely, sonicate the flask in a room temperature sonication bath and/or warm the flask slightly in warm tap water and agitate by swirling. When the solid is dissolved, dilute to the flask mark with purified water, stopper and invert the flask to mix the solution. Transfer the solution to a clean glass bottle. After correcting for standard purity, calculate, record and label the bottle with the exact concentration of CL 354,825 to three significant figures.

2. Fortification Standard Solutions:

   a. Fortification Standard A. Pipet into a 100-mL volumetric flask a volume of the CL 354,825 Stock Standard (D.1.a) to deliver 2500 mcg of standard. Dilute to the flask mark with 10% Methanol/Water, stopper and mix by inversion. Transfer the solution to a small amber glass bottle and label with solution identification and concentration (25.0 mcg/mL CL 354,825).

   b. Fortification Standard B. Pipet into a 100-mL volumetric flask 10 mL of the Fortification Standard A (D.2.a). Dilute to the flask mark with 10% Methanol/ Water, stopper and mix by inversion. Transfer the solution to a small amber glass bottle and label with solution identification and concentration (2.5 mcg/mL CL 354,825).

   c. Fortification Standard C. Pipet into a 100-mL volumetric flask 10 mL of the Fortification Standard B (D.2.b). Dilute to the flask mark with 10% Methanol/ Water, stopper and mix by inversion. Transfer the solution to a small amber

glass bottle and label with solution identification and concentration (0.25 mcg/mL CL 354,825).

3. Chromatography Standard Solutions:

   a. Chromatography Standard A. Pipet into a 100-mL volumetric flask a volume of the CL 354,825 Stock Standard (D.1.a) to deliver 1000 mcg of standard. Dilute to the flask mark with 10% Methanol/Water, stopper and mix by inversion. Transfer the solution to a small amber glass bottle and label with solution identification and concentration (10.0 mcg/mL CL 354,825).

   b. Chromatography Standard B. Pipet into a 100-mL volumetric flask 10 mL of the Chromatography Standard A (D.3.a). Dilute to the flask mark with 10% Methanol/Water, stopper and mix by inversion. Transfer the solution to a small amber glass bottle and label with solution identification and concentration (1.0 mcg/mL CL 354,825).

   c. Chromatography Standard C. Pipet into a 100-mL volumetric flask 2.5 mL of the Chromatography Standard B (D.3.b). Dilute to the flask mark with 10% Methanol/Water, stopper and mix by inversion. Transfer the solution to a small amber glass bottle and label with solution identification and concentration (0.025 mcg/mL CL 354,825).

   d. Chromatography Standard D. Pipet into a 100-mL volumetric flask 5 mL of the Chromatography Standard B (D.3.b). Dilute to the flask mark with 10% Methanol/Water, stopper and mix by inversion. Transfer the solution to a small amber glass bottle and label with solution identification and concentration (0.05 mcg/mL CL 354,825).

   e. Chromatography Standard E. Pipet into a 100-mL volumetric flask 10 mL of the Chromatography Standard B (D.3.b). Dilute to the flask mark with 10% Methanol/Water, stopper and mix by inversion. Transfer the solution to a small amber glass bottle and label with solution identification and concentration (0.10 mcg/mL CL 354,825).

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E. HPLC Conditions

Operating conditions described below are provided for use as a guide in establishing actual operating conditions and should be adjusted as necessary to obtain peak shape and resolution from background peaks that are equivalent to or better than the chromatograms shown in Figure 2. If an interfering absorbance peak that elutes at the same retention time as CL 354,825 is found in a control soil sample, an alternate HPLC mobile phase may be used for analysis. An alternate mobile phase is suggested in the Method Notes, Section N.

1. Instrument:

   a. HPLC Pump. Hewlett Packard Series 1050 Liquid Chromatograph.

   b. Detector. Hewlett Packard Series 1050 Variable Wavelength Detector.

   c. Data System. Hewlett Packard HPLC 3D Chemstation Software Version G1307A Rev.A.02.02 on an HP Vectra 486/33 Computer.

   d. Autoinjector. Hewlett Packard Series 1050 Autosampler.

   e. Column Oven. Hewlett Packard Series 1050.

