Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Hydramethylnon (Amdro)

MRID #: 433452-01

Matrix: Soil

Analysis: HPLC/UV

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AGRICULTURAL RESEARCH DIVISION
HUMAN AND ENVIRONMENTAL SAFETY
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Recommended Method of Analysis - M 2266

AMDRO® (CL 217,300): HPLC Method for the Determination of CL 217,300 Residues in Soil.

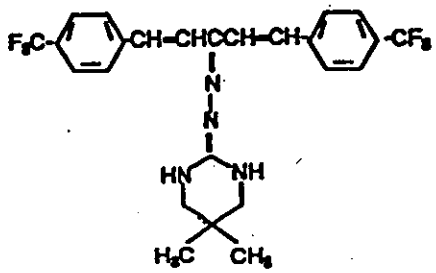
A. Principle

Residues of CL 217,300 are extracted from soil with methylene chloride-methanol. Cleanup is achieved using solvent partitioning and solid phase extraction techniques. CL 217,300 residues are measured using HPLC equipped with a UV detector (400nm). Results are calculated as CL 217,300 by direct comparison of peak heights to those of external standards. The validated sensitivity of the method is 10 ppb.

B. Reagents (Items from manufacturers other than those listed may be used provided they are functionally equivalent.)

1. Analytical Standard: CL 217,300, analytical grade of known purity. Obtained from American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08543-0400.

   a. CL 217,300: tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene]hydrazone



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2. Solvents: B & J Brand High Purity Solvents, Baxter, Burdick and Jackson.

   a. Methylene Chloride
   b. Methanol
   c. Acetonitrile (UV Grade)
   d. Acetone

3. Water, Deionized: Water passed through Millipore's Milli-Q Plus Ultra Pure Water System. Use this water for all steps.

4. Reagents: "Baker Analyzed" Reagents, J.T. Baker Company.

   a. Triethylamine
   b. Concentrated Hydrochloric Acid (HCl)

5. Solutions:

   a. Extraction Solvent: Dilute 333 mL methanol to 1 liter with methylene chloride. Mix well.

   b. Wash Solvent: Dilute 300 mL deionized water to 1 liter with acetone. Mix well.

   c. Elution Solvent: Dilute 2.5 mL triethylamine to 1 liter with acetonitrile. Mix well.

   d. HPLC Dilution Solvent: Dilute 200 mL deionized water to 1 liter with acetonitrile. Mix well.

   e. Liquid Chromatographic Mobile Phase: Mix 5 mL triethylamine with 150 mL deionized water in a 1000-mL graduated mixing cylinder. Dilute to 1 liter with acetonitrile and shake to mix. Filter the mobile phase through a Rainin Nylon 66 (0.45µm) filter or equivalent.

   f. 1 N HCl: Add 82.5 mL concentrated HCl to 500 mL deionized water. Dilute to 1 liter with deionized water. Mix well.

   g. 0.05 N HCl: Dilute 50 mL of 1 N HCl (Reagent Solution B.5.f.) to 1 liter with deionized water. Mix well.

C. Apparatus (Items from other manufacturers other than those listed may be used provided they are functionally equivalent.)

1. Balance, Analytical: Sartorius Research R200D, precision ± 0.05 mg.

2. Balance, Pan: Sartorius, Model L610, precision ± 5 mg.

3. Soil Extraction Bottles: Nalgene, 500-mL capacity, narrow-mouth, polypropylene, Nalge Company, Cat. No. 2002-9016.

4. Assorted Glassware: General laboratory.

5. Filtering Funnel: Buchner, porcelain, 9-cm diameter.

6. Filter Paper: 9-cm diameter, glass-fiber, 934-AH, Whatman, Inc.

7. Reciprocating Shaker: Eberbach, Model 6010, equipped with a Utility Carrier Box, Model 6040, Eberbach Corporation.

8. Flash Evaporator: Buchler Instruments, Model PF10DN, equipped with a heated waterbath maintained at approximately 30° C.

