Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Primisulfuron-Methyl

MRID #: 432632-05

Matrix: Soil

Analysis: HPLC/UV

Appendix II

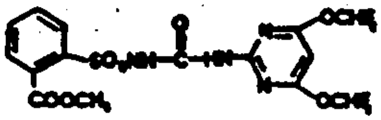
Method AG-498

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 1 of 15	METHOD NO. AG-490	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION -/24/86		
SUBMITTED BY: W. T. Beidler, R. P. Shoffner		
		APPROVED BY:

1.0 SCOPE

This method is used for the determination of residues of CGA-136872, structure shown below, in soil. The detection limit for the method is 0.01 ppm of CGA-136872.



CGA-136872

2.0 PRINCIPLE

Residues of CGA-136872 are extracted from soil by shaking for one hour at room temperature with acetonitrile:water:concentrated ammonium hydroxide, 90:8:2. An aliquot of the extract is evaporated to a small volume and diluted with 0.1N sodium carbonate. The alkaline aqueous solution is partitioned with toluene, then the aqueous portion is acidified with dilute phosphoric acid and partitioned again with dichloromethane. The dichloromethane is evaporated and the contents of the flask are dissolved in acetonitrile and the solvent evaporated again to remove any residual water. Final cleanup is performed with an Alumina-A Sep-Pak. Residues of CGA-136872 are determined by HPLC on a Zorbax-ODS column using a mobile phase comprised of 65% acetonitrile:28% 0.02M monobasic hydrogen phosphate:7% 0.02M phosphoric acid and UV detection at 234 nm. A flow diagram for the method is presented in Figure 1.

3.0 APPARATUS

3.1 Bottle, Boston round, 8-oz.

3.2 Bottle, Nalgene (polyethylene), 8-oz., wide-mouth.

3.3 Centrifuge (Sorvall RC2-B, equipped with a Type GSA rotor or equivalent).

3.4 Filter paper, Whatman 2V, 24-cm.

PAGE 2 of 15	METHOD NO. AG-498	SUBJECT
EDITION		DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
	APPROVED BY:	

3.5 Flask, round bottom, 100-ml and 50-ml.

3.6 Funnel, filter, 10-cm.

3.7 Funnel, separatory, 250-ml.

3.8 Graduated cylinder, 50-ml.

3.9 ISS-100 Microvial (Perkin-Elmer) or equivalent.

3.10 Mechanical shaker (Eberbach) or equivalent.

3.11 Rotary evaporator (Buchi) or equivalent.

3.12 Sep-Pak, Alumina-A (Waters Assoc.).

3.13 Syringe, Luer-Lok, 20-ml.

4.0 REAGENTS

4.1 Acetonitrile, HPLC grade.

4.2 Acetonitrile:water:conc. NH_4OH, 90:8:2.

4.3 Ammonium hydroxide, Conc., Reagent grade.

4.4 Dichloromethane, HPLC grade.

4.5 Methanol, HPLC grade.

4.6 Phosphoric acid, Reagent grade, 0.02<u>M</u> in deionized water.

4.7 Phosphoric acid, Reagent grade, 1.2M in distilled water.

4.8 Potassium dihydrogen phosphate, Reagent grade, 0.02M in deionized water.

4.9 Sodium carbonate, Reagent grade, 0.1M in distilled water.

PAGE 3 of 15	METHOD NO. AG-498	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION		
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		APPROVED BY:

4.10 Standard CGA-136872 (available from CIBA-GEIGY Corp., P.O. Box 18300, Greensboro, NC 27419).

4.11 Toluene, HPLC grade.

4.12 Water, distilled.

4.13 Water, distilled, deionized.

5.0 PROCEDURE

5.1 Extraction

5.1.1 Weigh a 25-gram subsample from a well-homogenized, stone-free soil sample into an 8-oz. Nalgene bottle. Add 125 ml of the acetonitrile:water:NH_4OH (90:8:2) extraction mixture and shake for one hour at room temperature using a mechanical shaker.

5.1.2 Centrifuge for 10 minutes at 5,000 RPM using a Type GSA rotor.

5.1.3 Filter the sample through a Whatman 2V filter paper into an 8-oz. Boston round bottle.

5.2 Partition

5.2.1 Measure a 25-ml aliquot (5-g equivalent) of the extract from Step 5.1.3 into a graduated cylinder, pour the aliquot into a 100-ml round bottom flask and evaporate the solvent on a rotary evaporator until acetonitrile stops distilling. (There may be 1-2 ml of water remaining depending on the moisture content of the soil.)

