Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Difenzoquat Methyl

MRID #: 438043-02

Matrix: Soil

Analysis: GC/NPD

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Recommended Method of Analysis

AVENGE* difenzoquat methyl sulfate (CL 84,777): GC Method for the Determination of CL 84,777 Residues in Soil

A. Principle

CL 84,777 is extracted from soil with hydrochloric acid in methanol, after the soil was mixed with sea sand and packed in a chromatographic column. The acid-methanol extract is concentrated, coextractives are removed with hexane and then CL 84,777 is partitioned into methylene chloride. Final cleanup is achieved with alumina column chromatography. The eluent is evaporated and the residue is dissolved in a measured volume of acetone. The quantitation of CL 84,777 is effected by gas chromatography using a nitrogen-sensitive detector and the external standardization technique. The validated sensitivity of the method is 0.05 ppm.

B. Reagents

1. CL 84,777: [Pyrazolium methyl sulfate,1,2-dimethyl-3,5-diphenyl]. Analytical standard, obtainable from American Cyanamid Company, Agricultural Research Division, P. O. Box 400, Princeton, New Jersey. TOXIC, HANDLE WITH CARE

*Trademark of American Cyanamid Company

2. Solvents: Specially purified, "Distilled in Glass", Burdick and Jackson Laboratories, Incorporated, or equivalent.

   a. Methanol
   b. Methylene chloride
   c. Hexane
   d. Toluene
   e. Acetone

3. Hydrochloric Acid (37%): Reagent grade, Mallinckrodt Company.

4. Alumina: Cat. No. A-540, Fisher Scientific Company.

5. Sand, Purified: Cat. No. 44-163, J. T. Baker Chemical Company.

6. GC Packing: 10% OV-101 on 80/100 mesh Supelcoport, Supelco, Incorporated, Cat. No. 1-1753.

7. Solutions:

   a. 4% HCl in Methanol: Mix 40 ml of HCl and 960 ml of methanol.

   b. 10% Methanol in Toluene: Mix 100 ml of methanol and 900 ml of toluene.

   c. 10% Methanol in Methylene Chloride: Mix 100 ml of methanol and 900 ml of methylene chloride.

C. Apparatus

1. Gas Chromatograph: Tracor Model 550 equipped with a Model 702 nitrogen-phosphorus detector.

2. Gas Chromatographic Column: 91 cm x 2 mm ID glass, packed with 10% OV-101 on 80/100 mesh Supelcoport. The column was packed using vacuum. Silylated glass wool plugs were inserted at each end. The column was conditioned overnight at 250°C with a helium flow rate of 30 ml/min.

3. Recorder: Hewlett Packard Model 3380A recording integrator.

4. Micro Syringe: Hamilton #701, microliter type, 0-10 mcl range.

5. Analytical Balance and Triple Beam Balance.

6. Flash Evaporator: Buchler Instruments, Model PF-10DN or equivalent, equipped with a heated water bath in which evaporation flasks can be partially submerged. Maintain water bath at 35°C.

7. Filter Paper: Glass fiber, No. 934AH, 9 cm, Ace Scientific Company, Incorporated, Cat. No. 12-5425-07.

8. Flasks, Round-Bottom: Ⅷ 24/40, Kontes Glass Company, No. K-601000, 500-ml, 1,000-ml and 300-ml.

9. Flasks, Volumetric: Kontes Glass Company, No. K-621500, 100-ml and 50-ml.

10. Funnels, Separatory: Squibb-type with Teflon stopcock, Kontes Glass Company, No. K-636030, 250-ml.

11. Graduated Cylinders: Kontes Glass Company, No. K-481500, 250-ml and 100-ml.

12. Flasks, Filtering, Heavy Wall with Side Tubulation: Ace Scientific Company, No. 12-6616-81, 500-ml.

13. Chromatographic Columns: Chromaflex column, Teflon stopcock, Kontes Glass Company, Cat. No. K-420280, sizes 222 and 223.

