ORGANICS LABORATORY

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Method No.	Edition	Revision
C30023	2	2

Subject:

Determination of CGA-163935 and CGA-179500 in Soil using High Performance Liquid Chromatography

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References: Developed at MVTL and ABC labs

1.0 SCOPE

This method describes the procedure for extracting and analyzing soil samples for CGA-163935 and CGA-179500 residues. The screening limit for this residue is 0.01 ppm in soil.

2.0 PRINCIPLE

CGA-163935 and CGA-179500 residues are extracted from soil by shaking with an extracting solution of 30% methanol/70% phosphate buffer. The soil extracts are filtered and pH is adjusted to about 3. CGA-163935 and CGA-179500 are removed from the soil extracts by partitioning the extracts against dichloromethane. The dichloromethane is evaporated to near dryness and the sample is redissolved in Final Sample Solution. The resulting extract is then analyzed by HPLC.

3.0 CHEMICALS AND SOLUTIONS

3.1 Chemicals

3.1.1 Acetic acid, glacial
3.1.2 Acetonitrile, analytical grade
3.1.3 CGA-163935 and CGA-179500 Standard Reference Material
3.1.4 Dichloromethane, analytical grade
3.1.5 Isopropanol, analytical grade
3.1.6 Methanol, HPLC grade or equivalent
3.1.7 Phosphoric acid
3.1.8 Potassium phosphate monobasic
3.1.9 Sodium hydroxide, reagent grade
3.1.10 Sodium phosphate dibasic
3.1.11 Water, deionized/reverse osmosis

3.2 Solutions

3.2.1 1N Sodium Hydroxide Solution: 4 grams NaOH dissolved in 100 mL water.

3.2.2 Extraction buffer: 5.82 grams Na_2HPO_4 and 3.81 grams KH_2PO_4 dissolved in 1 liter water. This corresponds to a 0.041M Na_2PO_4 and 0.028M KH_2PO_4 solution.

3.2.3 Extraction Solution: Mix 300 mL methanol and 700 mL extraction buffer. Adjust pH to 8.0 with 1N NaOH.

3.2.4 Buffer: Dissolve 136.09 grams KH_2PO_4 in 1 liter water. Add 50 mL H_3PO_4.

3.2.5 HPLC Mobile Phase: Mix 450 mL methanol, 546 mL water, 3 mL glacial acetic acid and 1 mL phosphoric acid. Filter under vacuum through a 0.45 micron filter.

3.2.6 Sample dilution Solvent: Mix 690 mL water, 300 mL methanol and 10 mL glacial acetic acid.

3.2.7 Methanol/Water Solution: Mix 500 ml methanol with 500 ml water.

3.2.8 Standard Dilution Solvent: Mix 699 ml water with 300 ml methanol and 1 ml glacial acetic acid.

4.0 APPARATUS

4.1 Equipment

4.1.1 Centrifuge, capable of holding 200 ml bottles and capable of 1500 rpm
4.1.2 Glass Wool, silanized
4.1.3 Graduated Cylinder, 100 ml
4.1.4 pH meter
4.1.5 Rotary Film Evaporator
4.1.6 Shaker, reciprocating, capable of 200 rpm
4.1.7 Teflon Stir Bar and Magnetic Stirrer
4.1.8 Ultrasonic Cleaner, Branson 2200 or equivalent

4.2 Glassware

Standard laboratory glassware

4.3 Preparation

Glassware for this method should be prepared by MVTL SOP 21-18, Silanizing Glassware.

4.4 HPLC

4.4.1 Column: Supelco LC8DB, 15cm X 4.6mm ID 5 micron particle size, catalog # 5-8347, or equivalent
4.4.2 Detector: Gilson 116 variable wavelength UV detector at 280 nm, or equivalent
4.4.3 Injector: Shimadzu SIL-6B, or equivalent
4.4.4 Pump: Shimadzu LC-6A, or equivalent
4.4.5 Recorder: Waters 860 data system, or equivalent

5.0 ANALYTICAL PROCEDURE

5.1 Sample Preparation

Samples for this method should be prepared for analyses by MVTL SOP 21-16.