9. Ultrasonic Cleaner: Branson Model 3200, Branson Ultrasonics Corporation.

10. Vac-Elut Processing Station: Analytichem International, Cat. No. A16000.

11. Solid Phase Extraction Cartridge: Analytichem International, Bond Elut C18/OH cartridge (1000 mg), Cat. No. 1225-6040.

12. Bond Elut Adapter: Analytichem International, Cat. No. 636001.

13. Reservoirs, Disposable: Varian, 25-mL capacity, Cat. No. 1213-1011.

14. Plastic Syringe, Disposable: Luer-Lok, 10-mL capacity, Becton Dickinson & Co., Cat. No. 9604.

15. Microfilter: Millex-SR 0.5µm Filter Unit, Millipore Products, Cat. No. SLSR025NB.

16. Microliter Syringe: 1-mL Glenco syringe for Rheodyne valves, Cat. No. 5-8678.

17. HPLC Column: 25-cm x 4.6-mm ID, REXCHROM S5-100-ODS (octadecyldimethylsilyl), Regis Chemical Co., Cat. No. 728218.

18. Liquid Chromatograph:

    a. Pump: Applied Biosystems Spectroflow 400 solvent delivery system.

    b. Detector: Applied Biosystems Spectroflow 783 UV absorbance detector.

    c. Sample Injector: Rheodyne valve, Model 7125 with a 200-µL loop.

    d. In-line Frit Filter: Supelco, Inc., Cat. No. 5-8420. Additional 0.5µm replacement frits, Supelco, Inc., Cat. No. 5-9037.

19. Recorder: Spectra-Physics, SP 4290 Recording Integrator.

D. Preparation of Standard Solutions (store in amber bottles)

1. Stock Solution: (prepare monthly, store in refrigerator)

a. Weigh accurately a known amount (approx. 10 mg) of CL 217,300 into a 100-mL volumetric flask. Dilute to the mark with acetonitrile and mix well. Calculate and record the exact concentration of CL 217,300. This solution contains approximately 100 mcg/mL.

NOTE: Resulting concentration of the standard stock solution must be corrected for purity.

2. Standard Fortification Solutions: (prepare weekly)

a. Pipet into a 100-mL volumetric flask, an appropriate amount of stock solution D.1.a. to deliver 1000 mcg of CL 217,300. Dilute to the mark with acetonitrile and mix well. This solution contains 10 mcg/mL CL 217,300.

b. Pipet into a 100-mL volumetric flask, a 50-mL aliquot of stock solution D.2.a. Dilute to the mark with acetonitrile and mix well. This solution contains 5 mcg/mL CL 217,300.

c. Pipet into a 100-mL volumetric flask, a 5-mL aliquot of stock solution D.2.a. Dilute to the mark with acetonitrile and mix well. This solution contains 0.5 mcg/mL CL 217,300. Prepare this solution daily.

3. HPLC Calibration Standard Solutions: (prepare daily)

a. Pipet into a 100-mL volumetric flask, a 1-mL aliquot of stock solution D.2.a. Dilute to the mark with HPLC Dilution Solvent (Reagent Solution B.5.d.) and mix well. This solution contains 0.1 mcg/mL CL 217,300.

b. Pipet into a 100-mL volumetric flask, a 10-mL aliquot of stock solution D.2.c. Dilute to the mark with HPLC Dilution Solvent (Reagent Solution B.5.d.) and mix well. This solution contains 0.05 mcg/mL CL 217,300.

c. Pipet into a 100-mL volumetric flask, a 5-mL aliquot of stock solution D.2.c. Dilute to the mark with HPLC Dilution Solvent (Reagent Solution B.5.d.) and mix well. This solution contains 0.025 mcg/mL CL 217,300.

The 0.1 mcg/mL, 0.05 mcg/mL, and 0.025 mcg/mL CL 217,300 standard solutions are used for the linearity check. The 0.05 mcg/mL CL 217,300 standard is used for quantitation.

E. Liquid Chromatographic Conditions

1. Instrument:

a. Pump: Applied Biosystems Spectroflow 400.

b. Detector: Applied Biosystems Spectroflow 783 UV absorbance detector.

2. Column:

a. Column: REXCHROM S5-100-ODS, 25 cm x 4.6 mm ID.

b. In-line Frit Filter: Supelco 0.5 µm in-line frit filter placed just before the column. Do not use a guard column.