D. Preparation of Standard Solutions

1. Accurately weigh by difference using an analytical balance, 10 mg (±1 mg) of CL 84,777 standard of known purity into a 100-ml volumetric flask. Dissolve the material in 100 ml of acetone and mix well. Designate this solution which contains approximately 100 mcg of CL 84,777/ml as Standard Solution A.

2. Transfer by pipet, a 25-ml aliquot of Standard Solution A to a 100-ml volumetric flask. Dilute to the mark with acetone and mix. Designate this solution, which contains approximately 25 mcg of CL 84,777/ml as Standard Solution B.

3. Transfer by pipet, a 10-ml aliquot of Standard Solution B to a 50-ml volumetric flask. Dilute to the mark with acetone and mix. Designate this solution, which contains approximately 5 mcg of CL 84,777/ml as Standard Solution C.

4. Transfer by pipet, a 10-ml aliquot of Standard Solution C to a 50-ml volumetric flask. Dilute to the mark with acetone and mix. Designate this solution, which contains approximately 1 mcg of CL 84,777/ml as Standard Solution D.

E. Gas Chromatographic Conditions

1. Instrument: Tracor Model 550.

2. Detector: N-P detector.

3. Column: 91 cm x 2 mm ID glass, packed with 10% OV-101 on 80/100 mesh Supelcoport.

4. Instrument Conditions

Column temperature	205°C
Injection port temperature	300°C
Detector temperature	290°C
Hydrogen flow rate	2.5 ml/min
Helium flow rate	30 ml/min
Air flow rate	120 m/min
Retention time	3-4 minutes

5. Recording Integrator: 0.5 cm/min chart speed, area reject 1,000 and slope sensitivity of 0.3 mv/min.

6. Sensitivity: Electrometer sensitivity set to obtain a peak height of approximately 30% FSD (full-scale deflection) for a 10 ng injection of CL 84,777. Several injections of the 100 mcg/ml standard solution and sample extracts should stabilize the response.

F. Linearity Check

The gas chromatograph should be checked for linearity at least weekly and whenever the column, new or used, is newly installed in the instrument.

1. Transfer 1, 2 and 4 ml of Standard Solution B to 50-ml volumetric flasks. Dilute to volume with acetone. These solutions will have concentrations of CL 84,777 of 0.5, 1.0 and 2.0 mcg/ml, respectively.

2. Inject 10-mcl aliquots of each solution.

3. Plot the height for each peak versus the nanograms injected to demonstrate the linearity of the response. Significant departure from linearity over this range indicates instrumental difficulties which should be corrected before proceeding.

G. Sample Preparation

Spread the soil sample in a shallow alumium tray and air-dry in a hood (24-48 hours depending upon soil type and moisture content). Remove large stones and vegetative matter and using a pestle, break up lumps to obtain a fairly homogeneous sample. Spread the sample on a large piece of paper and using a large spatula, separate the sample into quarters. Remove two opposite quarters and mix the two remaining quarters thoroughly. Repeat this quartering step until 400-500 grams of soil remain.

H. Recovery Test

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. A fortified sample should also be processed with each set of samples analyzed.

1. Weigh a representative 25-gram portion of the control soil, transfer it to a 250-ml glass container provided with a stopper.

2. Add by pipet a volume (1-3 ml) of the respective standard solutions appropriate to the fortification level to be tested. Add the solution dropwise and spread it over the surface of the sample.

3. Add 75 grams of sea sand.

4. Mix the contents by hand shaking and continue from Step 2 of Section I.

I. Extraction of Soil

1. Weigh a 25-gram portion of a sample, transfer it to a one-pint cardboard container and add 75 grams of sea sand. Mix the contents by hand shaking.

2. Pack a chromatographic column as follows: Close the stopcock, place a glass wool pledget at the bottom of a chromatographic column (size 232, 19 mm x 300 mm). Pour the prepared soil sample into the column, tap the column gently and place a pledget of glass wool on top of the column.

3. Add 250 ml of 4% acid-methanol solvent. Open the stopcock and allow the solvent to percolate through the column at the rate of 4 ml/min into a 1,000-ml round-bottom flask.