5.2.2 Add 50 ml of 0.1M Na_2CO_3 to the 100-ml round bottom flask, then transfer to a 250-ml separatory funnel.

5.2.3 Partition the aqueous solution with 50 ml of toluene by shaking vigorously for 30 seconds, then, after the layers separate, drain the lower layer into another 250-ml separatory funnel. Discard the toluene.

5.2.4 Add 10 ml of 1.2M H_3PO_4 to the separatory funnel containing the lower layer from step 5.2.3. and shake carefully with frequent venting until most of the CO_2 has dissipated.

5.2.5 Partition the acidified aqueous solution (pH should be 2-3) with two 25-ml portions of dichloromethane shaking vigorously each time for 30 seconds.

5.2.6 Collect both the dichloromethane portions in a 100-ml round bottom flask and evaporate on a rotary evaporator at a bath temperature of 40-45°C. When the dichloromethane no longer distills (there will usually be several water droplets left on the walls of the flask) stop the evaporation, immediately add 5 ml of fresh acetonitrile to the flask, swirl thoroughly and evaporate again to dryness. It is important not to leave the flask on the rotary evaporator for prolonged periods, especially before the acetonitrile evaporation (which removes any residual water) is performed.

5.3 <u>Cleanup</u>

5.3.1 Fit a 20-ml Luer-Lok syringe with an Alumina-A Sep-Pak and wash the Sep-Pak first with 5 ml of 15% methanol/acetonitrile, then with 5 ml of 100% acetonitrile. It may be necessary to start the solvent flow through the Sep-Pak by applying pressure with a pipette bulb or pressurized air.

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 5 of 15	METHOD NO. AG-498	SUBJECT
EDITION		DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

5.3.2 Dissolve the residue in the flask from Step 5.2.6 in 5 ml of acetonitrile and pipette into the syringe. Once the flow is started, allow the solvent to drain by gravity. When flow stops, discard the acetonitrile.

5.3.3 Elute the Sep-Pak with 15 ml of 15% methanol/acetonitrile, collecting the eluant in a 50-ml round bottom flask. Evaporate the solvent on a rotary evaporator.

5.3.4 Dissolve the contents of the 50-ml round bottom flask in 0.5 ml of acetonitrile or some multiple of 0.5 ml for higher residue levels.

5.3.5 Pipette the sample into a microvial for HPLC analysis.

6.0 HPLC ANALYSIS

6.1 Preparation of Standard CGA-136872

6.1.1 Weigh 100.0 mg of CGA-136872 analytical standard into a 100-ml volumetric flask and dilute the flask to the mark with acetonitrile.

6.1.2 Make serial dilutions of the 1 mg/ml standard solution with acetonitrile to give a series of injection standards in a range of 0.05 to 2.0 ng per µl.

6.2 Standardization

6.2.1 Standardize the HPLC under the conditions listed in Table I by making 20-µl injections in the range of 1 to 40 ng, depending on the concentrations being determined.

6.2.2 Measure the peak heights of the injected standards. Typical chromatograms for standards are shown in Figure 2 and

PAGE 6 of 15	METHOD NO. AG-498	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION		
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

standardization data generated from the chromatograms are listed in Table II.

6.2.3 Construct a standard curve by plotting, either manually or by computer, the detector response versus nanograms injected, or enter the data into an appropriate electronic calculator to obtain a least squares regression line.

6.3 Detection of Sample Residues

6.3.1 Inject a 20-µl aliquot of the sample from Step 5.3.5 into the HPLC under the same conditions employed for standards. Make dilutions of samples, as necessary, to maintain peak heights within the range of the standard curve. Compare the peak heights of the unknown samples with the standard curve or enter into the least squares program to determine the nanograms of CGA-136872 present in the injected aliquot.

6.3.2 Calculate residue results in terms of ppm CGA-136872 by the following equation:

$$\text{PPM} = \frac{\text{CGA-136872 Found (ng)}}{\text{Mg Soil Injected}} \times \left[\frac{V+(W \times M/100)}{V}\right]^{*} \times \frac{100}{(100-M)} \times \frac{100}{R}$$

Where V = volume of extraction solvent (125 ml), W = weight of the soil sample (25 grams), M = percent moisture in soil and 100 = conversion factor. R = percent recovery based on fortified controls taken through the procedure.