NOTE: Replace frit as needed when mobile phase pressure significantly rises due to clogging of the frit by sample matrix.

3. Instrument Conditions:

a.	Column Temperature:	Room Temperature (approx. 24° C)
b.	Mobile Phase:	Acetonitrile : Water : Triethylamine 845 : 150 : 5
c.	Flow Rate:	0.80 mL/minute
d.	Detector Wavelength:	400 nm
e.	Detector Range:	0.001 AUFS
f.	Sample Loop:	200 mcL
g.	Recorder:	0.5 cm/minute chart speed, 10 mV
h.	Attenuation:	16
i.	Retention Time:	approx. 8.7 minutes

4. Sensitivity: Attenuation on the recording integrator should be set so that 10 ng of CL 217,300 gives a peak height of approximately 20-30% full-scale deflection.

F. Linearity Check

The liquid chromatograph should be checked for linearity of response at the beginning of the study and whenever a new column or instrument is used.

1. Adjust the HPLC conditions to attain peak heights of approximately 20-30% full-scale deflection for a 10-ng injection of CL 217,300.

2. Inject 200-mcL aliquots of the analytical standard solutions prepared in Sections D.3.a., D.3.b., and D.3.c.

3. Plot the height for each peak versus the nanograms injected to show the linearity of response. Significant departure from linearity over this range indicates instrumental difficulties which should be corrected before proceeding.

G. Sample Preparation

1. Refer to American Cyanamid SOP's R-05-08, R-05-09, and R-05-10.

H. Recovery Test

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. A fortified sample should also be processed with each set of samples analyzed. Refer to American Cyanamid SOP R-03-05.

1. Weigh a 50-g sample of control soil into a 500-mL, plastic, narrow-mouth bottle.

2. Add by pipet, a volume (usually 1 to 5 mL) of standard fortification solution appropriate to the fortification level to be tested.

3. Proceed with the extraction and cleanup steps.

I. Extraction

NOTE: All soil samples should be run completely through the method and injected on HPLC within one working day once the initial extraction has been started. Do not allow sample extracts to sit overnight before analysis.

1. Weigh 50 g of soil into a 500-mL, plastic, narrow-mouth bottle.

2. Add 15 mL deionized water. Add 250 mL Extraction Solvent (Reagent Solution B.5.a.) and shake on "high" speed on the reciprocating shaker for one hour.

3. Filter the extract by vacuum into a 500-mL vacuum flask using a Whatman 934-AH glass fiber filter positioned on a 9-cm Buchner funnel.

4. Rinse the extraction bottle with 10 mL methylene chloride and pass the rinse through the filter.

5. Pour the extract into a 250-mL graduated mixing cylinder and allow it to come to room temperature (approx. 30 minutes).

6. When at room temperature, bring the total volume up to 250 mL with methylene chloride. Shake to mix.

J. Partitioning

1. Transfer a 100-mL aliquot of the extract to a 250-mL separatory funnel.

2. Add 50 mL 0.05 N HCl (Reagent Solution B.5.g.) and shake vigorously for 30 seconds. Draw off the lower, methylene chloride layer into a 200-mL pear-shaped flask.

3. Add another 25 mL methylene chloride to the separatory funnel and shake vigorously for 30 seconds. Draw off the lower, methylene chloride layer into the 200-mL pear-shaped flask and discard the aqueous, upper layer.

4. Add 1 mL deionized water to the flask. Evaporate all the methylene chloride down to the 1 mL of water on a flash evaporator with a waterbath temperature set at approximately 30° C.

   NOTE: Be sure to add the 1 mL deionized water and do not allow the sample to go to dryness.

5. Add 10 mL acetonitrile and stopper the flask. Sonicate for 30 seconds on the ultrasonic cleaner, tilting the flask on its side and constantly turning it by hand.

6. Add 10 mL deionized water and swirl to mix.

K. Solid Phase Extraction Cleanup

1. Prepare a 1000-mg Bond-Elut C18/OH cartridge using an Analytichem Vac-Elut Processing Station. By vacuum, wash the cartridge with 5 mL methanol, then 2 x 5 mL deionized water. Do not allow the cartridge to go dry between wash additions or after the final addition of water.

