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DETERMINATION OF DIQUAT RESIDUES
IN SOIL BY GAS CHROMATOGRAPHY
METHOD RM-5G-1

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INTRODUCTION

Method RM-5G (dated May 22, 1980) describes a gas chromatographic procedure for the analysis of low levels of diquat residues in soil. This revision of the method describes updated gas chromatography parameters and revised sample cleanup procedures designed to shorten the sample preparation time by analysis of aliquots of the acid hydrolysate instead of the total sample as described in the original method.

In summary the method involves extraction by acid hydrolysis, cleanup and concentration by ion exchange chromatography followed by reduction with sodium borohydride and measurement of the diquat reduction product by gas chromatography using a nitrogen/phosphorous-flame ionization detector. The reduction procedure is a modification of a procedure described by Ukai, et al (1).

APPARATUS

Round-bottom flask, 50 and 1000 ml, standard taper joints

Reflux condensers, standard tapered joint

Heating mantels for 1000 ml flasks

Columns, 1 x 20 cm, equipped with a teflon stopcock and a reservoir at the top (A 25 ml burette may be used)

Water bath, 65-70° C

Separatory funnel with Teflon® stopcock, 125 ml capacity

Vacuum rotary evaporator equipped with an ambient temperature water bath

Buchner funnel

Filter flask

Filter paper, Whatman No. 42, 15 cm

Beakers, 1000 and 100 ml

Gas chromatograph (Hewlett Packard 5890 or equivalent) equipped with a nitrogen/phosphorous-flame ionization detector and an automatic liquid sampler (HP 7673A) and the following operating parameters:

Column: 15 m x 0.53 mm i.d., methylsilicone (DB-1, J&W Scientific)
Injector temperature: 250° C
Column oven temperature: 150° C
Detector temperature: 300° C
Carrier gas flow rate: 10 ml/min (He)
Make-up gas flow rate: 20 ml/min (He)
Hydrogen flow rate: 3.5 ml/min
Air flow rate: 110 ml/min
Retention time: 3.7 min (See Figure 1)

REAGENTS

Deionized water

Diethyl ether, A.R., Mallinckrodt Cat. #0805

Hexane, Pesticide Quality

Sodium Borohydride, Eastman #5066

Methanol, Pesticide Quality

Sulfuric acid, 18N

Hydrochloric acid, 1N and 2N

Sodium hydroxide, 5N and 10N

Ammonium chloride, saturated solution (approximately 5M)

Ammonium chloride, 1/10 saturated solution (approximately 0.5M)

Cation exchange resin, Dowex 50W-X8 or AG 50W-X8, 200-400 mesh, hydrogen form. (an analytical grade cation exchange resin) available from Bio-Rad Laboratories, Richmond, CA. If Dowex 50W is used, it should be placed in a large chromatography column and backwashed with water to remove fine particles.

Sodium chloride, saturated solution

Diquat dibromide monohydrate reference standard, (51.05% diquat cation) available from Chevron Chemical Company, 15049 San Pablo Avenue, Richmond California 94804

Diquat standard stock solutions, 2.5 and 0.50 mg/ml diquat cation in water

Diquat fortifying solution, 5.0 µg/ml diquat cation in water; prepare daily in a plastic volumetric flask

Diquat reference solution, 5.0 µg/ml diquat cation in saturated ammonium chloride

EXTRACTION

Transfer 50 g of sample to a 1000 ml boiling flask. Fortify a control sample with 1.0 ml 5.0 µg/ml diquat cation in water for recovery purposes. Add 100 ml of 18 N sulfuric acid and reflux for 5 hours. (If foaming occurs, add a few ml of caprylic alcohol and shake the flask until the soil is completely wetted.) Cool, then add 100 ml water to the flask. Filter the mixture through filter paper with suction using a Buchner funnel. Wash the boiling flask and filter cake with two 100 ml portions of water. Transfer the sample to a 500 ml graduated cylinder and adjust the volume to 500 ml with water. Mix, then transfer the sample to a plastic container with a screw cap lid.

Weigh 10 grams of soil into a tared moisture dish. Dry in the oven at 110° C overnight, then reweigh. Calculate the percent moisture in the soil.

ION EXCHANGE COLUMN CLEANUP

Place a plug of glass wool in the bottom of the ion exchange column, add 4 ml of settled resin in water and cap with another plug of glass wool. Keep the column covered with water at all times. Use a freshly prepared column for each sample.