2. Column: Waters Symmetry C8, 4.6 mm I.D. x 150 mm length cartridge column with an integrated Symmetry C8 guard column.

3. Instrument Conditions:

a.	Mobile Phase. (Isocratic)	Acetonitrile/Water/Formic acid 32:66:2 v/v/v
b.	Flow Rate.	1 mL/minute
c.	Column Temperature.	30°C controlled with column heater
d.	Injection Volume.	100 microliter, autoinjector or manual loop injector
e.	Detector Wavelength.	335 nm
f.	Integration Parameters.	
	Integration Start	5 minutes
	Attenuation	Approx. 3 milliabsorbance units full scale
	Peak Width	0.2 minutes
	Threshold	-5.0
	Offset	10% of full scale
	Stop Time	10 minutes
	Injection Delay	6 minutes
g.	Retention Times.	Approximately 6.4 minutes for CL 354,825

F. Linearity Check

The HPLC must be checked for linearity of response at least once for each related group of analyses. Linearity must also be confirmed following any change of column, modification of the instrument or significant alteration of chromatographic conditions. The linearity of response is checked by injection of at least three standards of different known concentrations. The response ratio for each standard injection is calculated and compared to the average response ratio.

1. Set up the HPLC conditions described above and obtain a stable chromatographic response for injection of 100 mcL of the Chromatography Standard D (0.05 mcg/mL CL 354,825).

2. Adjust the integrator to attain a peak height of approximately 40% full-scale deflection for the CL 354,825 peak after injection of 100 mcL of the Chromatography Standard D, 0.05 mcg/mL, (5 ng CL 354,825 injected).

3. Inject and chromatograph 100 mcL of each of the Chromatography Standards C, D and E (0.025, 0.05 and 0.10 mcg/mL CL 354,825, respectively).

4. Manually measure the peak height with a ruler for the CL 354,825 peak for each standard chromatogram or use the integrated peak area or integrated peak height provided by the data system or integrator. When integrated values are used, inspect each peak to insure that the baseline is properly defined and/or that the peak start and stop tick marks are placed properly. Calculate the response ratio for each standard injected by dividing the peak response (height or area) by the amount (nanograms) of sample injected. Calculate the average response ratio. Significant departure from linearity as indicated by deviation of any response ratio from the average response ratio of greater than 15% indicates instrument or experimental difficulties which must be corrected before proceeding.

G. Fortification Recovery Test

The validity and performance of the procedure must always be demonstrated by recovery tests. At least one fortified sample must be processed with every six samples analyzed. The fortification levels chosen for a study should include the level of sensitivity (LOQ, Limit of Quantitation, 5 ppb) of this method and should bracket the sample concentration range expected or found.

1. Weigh a 50 gram sample of a control soil into a 250-mL centrifuge bottle.

2. Add by pipet a volume of a Fortification Standard that is appropriate to the fortification level to be tested.

3. Process and analyze this fortified sample as described in sections H, I, J, K, L and N.

H. Extraction and Initial Sample Clean-Up

Extraction:

1. Weigh a 50 gram sample of soil into a 250-mL centrifuge bottle (Nalgene, polypropylene). Add 200 mL of 0.5 N NaOH to the bottle, cap and shake on a reciprocating shaker for 30 minutes (see Method Note H.1, Section N).

2. Centrifuge the bottle at about 5000 x G (5500 RPM) for 15 minutes. Decant the supernatant solution into a graduated cylinder and measure 30 mL of the sample solution into a clean beaker. Add 30 mL of 0.5 N NaOH to the beaker.

Precipitation:

3. Titrate the sample solution to between pH 1.8 and pH 2.2 with 6 N HCl using a magnetic stirrer and a pH meter. If too much HCl is added and the pH goes below 1.8 then back-titrate the sample with 0.5 N NaOH to the proper pH range. Allow sample to sit for 30 minutes.

Filtration:

4. Add approximately 1 gram of Celite to each sample extract, stir to mix and filter the suspension through a Whatman disposable filter unit with a 934-AH glass fiber filter into a 250-mL vacuum flask using slight vacuum.

5. Rinse the sample beaker twice with 20 mL of 0.024 N HCl and use each rinse to resuspend and wash the precipitate/Celite residue in the filter (See Method Note H.5, Section N).

I. C18 SPE Fractionation

Vacuum Manifold Setup:

1. Set up the SPE vacuum manifold as shown in the attached Figure 1 and as described in Method Note I.1., Section N. The C18 cartridges will be attached to the SPE vacuum manifold by a length of silicone tubing to allow direct application of samples to the cartridges by vacuum.

C18 Preparation and Sample Loading:

2. Precondition one Sep-Pak C18 cartridge for each sample by washing it first with 10 mL of methanol followed by 10 mL of 0.024 N HCl using a 10-mL plastic syringe (see Method Note I.2., Section N).