This equation takes into account the volume increase in the extracting solvent due to water contained in the soil, the procedural recovery, and expresses the residue on the basis of soil dry weight.

*Generally, if the soil moisture is less than 10%, this portion of the equation may be omitted.

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 7 of 15	METHOD NO. AG-498	SUBJECT
EDITION		DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

6.4 **Fortification Experiments**

This method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified prior to extraction with 0.01 ppm or more of CGA-136872.

6.4.1 Add 0.25 ml of 1 µg/ml standard solution of CGA-136872 to 25 g of control soil prior to extraction (Step 5.1.1) for a 0.01 ppm fortification. Use correspondingly greater amounts of standards (volume not to exceed 2 ml) for higher fortifications. Analyze the control and fortified samples by the procedures of the method.

6.4.2 Calculate the final ppm value for the control and fortified samples according to the following equation:

$$\text{PPM} = \frac{\text{CGA-136872 Found (ng)}}{\text{Mg Soil Injected}} \times \frac{[V+(W \times M/100)]^{*}}{V}$$

*As in Section 6.3.2, this portion of the equation may be omitted if the soil moisture is less than 10%. The letters V, W and M have the same significance as in Section 6.3.2.

6.4.3 Correct the recovery value (ppm) by subtracting the ppm value, real or apparent, found in the control. Calculate the recovery factor in percent by the following equation:

$$R\ (\%) = \frac{\text{ppm Found (corrected)}}{\text{ppm Added}} \times 100$$

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 8 of 15	METHOD NO. AG-498	SUBJECT
EDITION		DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Weidler, R. P. Shoffner		
	APPROVED BY:	

7.0 DISCUSSION

This method has been used for the preliminary analysis of control, CGA-136872-fortified, and treated soil samples from soil dissipation studies conducted at three different locations. Fortification levels ranged from 0.01 to 1.00 ppm and recoveries averaged 89% with a standard deviation of 8% (n = 12). At a screening level of 0.01 ppm, no residues were found in any of the control samples. Results of these analyses are shown in Table III and reported in AG-A 9486-01. The results show rapid and extensive degradation of the compound in soil.

The method has also been applied to soils from an experimental field plot of corn which was sprayed with ϕ-^{14}C-CGA-136872. The results (Table IV and AG-A 9487-01) show good extractability and recovery of total residues as parent CGA-136872 with the zero-day sample and somewhat lower values for the aged (four-week) sample. Analytical values from aliquots of the final fraction determined by HPLC and by liquid scintillation counting are in agreement for both the zero-day and aged samples and indicate extensive degradation of the compound in field soil.

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 9 of 15	METHOD NO. AG-498	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION		
SUBMITTED BY: W. T. Beidler,	K. P. Shoffner	
		APPROVED BY:

TABLE I: <u>HPLC OPERATING CONDITIONS FOR DETERMINATION OF CGA-136872</u>

Instrument:	Perkin-Elmer Series 4 Liquid Chromatograph with an LC85B Variable Wavelength UV Detector, an ISS-100 Sampling System, and a Chromatographics 3 Data Handling System or an equivalent HPLC pump and UV detector with or without automated data acquisition
Column:	Zorbax-ODS, 4.6 x 250 mm (DuPont Instruments)
Mobile Phase:	65% acetonitrile:28% 0.02$\underline{M}$ KH_2PO_4:7% 0.02$\underline{M}$ H_3PO_4
Flow Rate:	1.0 ml/min.
Temperature:	Ambient
Attenuation:	4
Detection:	Variable Wavelength UV Detector set at 234 nm
Minimum Detection Limit:	1.0 ng
Injection Volume:	20 µl
Chart Speed:	1.0 cm/min. from 4.5 to 5.5 minutes; 0.5 cm/min. all other times
Retention Time:	5.2 ± 0.15*

*The range of retention times is due to variations in ambient temperature.

