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

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EDITION 12/31/86		ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-131036 IN SOIL BY COLUMN SWITCHING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: M. L. Edmonds		
		APPROVED BY:

1.0 SCOPE

This method is used for the determination of CGA-131036, N-(6-methoxy-4-methyl-1,3,5-triazin-2-yl-aminocarbonyl)-2-(2-chloroethoxy)benzenesulfonamide, in soil. This method is a modification of AG-493 wherein the Bond Elute CN column clean-up of AG-493 is replaced by the first of two HPLC columns. This modification allows the limit of determination of the method to be decreased to 0.01 ppm as established by the lowest fortification levels.

The chemical structure for CGA-131036 is presented in Figure 1.

2.0 PRINCIPLE

A representative soil sample is extracted by shaking with 1:1 methanol:sodium carbonate buffer (pH 9). After filtering, a 5-g aliquot of extract is diluted with water and acidified with phosphoric acid. Residues of CGA-131036 are partitioned into methylene chloride and determined by high performance liquid chromatography (HPLC) using a Lichrosorb - CN column coupled with an analytical Zorbax-ODS column and UV detection at 232 nm.

A flow diagram for the method is presented in Figure 2.

3.0 APPARATUS

3.1 Bottle, Boston round, narrow mouth, amber, 8-oz.

3.2 Filter paper, Reeve Angel® 802, and Whatman® 2V, 24-cm.

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

3.4 Funnel, long stem, 10-cm size.

3.5 Funnel, separatory, 250-ml. with Teflon® stop cock.

3.6 Glass wool.

3.7 Graduated cylinder, 50-ml, 100-ml.

3.8 Jar, square, amber wide mouth, 16-oz.

3.9 Mechanical shaker.

3.10 Rotary evaporator, Büchi® or equivalent.

3.11 Vials, Wheaton, 1-ml.

4.0 REAGENTS

4.1 Acetonitrile, HPLC grade.

4.2 25% Acetonitrile/75% 0.001M phosphoric acid containing 0.5% tetrabutylammonium bromide.

4.3 30% Acetonitrile/70% phosphate buffer containing 0.5% tetrabutylammonium bromide.

4.4 Carbonate buffer (pH 9): 10.5 grams sodium carbonate + 8.4 grams sodium bicarbonate in one liter of water (0.1M each reagent).

4.5 Disodium hydrogen phosphate (Reagent Grade).

4.6 1:1 Methanol:carbonate buffer.

4.7 Methanol, HPLC grade.

4.8 Methylene chloride, HPLC grade.

4.9 Phosphate buffer (pH 7): 1:1 mixture of 0.062M Na_2HPO_4:0.042M KH_2PO_4.

4.10 1.2M Phosphoric acid.

4.11 Phosphoric acid (85%), Reagent Grade.

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4.12 Potassium dihydrogen phosphate (Reagent Grade).

4.13 Sodium bicarbonate, reagent grade.

4.14 Sodium carbonate, reagent grade.

4.15 Standard CGA-131036 (available from CIBA-GEIGY Corporation, P.O. Box 18300, Greensboro, NC 27419).

4.16 Tetrabutylammonium bromide (Fluka AG).

4.17 Water, distilled.

4.18 Water, (distilled and deionized).

5.0 PROCEDURE

5.1 Extraction

5.1.1 Weigh a 20-gram subsample from a well-homogenized stone-free soil sample into a 16-oz. square amber jar. Add 200 ml of 1:1 methanol:carbonate buffer and cap the jar using a plastic liner to prevent solvent losses during shaking.

<u>Note</u>: A subsample for soil moisture determination should also be taken at this point.

5.1.2 Extract the soil sample by shaking on a mechanical shaker for two hours.

5.1.3 Filter the extracted sample through a filter consisting of a ball of glass wool inside a Reeve Angel filter paper, inside a Whatman 2V filter paper, into an 8-oz. Boston round bottle.

5.2 Partition

5.2.1 Transfer a 5-g aliquot (~ 51 ml, volume corrected for moisture content of soil) of the extract from Section 5.1.3 into a 250-ml separatory funnel.

Add 100 ml of water and 10 ml of 1.2$\underline{M}$ phosphoric acid. Check the pH of the solution, which should be about 4, with pH paper. Adjust the pH to ≤ 4 by addition of 1.2$\underline{M}$ phosphoric acid, if necessary.

5.2.2 Add 25 ml of methylene chloride to the separatory funnel and shake vigorously for 30 seconds. Allow the layers to separate, then drain off the lower, methylene chloride layer into a 100-ml round-bottom flask, being careful not to transfer any aqueous layer to the round bottom flask.

