ORGANICS LABORATORY

Minnesota Valley Testing Laboratories, Inc.
1126 North Front Street
New Ulm, MN 56073

Method No.	Edition	Revision
T75123	05/03/90	Two

Subject:
Determination of CGA-131036 in water by High Performance Liquid Chromatography

Submitted by: Judy Ibberson

Approved by: Kim Sjogren

References: none

1.0 SCOPE

This method describes the determination of CGA-131036, N-(5-methoxy-4-methyl-1,3,5-triazin-2-yl-aminocarbonyly)-2-(2-chloroethoxy) benzenesulfonamide, in water. The limit of detection is 0.10 ppb.

2.0 PRINCIPLE

A representative water sample is adjusted to pH 2.5 with 1% HCl and then extracted with methylene chloride. The methylene chloride is then evaporated to near dryness and the CGA-131036 is back-extracted into a carbonate:bicarbonate buffer solution. The pH of the buffer is then adjusted to 2.5 and the sample is cleaned up using C-18 solid phase extraction. The CGA-131036 concentration is determined using HPLC with a photo-conductivity detector.

3.0 APPARATUS

3.1 Bond Elut C-18 cleanup columns, 3-mL (Analytichem International, Inc., Harbor City, CA).

3.2 Extrelut reservoir.

3.3 Beaker, 250-mL and 100-mL.

3.4 Separatory funnel, 250-mL and 125/60 mL, with Teflon stop cock.

3.5 Flask, round bottom, 250-mL.

3.6 Rotary evaporator, Haaki Buchler PE-6 or equivalent.

3.7 pH meter, Orion model 701 or equivalent.

3.8 Vials, Wheaton, 2-mL.

3.9 Filter, 0.5 um solvent resist.

3.10 Test tube, screwcap with Teflon faced septa.

3.11 Solid Phase Extraction Vacuum Manifold, Supelco catalog #5-7030, or equivalent.

3.12 Vortex mixer.

3.13 Plastic adaptor.

3.14 Solvent guide needle.

4.0 REAGENTS

4.1 Cyclohexane, Fisher HPLC grade.

4.2 Methanol, Fisher HPLC grade.

4.3 Isopropanol, Fisher HPLC grade.

4.4 Methylene Chloride, Pesticide residue grade.

4.5 Ethyl Acetate, Fisher HPLC grade.

4.6 0.1 M Sodium carbonate, 0.1 M Sodium bicarbonate buffer (12.4 g Na_2CO_3 - H_2O/ 8.4 g $NaHCO_3$ in one liter deionized water).

4.7 Glacial Acetic acid.

4.8 Solution I (750 parts cyclohexane, 125 parts methanol, 125 parts 2-propanol).

4.9 Solution II (1000 parts 2-propanol, 1 part glacial acetic acid, 1 part deionized water).

4.10 HPLC mobile phase (920 parts Solution I, 80 parts Solution II).

4.11 1% Hydrochloric acid.

4.12 Sodium bicarbonate, reagent grade.

4.13 Sodium carbonate, reagent grade.

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

4.15 Water, deionized.

5.0 PROCEDURE

5.1 Sample Pretreatment

5.1.1 Filter the water sample through a Millipore HVLP 04700 filter.

5.1.2 Measure individual 100.0 gram samples, to the nearest 0.01 g, into a 250-mL beaker.

5.2 pH Adjustment

5.2.1 Adjust the pH of the sample to 2.5 +/-0.02 by dropwise addition of 1% HCl.

5.3 Methylene Chloride Partition

5.3.1 Transfer the sample from Section 5.2.1 to a 250-mL separatory funnel.

5.3.2 Rinse the 250-mL beaker with 50 mL of methylene chloride and pour it into the separatory funnel.

5.3.3 Shake the contents of the separatory funnel vigorously for one minute.

5.3.4 Drain the bottom methylene chloride layer into a 250-mL round bottom boiling flask.

5.3.5 Add another 50 mL portion of methylene chloride to the separatory funnel; repeat Sections 5.3.3, 5.3.4, and 5.3.5 two more times, for a total of 150 mL methylene chloride.

5.3.6 Evaporate the methylene chloride extracts using a rotary evaporator with a water bath temperature of 35°C until approximately 2-5 mL remains. DO NOT TAKE SAMPLE TO DRYNESS.

5.4 Aqueous Buffer Partition

5.4.1 Transfer the methylene chloride from the 250-mL boiling flask from Section 5.3.6 to a 60-mL separatory funnel.

5.4.2 Rinse the 250-mL boiling flask with three 1-mL portions methylene chloride. Combine all rinses into the separatory funnel.

5.4.3 Add 5 mL of carbonate:bicarbonate buffer to the separatory funnel. Shake moderately for one minute.

5.4.4 Drain the bottom methylene chloride layer into a 100-mL beaker. Drain the buffer solution into another 100-mL beaker.