3. Attach the C18 cartridge to the end of the vacuum manifold/tubing arrangement and place the cartridge into the sample in the vacuum flask (from step H.5.). Open the stopcock for the sample line and apply sufficient vacuum (about 100 to 200 mbar) to the manifold to achieve a flow of about two drops per second. The samples are drawn directly into the C18 cartridges. For details on the sample load procedure see Method Note I.3., Section N.

C18 Cartridge Wash:

4. When the entire sample has been loaded and air has been drawn into the cartridge for approximately 30 seconds, rinse the sides of the sample flask down with 10 mL of 0.024 N HCl and allow the rinse to be drawn into the cartridge.

5. When the rinse is completely loaded, add an additional 30 mL of 0.024 N HCl to the flask and draw into the cartridge by vacuum until air enters the cartridge (approximately 30 seconds) as in step I.4.

6. Add 40 mL of 5% methanol/0.024 N HCl to the flask and load onto the cartridge by vacuum until air enters the cartridge for a fraction of a minute (approximately 30 seconds).

7. Remove the C18 cartridge from the vacuum manifold/tubing and wash with a total of 30 mL of 100 mM potassium phosphate pH 5.0 using a 10-mL plastic syringe. Pass 10 cc of air through the cartridge.

8. Wash the cartridge in the same manner with 10 mL of 0.024 N HCl followed by 10 mL of 20% methanol/water. Pass 10 cc of air through the cartridge.

9. Wash the cartridge with 15 mL of hexane using a 20-mL glass syringe. Pass 10 cc of air through the cartridge.

Elution of CL 354,825 Fraction:

10. Elute the C18 cartridge into a clean 100-mL pear-shaped flask with a total of 20 mL of 0.5% formic acid in methylene chloride using a 20-mL glass syringe. This eluate contains the CL 354,825 residue.

Evaporation:

11. Evaporate the eluate in the pear-shaped flask to dryness on a rotary evaporator (approximately 35°C water bath).

12. Blow a stream of pure nitrogen gas into the flask for several minutes to evaporate the final traces of methylene chloride and formic acid.

13. Dissolve the sample in 20 mL of 0.024 N HCl to prepare it for loading on an SCX column. Sonicate from 30 seconds to a minute and swirl the sample in the flask to assure complete dissolution or resuspension of the dried residue in the flask.

J. SCX-SPE Fractionation

SCX Column Preparation and Sample Loading:

1. Attach one SCX column to a closed Luer stopcock on a vacuum manifold.

2. Precondition the SCX column by washing 2 times with 5 mL of methanol followed by 2 times with 5 mL of 0.024 N HCl. Close the stopcock between each 5-mL wash before air enters the column.

3. Attach an adapter and a 75-mL reservoir to the top of the SCX column.

SCX Column Sample Loading:

4. Pour the CL 354,825 fraction from step I.13. into the reservoir.

5. Rinse the flask with an additional 20 mL of 0.024 N HCl and add this to the sample in the reservoir.

6. Open the stopcock to apply vacuum and load the fraction onto the column at a rate of about two drops per second.

SCX Column Wash:

7. When air enters the column (approximately 30 seconds), after the sample loading, rinse the sides of the reservoir with 10 mL of 0.024 N HCl and draw the rinse through the column by vacuum.

8. Repeat this rinse two times with 10 mL of methanol and allow air to be drawn into the column for a fraction of a minute (approximately 30 seconds).

Elution of CL 354,825 Fraction:

9. Open the vacuum manifold and place a pear-shaped vacuum flask (or other suitable container) in the vacuum manifold in a position to collect the eluate from the SCX column.

10. Close the vacuum manifold and elute the compound from the SCX column into the pear-shaped flask with a total of 20 mL of saturated potassium chloride in methanol.

Evaporation:

11. Remove the collected sample fraction from the vacuum manifold and evaporate the sample to dryness on a rotary evaporator (approximately 35°C water bath). If an alternate container is used for eluate collection, pour the sample into a pear shaped flask, rinse the container with about 5 mL of methanol and add the rinse to the sample before evaporation. This fraction is partitioned into methylene chloride as described in the next step.

K. Methylene Chloride Partition

1. Dissolve the dried residue in the flask from step J.11. in 10 mL of 1% formic acid and pour into a 125-mL separatory funnel.

2. Rinse the flask with an additional 10 mL of 1% formic acid and add to the separatory funnel. Add 20 mL of methylene chloride to the separatory funnel, stopper the funnel, shake briskly for 15 seconds and allow the phases to separate.

3. Draw off the methylene chloride phase (bottom phase) into a clean 100-mL pear-shaped flask taking care not to allow any of the aqueous phase to enter the flask.