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 10 of 15	METHOD NO. AG-498	SUBJECT
EDITION		DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

TABLE II: TYPICAL STANDARDIZATION DATA FOR CGA-136872 (AGA-9486-01)

CGA-136872 Injected (µg)	Peak Height
1	0.6325
2	1.1481
5	2.1389
10	5.4154
20	10.7387
40	21.0088

Slope = 0.5239431

Intercept = 0.1231307

Correlation Coefficient = 0.99995 (calculated by TI-55 calculator)

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 11 of 15	METHOD NO. AG-498	SUBJECT
EDITION		DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
	APPROVED BY:	

TABLE III: SUMMARY OF RECOVERY DATA FOR SOILS FROM VARIOUS GEOGRAPHIC LOCATIONS FORTIFIED WITH CGA-136872 (AG-A 9486-01)

Location	Percent Recovery at Fortification (ppm) of:			
	0.01	0.05	0.20	1.00
Mississippi	88	87	95	94
Nebraska	101	89	89	[illegible]1
New York	86	77	72	94

Mean = 89%

S.D. = 8% (n = 12)

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 12 of 15	METHOD NO. AG-498	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION		
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

TABLE IV: ANALYSIS OF FIELD SOILS TREATED WITH φ-^{14}C-CGA-136872[1]

AGA No.:	9487-01	
Test No.:	M4-161-2P,2S	
Location:	NY	
Sample No.:	S5040857 (B-Shipment)	S5040860 (B-Shipment)
Interval:	0-day	28-day
Total ppm[2]:	0.0342	0.0227
Percent of total ^{14}C in extract:	92%	56%
Percent of total ^{14}C in AG-498 final fraction:	69%	19%
Percent (ppm) of total ^{14}C residue determined as CGA-136872 by AG-498:	70% (0.024)	(<0.01)

[1] Field plots of 4-week old corn were sprayed once with φ-^{14}C-CGA-136872 (50.2 µCi/mg) at a rate of 0.140 lb. ai/Acre. Six cores of 0-3" soil were taken, composited and homogenized. Combustion samples were air-dried overnight before analysis.

[2] Total ppm were calculated by combustion analysis of ^{14}C.

BIOCHEMISTRY DEPARTMENT
CIBA-GEIGY CORPORATION
GREENSBORO, N.C.

PAGE 13 of 15	METHOD NO. AG-498	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION		
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 1: FLOW DIAGRAM FOR ANALYTICAL METHOD AG-498

25 g soil

Shake for 1 hour with 125 ml of acetonitrile:H_2O:NH_4OH, 90:8:2.

Centrifuge and filter.

Evaporate the solvent from a 25-ml (5-g) aliquot.

Add 50 ml of 0. M Na_2CO_3 to the residue.

Partition with 50-ml toluene.

Toluene (discard)

Aqueous

Acidify with 10 ml 1.2M H_3PO_4.

Partition with dichloromethane (2x25 ml).

Dichloromethane

Aqueous (discard)

Evaporate solvent, add 5 ml acetonitrile and evaporate again. Dissolve residue in another 5 ml of acetonitrile.

Prewash Alumina-A Sep-Pak first with 15% MeOH/acetonitrile then with 100% acetonitrile.

Load the residue in acetonitrile onto the Sep-Pak.

Discard the prewash and load solvents.