5.2 Extraction

5.2.1 Weigh 50 grams (to +/- 0.01 g) of well homogenized soil into a 200 ml glass centrifuge bottle. **Be sure that the sample is maintained frozen prior to weighing and that the unused portion of the sample is returned to the freezer immediately after weighing.**

5.2.2 Add 100 ml of extracting solution to the centrifuge bottle and seal with an aluminum foil lined cap.

5.2.3 Shake for one half hour on a wrist action shaker and centrifuge for 15 min at 1500 rpm.

5.2.4 Decant the resulting liquid through a funnel fitted with a glass wool (silanized) plug into a 400 ml beaker. Do not discard the soil.

5.2.5 Again add 100 ml of extracting solution to the centrifuge bottle containing the soil sample and replace the foil lined cap. Shake for one hour on the wrist action shaker, centrifuge for 15 min at 1500 rpm and decant the resulting liquid following step 5.2.4. This will result in combining the resulting liquids from this step and step 5.2.4.

5.2.6 Rinse the glass wool plug with 25 ml of extraction solution and allow the solution to drain into the 400 ml beaker.

5.2.7 Add a teflon coated stir bar to the beaker, place beaker on a magnetic stirrer at a moderate speed.

5.2.8 While continuously monitoring pH with a pH meter, add 25 ml-35 ml of buffer (3.2.4) then add 1.2M phosphoric acid until a pH of 3.0 (+/- 0.05) is reached.

5.2.9 Pour 25 ml of a 50/50 methanol/water solution into a 500 ml separatory funnel fitted with a teflon cap and stopcock. Add the acidic solution from step 5.2.8 to the separatory funnel.

5.2.10 Add 100 ml of methylene chloride to the separatory funnel and shake for one minute. Drain the methylene chloride layer (bottom layer) into a 500 ml boiling flask. If an emulsion layer forms during this step use a glass stir rod to break it up.

5.2.11 Add 50 ml of a 50/50 methanol/water solution to the separatory funnel. Repeat the methylene chloride partition from step 5.2.10 and again add the methylene chloride layer to the 500 ml boiling flask, combining the two methylene chloride extractions.

5.2.12 Add a third 100 ml portion of methylene chloride to the separatory funnel, and shake for one minute. Again drain the bottom layer into the 500 ml boiling flask, combining the three methylene chloride partitions.

5.2.13 Add 1 ml of the final sample solution to the 500 ml boiling flask. Remove the methylene chloride from the sample with a rotary film evaporator, evaporate to near dryness. The water bath of the evaporator should be maintained near 25° C. If sample becomes dry with a rotary film evaporator, add 1 mL of final sample solution and sonicate for 2 min. using an ultrasonic bath or equivalent.

5.2.14 Using a disposable pastuer pipette, add about 1 ml of the final sample solution to the boiling flask. Rotate the flask to redissolve as much of the sample as possible. Transfer the resulting solution to a 5 ml volumetric flask. Repeat this step until the 5 ml volumetric flask is brought to volume.

6.0 HPLC Analyses

The final determination of CGA-163935 and CGA-179500 is performed on a HPLC system equipped with a reverse phase column and a UV detector, both specified in section 4.4 of this method. The flow rate for the mobile phase is 2 ml/min. The mobile phase is specified in section 3.2 of this method.

6.1 Standardization

6.1.1 Make up standard solutions by serially diluting a known amount of the reference standard in Standard Dilution Solvent, section 3.2 of this method.

6.1.2 Inject constant volumes (250 uL) of standards of known concentration on the HPLC.

6.1.3 Measure the standard peak areas.

6.1.4 Plotting peak area vs. concentration, calculate the best fit line using linear regression.

6.2 Residue Determination

6.2.1 Inject the sample from 5.2.13, using the same volume as the standard injections.

6.2.2 Measure the peak area.

6.2.3 Determine the concentration (ppm) of CGA-163935 and CGA-179500 in the sample aliquot injected by inserting the peak area into the equation of the line obtained in 6.1.4.

6.2.4 Calculate the residue as follows:

$$\text{ppm(sample)} = \frac{(\text{extraction conc ug/ml})\,(\text{dilution ml})\,(\text{dilution factor})}{(\text{grams of sample})}$$

7.0 NOTES AND COMMENTS

7.1 Recovery of near screening level samples

Samples which are spiked for recovery in the 0.01 to 0.02 ppm in soil range will have a less than normally accepted percent recovery for the CGA-179500 analyte in some cases. Since these results are reproducible in the range of 50-70%, it has been requested by Ciba-Geigy to continue collecting and reporting data to 0.01 ppm.