4. Pour another 250 ml of 4% acid-methanol into the column and let it percolate through the column.

5. Concentrate the extract to 50-60 ml using a rotary-film evaporator with a water bath set at 35°C.

J. Solvent Partitioning

1. Transfer the extract (50-60 ml) into a 250-ml separatory funnel, wash the flask with 30 ml of distilled water and pour washings into the funnel.

2. Add 50 ml of hexane to the separatory funnel and shake for 3-4 seconds.

3. Allow the phases to separate and then drain the aqueous phase into another 250-ml separatory funnel. Discard the upper hexane phase.

4. Add another 50 ml of hexane, shake, allow the phases to separate and drain the bottom phase (the aqueous phase) into another 250-ml separatory funnel. Discard the upper hexane phase.

5. Add 100 ml of methylene chloride to the 250-ml funnel, stopper and shake vigorously for 45 seconds. Allow the phases to separate and drain the bottom phase into a 500-ml round-bottom flask.

6. Reextract the acid solution with a fresh portion of 100 ml of methylene chloride and after the phases separate, drain the methylene chloride into the 500-ml round-bottom flask.

7. Evaporate the combined methylene chloride phases just to dryness using a rotary-film evaporator with a water bath set at 35°C.

K. Cleanup on Alumina

1. Place a glass wool pledget at the bottom of a 14.5-mm x 250-mm (size 222) chromatographic column and add 50 ml of methylene chloride.

2. Slowly pour 15 grams of alumina into the column.

3. After the adsorbent has settled, drain the methylene chloride at a rate of 4 ml/min to within 1 cm of the top of the alumina.

4. Dissolve the contents of the evaporating flask (Section J.7) in 5 ml of methylene chloride and transfer quantitatively to the alumina column.

5. Position a 250-ml beaker beneath the column and open the stopcock to provide a flow of 3-4 drops per second.

6. When the liquid level drains to within 1 cm of the top of the column, close the stopcock.

7. Rinse the flask two times more with 5 ml of methylene chloride and transfer to the column.

8. Each time open the stopcock to provide a flow of 3-4 drops per second.

9. When the liquid level drains to within 1 cm of the top of the packing, wash the column with 100 ml of 10% methanol-toluene using a flow rate of 3-4 drops per second.

10. When the liquid level drains to within 1 cm of the top of the packing, close the stopcock.

11. Replace the beaker below the column with a 300-ml round-bottom flask and elute the column with 150 ml of 10% methanol-methylene chloride using an effluent rate of 2-3 drops per second.

12. When the flow ceases, transfer the flask to a rotary evaporator and evaporate to dryness.

13. Add two 20-ml portions of methanol and evaporate each time to dryness to remove any traces of methylene chloride that might enhance the GC response.

14. Dissolve the residue in 1 ml of acetone for GC analysis.

L. Gas Chromatographic Analysis

1. After obtaining a stable GC response for the standard, inject a 5-mcl aliquot of the sample.

2. If the sample peak goes off scale, dilute to an appropriate volume and reinject.

3. Compare the peak height with that obtained from 10 ng injection of standard CL 84,777.

4. Make a standard injection after every second sample and use the average peak height of the standards injected before and after the samples for the calculations.

M. Calculations

For each sample calculation use the sample peak height and the average peak height of the external standard obtained before and after the sample injection as follows:

$$\text{ppm (CL 84,777)} = \frac{(R1) \times (V1) \times (V3) \times (C) \times (V5)}{(R2) \times (W) \times (V2) \times (V4)}$$

Where:

R1 = Peak height of sample.

R2 = Average peak height of standard.

W = Weight of sample taken for analysis in grams, on dry basis.

V1 = Volume of extraction solvent added to sample in milliliters.

V2 = Aliquot of extract taken for analysis in milliliters.

V3 = Volume of acetone added to dissolve final residue for chromatographic analysis in milliliters.

V4 = Volume of sample solution injected in microliters.

V5 = Volume of working standard solution injected in microliters.

C = Concentration of working standard solution in micrograms per milliliters.

See Figure M-1129.A for typical chromatograms of control and fortified soil.

Figure M-1129.A: Typical Chromatograms for the Determination of CL 84,777 Residues in Soil