5.2.3 Repeat Section 5.2.2 and combine the methylene chloride fractions in the 100-ml round bottom flask.

5.2.4 Evaporate the contents of the round bottom flask to approximately 5 to 10 ml and quantitatively transfer the sample to a 50-ml round bottom flask. (The 50-ml round bottom flask is used in order to reduce the surface area available when dissolving the residue before HPLC analysis). Re-evaporate the sample to dryness at 40°C. If any moisture remains after evaporation, add 1-2 ml of acetonitrile to the flask and re-evaporate.

6.0 DETERMINATION OF CGA-131036

6.1 Preparation of Standard CGA-131036

6.1.1 Weigh 100.0 mg of CGA-131036 analytical standard into a 100-ml volumetric flask and dilute the contents of the flask to the mark with acetonitrile. Prepare serial dilutions of the 1.0 mg/ml standard solution in acetonitrile to give a series of fortification standards.

6.1.2 Prepare serial dilutions of the 1.0 mg/ml standard solution in 25:75 acetonitrile:water to give a series of injection standards in a range of 0.0125 to 0.25 ng/µl of CGA-131036.

6.2 Column-Switching HPLC System

6.2.1 Install the HPLC System according to Table I and Figure 3. The valve used in this system is a Valco Model EC6W 6-port injection valve with electric actuator (Valco Instrument Co., Inc., Post Office Box 55603, TX 77255). The valve is controlled using a Kratos Spectroflow Model 783G Programmable Absorbence Detector and the program is started by a signal from the WISP injector.

Control of the switching valve can be accomplished manually or by use of computer or other time programming equipment. Other controller and valve combinations used in this laboratory have been the Waters Model 590 Solvent Delivery System which time-programs the valve of a Waters WAVS and a valve unit and a Chrontrol Model CD-4S Timer (Lindberg Enterprises, Inc., 9707 Candida St., San Diego, CA 92126) which can control the Valco Model EC6W valve.

6.2.2 Determine the retention time of CGA-131036 on Column 1 by connecting Column 1 directly to the detector and injecting 50 ng of the standard. (Inject 200 µl of the 0.25 ng/µl standard solution prepared in Section 6.1.2.)

6.2.3 Reconnect the system as shown in Figure 3. Program the valve to start the CUT mode at about one-half minute before the retention time of CGA-131036 and return to the NORMAL mode at about one-half minute after the retention time.

6.2.4 Inject 50 ng of CGA-131036 and determine its retention time through the two columns and to confirm that the valve time programming is correct.

6.3 Standardization

6.3.1 Standardize the high performance liquid chromatograph by injecting 200-µl aliquots of injection standards under the conditions specified in Table I. This repreents a working range of 2.5 to 50 ng of CGA-131036.

6.3.2 Measure the peak heights of the injected standards either electronically or manually. Typical standard chromatograms are shown in Figure 4 and typical standardization data are shown in Table II. A typical standard curve is shown in Figure 5.

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

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6.4 Determination of Sample Residues

6.4.1 Dissolve the flask residue from Section 5.2.4 in 2.0 ml of 75:25 acetonitrile:water and ultrasonicate. Swirl and place a portion of the dissolved sample into a 3-ml HPLC autosampler vial.

6.4.2 Inject a 200-µl aliquot of the sample from Section 6.4.1 into the HPLC under the same conditions as for standards. Make appropriate dilutions of the samples to bring the 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-131036 present in the injected aliquot. Typical chromatograms for control and procedural recovery soil samples are shown in Figures 4-8.

6.4.2 Calculate residue results in terms of ppm CGA-131036 by the following equation:

$$\text{PPM} = \frac{\text{ng CGA-131036 Found}}{\text{mg Soil Injected}} \div \text{R}$$

The recovery factor (R) is determined using a fortified control sample carried through the procedure and is expressed as a decimal (100% = 1.00, etc.)

If results are to be reported in a dry weight basis use the following equation:

$$\text{Dry weight (ppm)} = \text{wet weight (ppm)} \div \frac{100 - \text{soil moisture (\%)}}{100}$$

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TABLE I: HPLC CONDITIONS FOR ANALYSIS OF CGA-131036

Instrument:	Waters WISP 712B Sample Injector using two Kratos Spectroflow 400 Solvent Delivery Systems or equivalent
Switching Valve Unit:	Valco Model EC6W 6-port sample injection valve with electric actuator
Columns*:	#1 Lichrosorb-CN, 250x4 mm, ~ 10 μm #2 Zorbax-ODS, 4.6x250 mm, ~ 5-6 μm
Mobile Phase:	Column #1 - 0.5% Tetrabutylammonium bromide in 25% acetonitrile:75% 0.001M phosphoric acid Column #2 - 0.5% Tetrabutylammonium bromide in 30% acetonitrile:70% pH:7 phosphate buffer
Flow Rate:	1.0 ml/min. for both pump flows
Column Switching Cut Time:	7.4-8.4 min.
Temperature:	Ambient
Detector:	Spectroflow 783G Variable Wavelength UV Detector or equivalent. This detector is used to time program the valve.
Wavelength:	232 nm
Attenuation:	0.10 AUFS - 0.0 min. to ~ 5.0 min. 0.01 AUFS - ~ 15.0 min. to ~ 25 min.