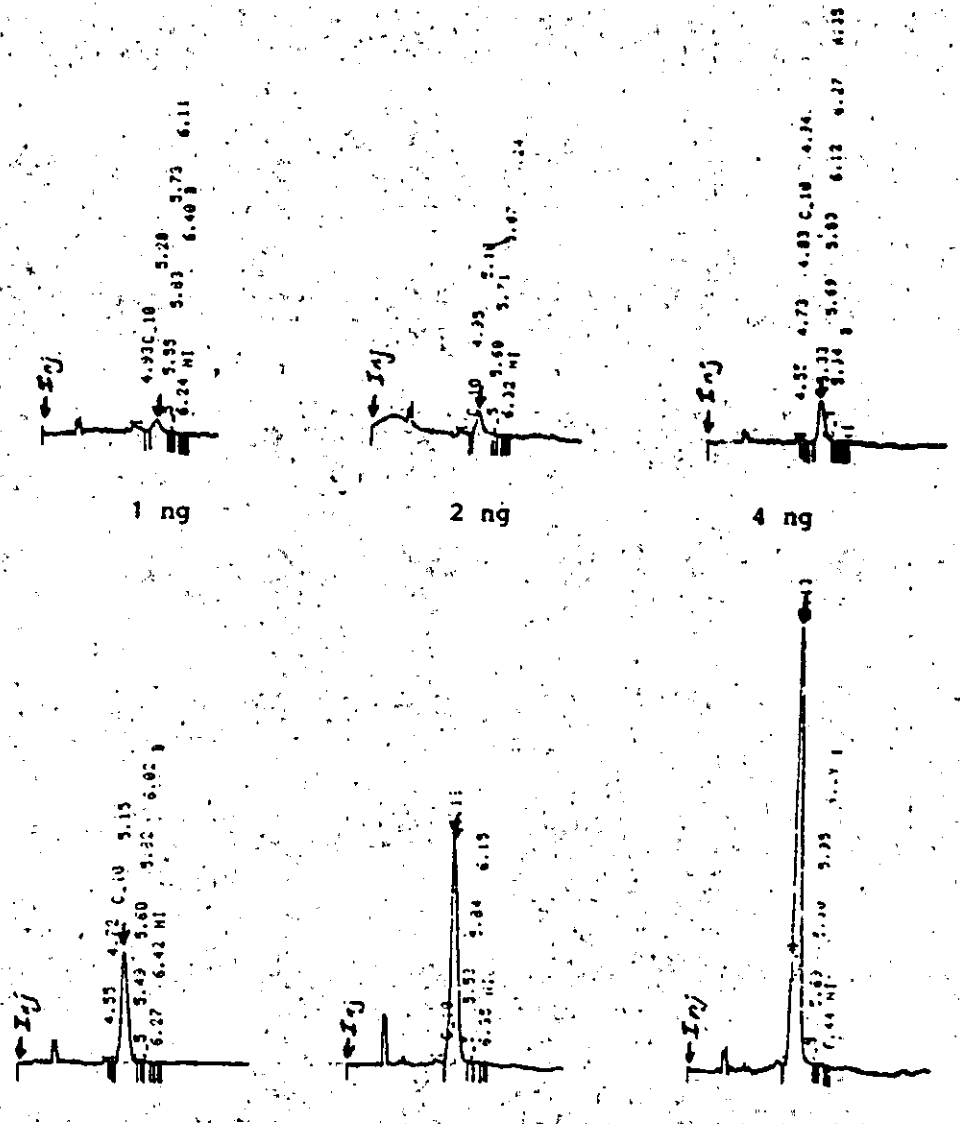
Elute with 15 ml of 15% MeOH/acetonitrile.

Collect the eluant and evaporate the solvent. Dissolve residue in 0.5 ml acetonitrile.

HPLC

PAGE 14 of 15	METHOD NO. AG-498	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION		
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 2: TYPICAL CHROMATOGRAMS OF CGA-136872 STANDARDS



1 ng 2 ng 4 ng

10 ng 20 ng 40 ng

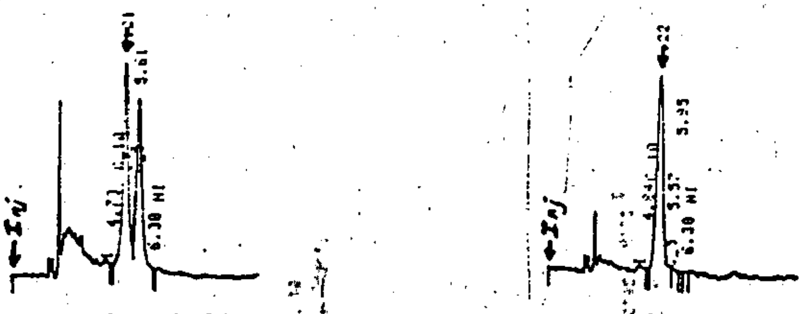
PAGE 15 of 15	METHOD NO. AG-498	SUBJECT DETERMINATION OF CGA-136872 IN SOIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
EDITION		
SUBMITTED BY: W. T. Beidler, K. P. Shoffner		
		APPROVED BY:

FIGURE 3: TYPICAL CHROMATOGRAMS FOR NEBRASKA SOIL (AG-A 9486-01)

Control Soil*
200 mg injected
<1 ng CGA-136872
<0.01 ppm

Control + 0.01 ppm
200 mg injected
1.95 ng found
101% recovery

Control + 0.05 ppm
200 mg injected
8.60 ng found
89% recovery



Control + 0.20 ppm
100 mg injected
17.2 ng found
89% recovery

Control + 1.00 ppm
20 mg injected
17.6 ng found
91% recovery

*Moisture content of control soil was 17%.

Note: The peak at 5.6 minutes occurs intermittently in samples but does not interfere with quantitation.