4. Repeat this partition two additional times with 20 mL of methylene chloride and draw off the methylene chloride phases (bottom phase) and add to the pear-shaped flask.

Evaporation:

5. Evaporate the combined methylene chloride phases with a rotary evaporator with the water bath set at approximately 35°C (see Method Note K.5., Section N.).

6. Dissolve the sample in 1 mL of 5% Methanol/0.024 N HCl and analyze by HPLC as described in section L.

L. HPLC Analysis

1. Set up the HPLC instrument according to the conditions listed in section E and obtain a satisfactory baseline and chromatographic response for a 100-microliter injection of the Chromatography Standard D (0.10 mcg/mL). Set the integrator attenuation to obtain about 40% full-scale deflection for the CL 354,825 peak of this standard. These conditions may be adjusted slightly to obtain equivalent or better peak shape and resolution as the chromatograms in Figure 2. Chromatograph 100 microliters of each of the Chromatography Standards C, D, and E (0.025, 0.05 and 0.10 mcg/mL, respectively) for a linearity check as described in section F.

2. Inject and chromatograph 100 microliters of each processed soil sample and intersperse injections of Chromatography Standard D (0.05 mcg/mL, working standard) before the first sample injection and after a maximum of every two sample injections.

3. Identify the peak of interest (CL 354,825) in each sample chromatogram and standard chromatogram and measure the peak response (peak height in millimeters or use the integrated peak area or integrated peak height provided by the data system or integrator). When integrated values are used, inspect each peak to insure that the baseline is properly defined and/or that the peak start and stop tick marks are placed properly. If the sample peak response is greater than the highest value from the linearity check, then dilute the sample to an appropriate volume with 5% Methanol/0.024 N HCl and rechromatograph the diluted sample. If an interfering absorbance peak that elutes at the same retention time as CL 354,825 is found in control soil samples, an alternate HPLC mobile phase may be used for analysis. An alternate mobile phase is suggested in the Method Note L3., Section N.

M. Calculations

Calculate the apparent CL 354,825 residue in the injected samples (in ppb) from the sample peak response and the average peak response of the standard chromatograms obtained before and after the sample injection as follows:

$$ppb = \frac{R(SAMP) * V1 * V3 * C(STD) * V5 * D.F.}{R(STD) * W * V2 * V4} * 1000$$

Where:

R(SAMP) = Sample Response (chromatographic response of the sample peak of interest).

R(STD) = Average Standard Response (average chromatographic response for the peak of interest in the working standard chromatograms before and after the sample chromatogram).

W = Weight of sample taken for analysis, in grams (i.e. 50 g).

V1 = Volume of extraction solvent used, in milliliters (i.e. 200 mL).

V2 = Aliquot of extract taken for analysis, in milliliters (i.e. 30 mL).

V3 = Volume of solvent added to dissolve final residues for HPLC analysis, in milliliters (i.e. 1.0 mL).

V4 = Volume of sample solution injected for HPLC analysis in microliters, (i.e. 100 mcL).

V5 = Volume of working standard solution injected for HPLC analysis, in microliters (i.e. 100 mcL)

C(STD) = Concentration of working standard solution injected in micrograms per milliliter (0.05 mcg/mL)

D.F. = Dilution factor

1000 = Conversion Factor for ng/mcg

Calculate the percent recovery for fortified samples as follows:

$$\text{Percent Recovery} = \frac{\text{ppb Found}}{FV * FC/W} * 100 = \frac{\text{ppb Found}}{\text{ppb Added}} * 100$$

Where:

FV = Fortification Volume, in milliliters.

FC = Concentration of fortification solution, in ng/mL.

W = Weight of sample taken for analysis, in grams (i.e. 50 g).

Typical chromatograms for the determination of CL 354,825 in soil are shown in Figure 2.

N. Method Notes

These method notes provide additional detail and observations that may be useful to the analyst. They are numbered with the section and step to which they apply:

C.12 With repeated sample analysis, occasionally oily films have been left on the pear shaped flasks even after washing. This oily film may be removed using the following wash procedure. 1) Soak flasks overnight in a solution of detergent PCC-54 (available from Pierce). 2) Rinse with water. 3) Soak flasks in 10% Nitric acid for 10 minutes. 4) Rinse with water. 5) Finally, rinse flasks with methanol as called for in the method.

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H.1 The Eberbach shaker described in Section C provides 280 reciprocations per minute with a linear reciprocation throw of 1.5 inches when set on high. To achieve the most thorough agitation of the soil, the bottle should be placed on the shaker in a horizontal position with the direction of reciprocation parallel to the long dimension of the bottle.