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TABLE I: HPLC CONDITIONS FOR ANALYSIS OF CGA-131036 (continued)

Minimum Detection Limit:	2.5 ng
Injection Volume:	200 µl
Chart Speed:	0.5 cm/min.
Retention Time:	~7.5 minutes for column 1 and 20.0 minutes for both.[1]

[1] Retention times and column switching times are subject to slight variations depending on the mobile phase preparation or HPLC system.

NOTE: Determine column switching time for every new batch of mobile phase or equivalent HPLC system.

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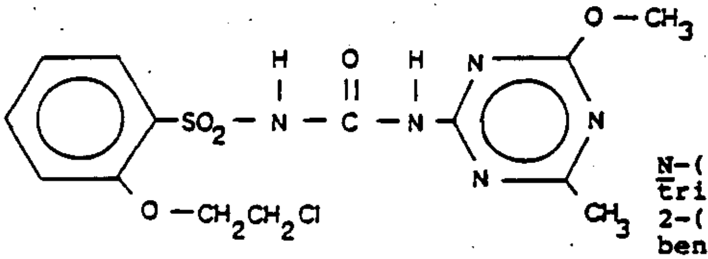
FIGURE 1: CHEMICAL NAME AND STRUCTURE



CGA-131036

N-(6-methoxy-4-methyl-1,3,5-triazin-2-yl-amino-carbonyl)-2-(2-chloroethoxy) benzenesulfonamide

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FIGURE 2: FLOW DIAGRAM FOR ANALYTICAL METHOD AG-509

20 g soil sample
↓
Shake 2 hrs. with 200 ml of 1:1 methanol:carbonate buffer
↓
Filter
↓
Dilute a 5-g aliquot with 100 ml of water
+ 10 ml of 1.2M phosphoric acid
↓
Partition with 2x25 ml methylene chloride
↓

↓
Aqueous (Discard)

↓
Methylene chloride
↓
Evaporate to dryness
↓
Add 2.0 ml of 25:75
acetonitrile:water
↓
Column switching HPLC
(see Figure 3)

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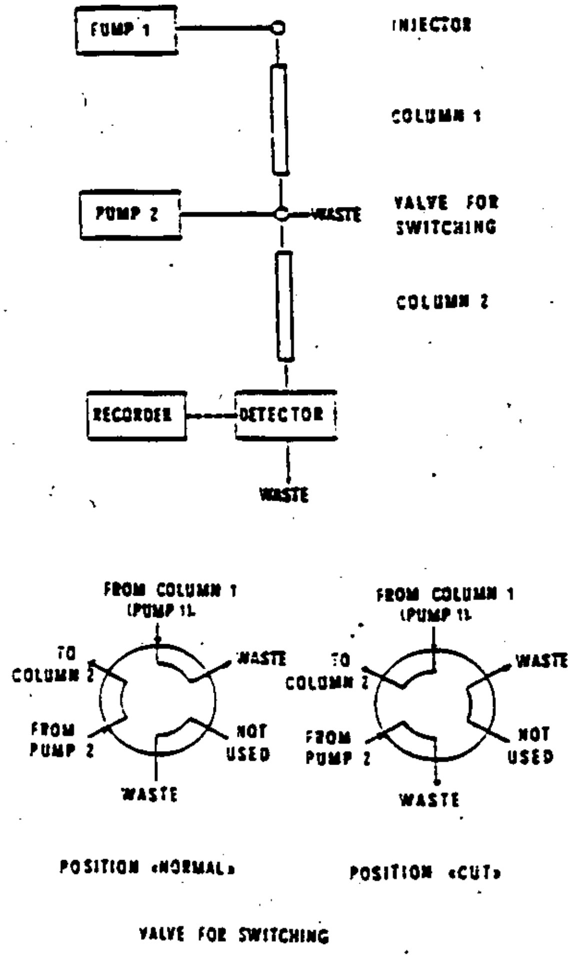
FIGURE 3: CONFIGURATION OF TWO COLUMN SWITCHING HPLC SYSTEM



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EDITION 2/13/87	AMENDMENT 1	ANALYTICAL METHOD FOR THE DETERMINATION OF CGA-131036 IN SOIL BY COLUMN SWITCHING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
SUBMITTED BY: M. L. Edmonds		
		APPROVED BY:

This amendment is issued to correct a typographical error in Section 6.4.1, page 7 of 9. The first sentence should read -

> "Dissolve the flask residue from Section 5.2.4 in 2.0 ml of 25:75 acetonitrile:water and ultrasonicate."

instead of

> "Dissolve the flask residue from Section 5.2.4 in 2.0 ml of 75:25 acetonitrile:water and ultrasonicate."