5.4.5 Add the methylene chloride layer back to the separatory funnel and repeat Sections 5.4.3 and 5.4.4 two more times, rinsing the 100-mL beaker with the carbonate:bicarbonate buffer each time.

5.5 Solid Phase Cleanup

5.5.1 Wash a C-18 Bond Elut cartridge with 5 ml of methanol followed by 5 ml deionized water using the vacuum manifold. Do not allow the cartridge to go dry.

5.5.2 Adjust the pH of the carbonate:bicarbonate extract from Section 5.4.5 to 2.5 by adding 1% HCl dropwise. Some foaming may occur during this pH adjustment.

5.5.3 Attach a reservoir to the top of the C-18 cartridge and pour the extract from Section 5.5.2 into the reservoir. Draw the sample through the cartridge at a rate of 2 drops per second. Rinse the beaker with 2 ml of DI water. Transfer, and drain through cartridge.

5.5.4 Elute the cartridge with 5 mL ethyl acetate into an appropriate collection vessel.

5.5.5 Transfer the ethyl acetate fraction to a screwcap test tube and evaporate to dryness using a gentle stream of nitrogen and a warm water bath (40°C).

5.5.6 Cap the test tube and place in a refrigerator until analyses by HPLC.

6.0 DETERMINATION OF CGA-131036

6.1 Preparation of Standard CGA-131036

6.1.1 Weigh 10.0 mg of CGA-131036 analytical standard into a 100 mL volumetric flask and dilute the contents of the flask to the mark with methylene chloride. Pipette 1.0 mL of the stock standard into another 100 mL volumetric flask and gently evaporate to dryness with a stream of nitrogen. Dilute to volume with Solution II and mix well. This represents a 1 ug/mL working standard.

6.1.2 Prepare serial dilutions of the 1 ug/mL standard solution at 0.50, 0.20, 0.10, 0.05, 0.02, 0.01 ug/mL using Solution II as a diluent.

6.2 Standardization

6.2.1 Standardize the high performance liquid chromatograph by injecting 10 uL aliquots of injection standards under the conditions specified in Table I. This represents a working range of 0.10 to 10.0 ng of CGA-131036.

6.2.2 Measure the peak heights of the injected standards and construct a standard curve by plotting peak heights vs nanograms injected.

6.3 Determination of Sample Residues

6.3.1 Pipette 1.0 mL of Solution II into the test tube containing the sample, cap using a Teflon faced septa, and vortex to dissolve the residue. Assemble a syringe fitted with a 0.5 um solvent resistant membrane filter and filter the sample into a 2 mL vial. Seal the vial with a Teflon faced septa.

6.3.2 Inject a 10 uL aliquot of the sample from Section 6.3.2 into the HPLC under the same conditions as for standards. Compare the peak heights of the unknown samples with the standard curve to determine the nanograms of CGA-131036 present in the injected aliquot.

6.3.3 Calculate residue results in terms of ppb CGA-131036 by the following equation:

$$PPB = \frac{\text{ng CGA-131036 Found} \times \frac{1.0\ \text{mL}}{0.01\ \text{mL}}}{\text{grams sample}}$$

TABLE I: HPLC CONDITIONS FOR ANALYSIS OF CGA-131036

Instrument:	Kratos Spectroflow 400 Solvent Delivery System, Rheodyne Model 7125 Injection Valve with a 20 uL loop
Column:	Waters u-porasil (30 cm x 3.9 mm) Catalog #27477
Mobile Phase:	920 parts Solution I, 80 parts Solution II The use of helium sparging is strongly recommended
Flow rate:	0.5 mL/minute
Detector:	Tracor Model 965 Photoconductivity Detector equipped with a mercury lamp. The detector is modified by removing the solvent pump and the zinc lamp transformer. Range setting of 1 and recorder attenuation set to accommodate the amount of analyte injected
Temperature:	35°C using a column heater (Eldex Catalog #725-1010), or equivalent
Recording Device:	Kipp and Zonen strip chart recorder Model BD40 at a chart speed of 0.2 mm/sec and input voltage of 10 mv
Minimum Detection Limit:	0.1 ng
Retention Time:	10.5 minutes

NOTES: After repeated injections of samples onto the column, a reduction in sensitivity and instability of the baseline will occur. To correct this problem the column is reconditioned with a mobile phase consisting of 10 parts 2-propanol, 10 parts methanol, 5 parts glacial acetic acid and 1 part water at a flow rate of 1 mL/min and a temperature of 50°C for several hours. This conditioning solvent must be flushed from the column and then replaced with the normal operating mobile phase. Generally an hour of flushing at 0.5 mL/min will be sufficient. This same treatment will be necessary when a new column is installed.