2. Assemble a 25-mL disposable reservoir onto the top of the prepared cartridge using an adapter.

3. Using vacuum, pass the sample from Step J.6. through the C18/OH cartridge at a rate of approximately 2-3 drops per second and discard the eluate. Allow air to pass through the cartridge for 5 seconds.

4. Add 15 mL Wash Solvent (Reagent Solution B.5.b.) to the evaporation flask. Stopper the flask and sonicate for 30 seconds while tilting the flask on its side and constantly turning it by hand.

5. Using vacuum, pass the Wash Solvent through the C18/OH cartridge at a rate of approximately 1 drop per second and discard the eluate. Allow air to pass through the cartridge for 5 seconds.

6. Using vacuum, elute the C18/OH cartridge with 15 mL Elution Solvent (Reagent Solution B.5.c.) at a rate of approximately 1 drop per second and collect in a 30-mL beaker placed inside the Vac-Elut Processing Station.

L. Partitioning

1. Transfer the eluate from Step K.6. to a 125-mL separatory funnel. Add 10 mL 0.05 N HCl and 25 mL deionized water to the separatory funnel.

2. Add 25 mL methylene chloride to the 30-mL collection beaker, swirl, then transfer to the separatory funnel. Cap and shake vigorously for 30 seconds.

3. Allow the two fractions to separate completely then draw off the lower, methylene chloride layer into a 100-mL pear-shaped flask.

4. Add another 25 mL methylene chloride to the collection beaker, swirl, then transfer to the separatory funnel. Cap and shake vigorously for 30 seconds.

5. Begin evaporating the first methylene chloride fraction using a flash evaporator with the waterbath temperature set at approximately 30° C.

6. When the methylene chloride has evaporated to approximately 5 mL, draw the lower, methylene chloride fraction from the separatory funnel into the evaporation flask. Evaporate all the methylene chloride using a flash evaporator.

7. Reconstitute the residue by adding 4 mL HPLC Dilution Solvent (Reagent Solution B.5.d.) to the flask. Stopper the flask and sonicate for 30 seconds while tilting the flask on its side and constantly turning it by hand.

8. Attach a Millex-SR 0.5 µm filter unit onto a 10-mL Luer-Lok, disposable syringe. Push the sample through the filter unit and into an appropriate collection vial. Cap and label the vial for analysis by HPLC.

M. Liquid Chromatographic Analysis

1. After obtaining a satisfactory chromatographic response (as shown in Figure 1), inject, in sequence, a 200-mcL aliquot of the CL 217,300 working standard (0.05 mcg/mL), 200-mcL aliquots of up to two samples, and another 200-mcL aliquot of the working standard.

2. If a sample peak goes off-scale, dilute an aliquot of the sample with HPLC Dilution Solvent until the peak height of CL 217,300 falls within the range of linearity, established in Section F.3, and reinject. Refer to American Cyanamid SOP R-03-05.

3. Use the average peak height of the standards bracketing the samples for the quantitation.

N. Calculations

For each sample calculation, use the sample peak height and the average peak height measurements of the external standards before and after the sample as follows:

$$PPB = \frac{R(SAMP) \times (V1) \times (V3) \times C(STD) \times (V5) \times (DF)}{R(STD) \times (W) \times (V2) \times (V4)} \times 1{,}000$$

$$\% \text{ RECOVERY} = \frac{\text{PPB FOUND IN FORTIFIED CONTROL}}{\text{PPB ADDED}} \times 100$$

Where:

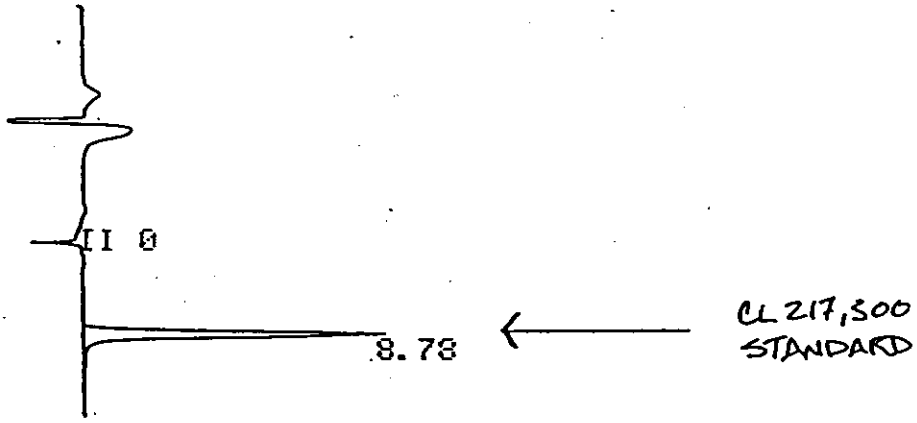
R(SAMP)	=	Peak height of sample in mm.
R(STD)	=	Average peak height of working standard in mm.
W	=	Weight of sample taken for analysis in grams (50 g).
V1	=	Total Volume of extraction solvent in mL (250 mL).
V2	=	Volume of extract taken for analysis in mL (100 mL).
V3	=	Volume of final solution used for HPLC analysis in mL (4 mL).
V4	=	Volume of sample solution injected in mcL (200 mcL).
C(STD)	=	Concentration of standard solution in mcg/mL (0.05 mcg/mL).
V5	=	Volume of standard solution injected in mcL (200 mcL).
DF	=	Dilution Factor (1 unless additional dilutions are needed).

Figure 1 shows typical chromatograms for the analysis of CL 217,300 residues in soil.

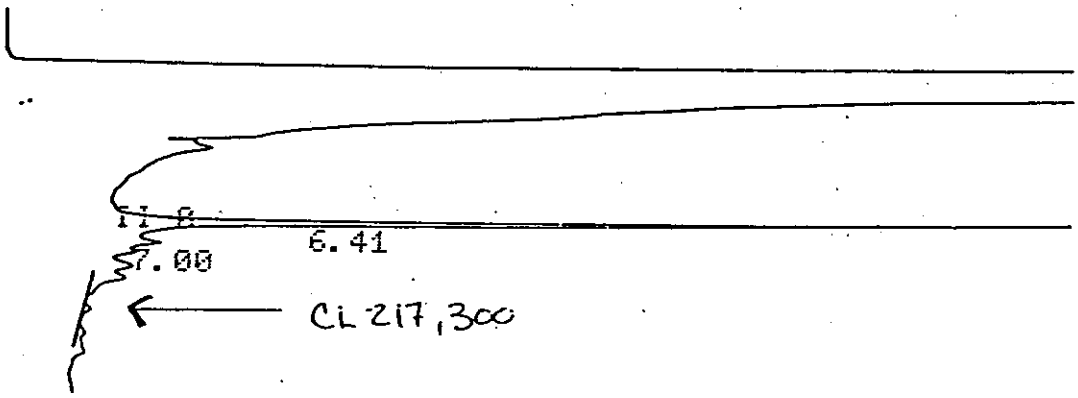
APPROVED: shahn fletcher 1/21/93
J. Shahn Fletcher Date
Author

Gerald L. Picard 1/21/93
Gerald L. Picard Date
Group Leader

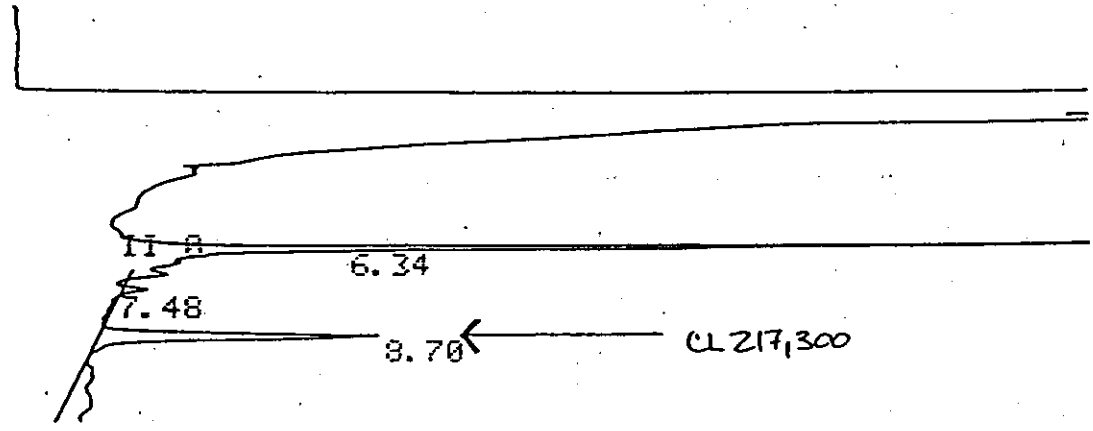
Figure 1: Typical Chromatograms for Analysis of CL 217,300 Residues in Soil.