H.5 To resuspend and wash the filtered precipitate, use a disposable plastic pipet to squirt the precipitate/Celite residue with the rinse solution and swirl the filter cup until the material is completely resuspended and dispersed. Apply a slight vacuum to draw the first rinse into the flask and repeat with the second 20 mL of rinse solution. The sample extract should be clear and yellow in color. If the extract is cloudy, the filter may have failed. Refilter the sample slowly through a new filter into a clean flask, rinse the filter as described in step H.5. and continue with Section I.

I.1 Set up the SPE vacuum manifold as shown in the attached Figure 1 and described as follows. Connect a vacuum source to a large side arm vacuum flask. The flask must be large enough to contain the total volume of all the samples and washes that will be processed without spillover into the vacuum system. Connect a vacuum line from the flask to the vacuum controller of the SPE vacuum manifold. Attach Luer stopcocks to the ports of the manifold and close the stopcocks. Attach twelve inch lengths of silicone tubing to the Luer stopcocks. The C18 cartridges will be attached to the end of the silicone tubing to allow direct application of samples to the cartridges by vacuum.

I.2 To pre-wash the cartridge, attach the cartridge to a 10-mL plastic syringe. Add 10 mL of methanol and push the methanol wash through the cartridge. Repeat this with a syringe and the dilute HCl wash. Do not introduce air into the cartridge during this procedure.

I.3 To load the sample onto the cartridge, connect the male Luer outlet of a preconditioned C18 cartridge to the end of the vacuum manifold/tubing arrangement and place the cartridge (connected with the tubing to the vacuum manifold) into the sample in the vacuum flask. Tilt each sample flask slightly and place the open end of the cartridge into the bottom corner of the flask to facilitate the removal of the entire sample. Repeat for each sample to be processed. Open the stopcock for each sample line and apply sufficient vacuum (about 100 to 200 mbar) to the manifold to achieve a flow of about two drops per second. The samples are drawn directly into the C18 cartridges.

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K.5 It may be necessary to evaporate each 20-mL partition fraction separately if bumping or splash-over occurs during evaporation. Additionally, if any aqueous phase has been carried into the pear-shaped flask, decant the methylene chloride carefully into a new pear-shaped flask leaving the aqueous phase behind. Rinse the old flask twice with 2 mL methylene chloride and decant the rinses into the new flask. Evaporate the sample in the pear-shaped flask to dryness on a rotary evaporator.

L.3 Alternate HPLC Mobile Phase: If an interfering absorbance peak that elutes at the same retention time as CL 354,825 is found in control soil samples an alternate HPLC mobile phase may be used for analysis. A useful alternate is 34% acetonitrile/65% water/1% formic acid v/v/v. Mix 340 mL acetonitrile, 650 mL purified water and 10 mL formic acid. This slight change has been observed to have a significant impact on the separation selectivity.

O. Modifications to Method M 2455:

The following minor modifications have been made to Method M 2455:

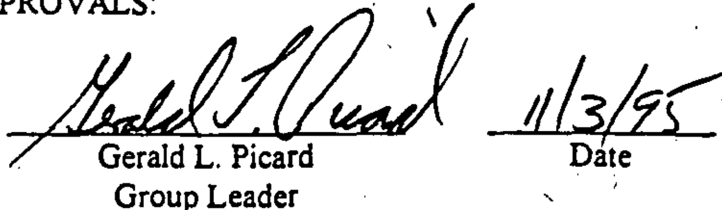
1. In Section H.2.,

   a) The sample measured out is 30 mL instead of 60 mL. This sample is then mixed with 30 mL of 0.5 N NaOH, prior to precipitation step H.3.

   b) Centrifuging is done at 5000 x G instead of 1000 x G.

2. In Sections D, F and L, the concentrations of the chromatography standards were reduced by a factor of 2 to accommodate the smaller aliquot taken.

APPROVALS:



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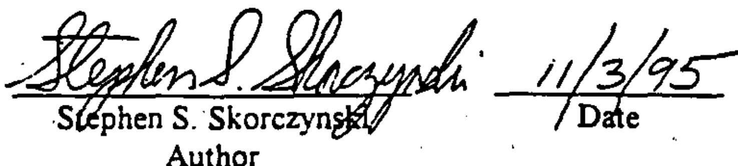
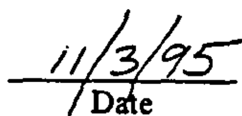
Date

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