

## ORGANICS LABORATORY

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Method No.	Edition	Revision
CHR-12111672	01/03/89	
Subject:		
Determination of GS-11526 Residues in Soil Using HPLC		
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References:		

### 1.0 SCOPE

This method describes the procedure for extraction, cleanup and quantitation of GS-11526 in soil. This method is sensitive to 0.01 ppm.

### 2.0 PRINCIPLE

GS-11526 is extracted from soil by Soxhlet extraction using methanol:water (80:20). Any parent compound in the extract is removed by the use of a hexane partition.

### 3.0 CHEMICALS AND SOLUTIONS

GS-11526 reference standard (CIBA-GEIGY Corporation)  
Hexane, Optima, Fischer Scientific or equivalent  
Methanol, Anhydrous, J.T. Baker Inc. or equivalent  
Methanol:Water (80:20)  
Sodium Citrate  
Octanesulfonic acid sodium salt  
Water, deionized  
Phosphoric Acid

#### 4.0 APPARATUS

##### 4.1 Equipment

Extraction thimble, Whatman cellulose,  
33 x 94 mm.  
Boiling chips  
C-18 Bond Elut cartridge, 6 cc,  
Analytichem Int.  
Vacuum box  
Rotary Film Evaporator

##### 4.2 Glassware:

Soxhlet extractor  
Boiling Flask, 500 mL  
Graduated Cylinders, 250 mL and 25 mL  
Volumetric flask, 10 mL  
Pipettes, Dispo Pasteur  
Separatory Funnel, 125 mL  
Vial, Auto Sampler (4 mL) with screw cap and  
teflon septum

##### 4.3 HPLC

Pump: Waters 6000 A Solvent Delivery  
System or equivalent.  
Autosampler: Waters WISP 710 B or equivalent.  
Column: Beckman Ultrasphere 4.6mm x 15cm  
reverse phase C-18 ion pairing  
5 um particle size.  
Detector: Gilson UV variable Wavelength  
or equivalent set at 240 nm and  
0.1 AUFS.  
Data System: Maxima Data System, Dynamic  
Solutions or equivalent.  
Mobile Phase: Methanol:Water (60:40), 0.01 M  
Sodium Citrate, 0.02 M  
Octanesulfonic Acid.  
pH = 4.0 with concentrated  
Phosphoric Acid.

#### 5.0 ANALYTICAL PROCEDURE

##### 5.1 Extraction

5.1.1 Weigh 50 g (to 0.001g) of soil into an  
extraction thimble. Place a cotton  
plug atop the thimble to hold soil in  
place.

5.1 Extraction (continued)

5.1.2 Add 250 mL of methanol:water (80:20) and boiling chips to a 500 mL boiling flask and assemble the Soxhlet apparatus with cooling water flowing through the condenser.

5.1.3 Extract 10-14 hours, depending on extractability of the particular soil type. Time the removal of the boiling flask and Soxhlet apparatus from mantle to minimize the extract solution retained in the boiling flask.

5.1.4 Evaporate the extract to less than 20 mL on the rotary evaporator. Transfer extract to a 25 mL graduated cylinder and adjust to 20 mL with deionized water.

5.1.5 Pour extract and water from the graduated cylinder into a 125 mL separatory funnel.

5.2 Sample Cleanup

5.2.1 Add 25 mL of hexane and shake the sample for one minute.

5.2.2 Drain off the aqueous layer into a 25 mL graduated cylinder.

5.2.3 Drain and discard the hexane layer.

5.2.4 Transfer the sample back to the 125 mL separatory funnel and repeat the wash with another 25 mL of hexane.

5.2.5 Place C-18 Bond Eluts on a vacuum box. Prime Bond Eluts by eluting 2 volumes of approximately 5 mL methanol, followed by 2 volumes of approximately 5 mL deionized water, making sure not to let the packing go dry.

5.2.6 Add the sample from the graduated cylinder to the Bond Elut, eluting the waste drop wise. Wash the sample with 10 mL of deionized water. Elut CS-11526 with approximately 7 mL of the mobile phase used in analysis. Adjust the volume with mobile phase to 10 mL.

## 5.2 Sample Cleanup (continued)

in a volumetric flask. Transfer to an auto sampler vial for analysis by HPLC.

## 6.0 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS

The final determination of GS-11526 is performed on an HPLC equipped with a reverse phase column and a UV detector set at 240 nm. The pump flow rate is set at 0.8 mL/min. and the column and precolumn are kept at 35 C by the use of a column heater.

### 6.1 Standardization

- 6.1.1 Make up standard solutions by serial dilutions of a known amount of the reference standard.
- 6.1.2 Inject constant volumes (100 uL) of known amounts of standard on the HPLC.
- 6.1.3 Measure the standard peak heights.
- 6.1.4 Construct a Linear Regression by plotting peak height against concentration.

### 6.2 Residue Determination

- 6.2.1 Inject the solution from 5.2.5 at the same volume used for the standards (100 uL).
- 6.2.2 Measure the peak height.
- 6.2.3 Determine the amount of GS-11526 in the sample by inserting the peak height into the equation of the line obtained from 6.1.4
- 6.2.4 Calculate sample residue as follows:

$$\text{ppm} = \frac{\text{ng residue found}}{\text{mg sample injected} \times (1-M) \times R}$$

M = soil moisture content

R = recovery factor based on fortified controls carried through procedure