CL 217,300 Standard, 10 ng Injected



Control Soil (AC 6794.91, Greenville, Mississippi), 1000 mg Injected, 0.27 ppb Apparent CL217,300 Found



Control Soil (AC 6794.91, Greenville, Mississippi), Fortified with CL 217,300 at 10.0 ppb, 1000 mg Injected, 9.86 ppb Apparent CL 217,300 Found, 99% Recovered

AM93PT01

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HAS

5 NG CL 217, 300
HEIGHT PERCENT TABLE

SAMPLE: S4231224.01 INST: 03 VIAL: F6 SEQ NUMBER: 007
DATE-TIME COLLECTED : 02/11/93 17:27:24
COLLECTION TIME : 11.00 DATE-TIME PROCESSED : 02/11/93 17:38:51
METHOD: AC066 / AC066 REV #: 00003 ANALYST: A217 SAMP RATE: 3.13
SAMPLE WT : 1.0000 STANDARD WT : 1.0000 DILUTION FACTOR : 1.0000

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001	9.043	1	CL217, 300	1.00000	11830
					11830

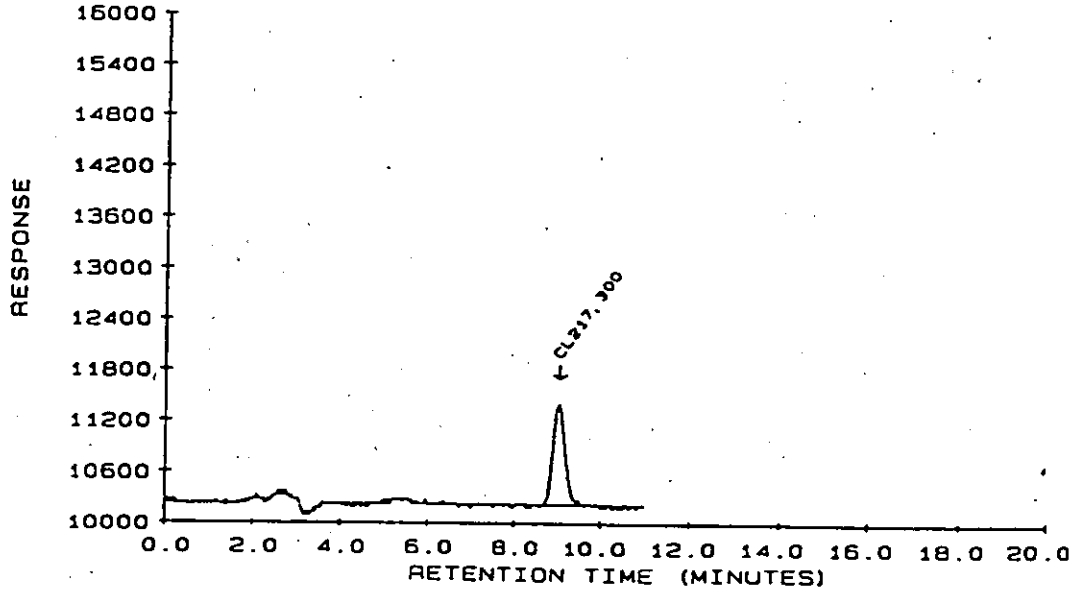

FIGURE 1

Huntingdon