Transfer a 100 ml aliquot of diluted sample extract to the column. Use air pressure to percolate the extract through the column at a maximum flow rate of 10 ml per minute. Back flush the resin bed with 25 ml of water and allow the resin to resettle. Wash the column with an additional 25 ml of water. Rinse the column with 10 ml of 2N hydrochloric acid followed by 25 ml of water. Rinse the column with 25 ml of 1/10 saturated ammonium chloride followed by 50 ml of water. Discard all column eluates.

Add a small quantity of solid ammonium chloride to the top of the column. Elute diquat from the column with saturated ammonium chloride at a flow rate of 0.5-1.0 ml per minute (10 to 12 drops per minute). Collect exactly 25 ml of eluate.

REDUCTION AND EXTRACTION

Add approximately one gram of sodium borohydride to a beaker containing 15 ml of 10N aqueous sodium hydroxide and mix. Add the column eluate slowly to the borohydride mixture with continuous stirring. (Liberation of ammonia occurs and it is recommended that the remainder of the reduction procedure be conducted in a fume hood.) Place the sample in a 65-70° C water bath for 10 minutes. Allow the sample to cool and transfer to a separatory funnel. Rinse the beaker and extract the sample with 25 ml diethyl ether. Discard the lower aqueous layer and extract the ether with 10 ml of 1N hydorchloric acid. Drain the acid into a second separatory funnel and add 3.0 ml 5N sodium hydroxide and 5.0 ml of saturated aqueous sodium chloride. Mix and extract the sample with 20 ml 50% hexane in diethyl ether. Transfer the lower aqueous layer to another separatory funnel and reextract with 10 ml of 50% hexane in diethyl ether. Combine the organic layers in a round-bottom flask and evaporate just to dryness using a vacuum rotary evaporator and an ambient temperature water bath. Dissolve the sample in 2.0 ml of basic methanol (2.6 ml 5N sodium hydroxide diluted to 50 ml with methanol). Proceed with the measurement.

REFERENCE STANDARD PREPARATION

Add 1.0 ml 5.0 µg/ml diquat in saturated ammonium chloride to 24.0 ml of saturated ammonium chloride. Proceed with the reduction and extraction as described previously. Dissolve the sample in 5.0 ml of basic methanol to obtain an equivalent diquat cation concentration of 1.0 µg/ml. Dilute the 1.0 µg/ml diquat standard with basic methanol to obtain diquat standards with concentrations of 0.05, 0.25 and 0.50 µg/ml (reference standard for measurement).

MEASUREMENT

Transfer the solutions to be measured to vials for use on the automatic liquid sampler. Load the sample tray in the following order: standard, standard, methanol wash, control sample, fortified sample, standard, methanol wash, sample, sample, standard..... Set the syringe to deliver 1.0 ul. The standard vials contain 0.50 µg/ml diquat as the reduction product).

CALCULATION

$$ppm = \frac{\text{Peak Height (sample)}}{\text{Ave. Peak Height Std.}} \times 0.5\ \mu g/ml \times 2.0\ ml \times 5 \times \frac{1}{50\ g} \times \text{Dilution factor}$$

LIMIT OF DETECTION

The limit of detection is approximately 0.01 ppm for diquat for a 50 g soil sample analyzed using the described procedure.

NOTES

1. All diquat standards must be stored in the dark when not in use. The dilute fortification standard in water is prepared daily in a plastic volumetric flask to minimize absorption.

2. A fortified sample must be analyzed concurrently with each set of samples. Method recovery should be between 70-120% to be acceptable. All samples analyzed with a fortified sample which did not have an acceptable recovery must be reanalyzed or the results in question approved by supervisory personnel.

3. For soil or sediment with high clay content, increase sulfuric acid reflux time to 10 hours if low recoveries of diquat cation are experienced when refluxing for 5 hours.

4. The linearity of the measurement system must be verified on a daily basis. The coefficient of variation of the response factors of the four standards prepared for analysis must be ±10% or less.

5. The coefficient of variation of the standard measurements must be ±5.0% or less to be acceptable. All deviations from this requirement must be approved by supervisory personnel.

6. The methanol wash solutions are included in the analysis sequence to prevent contamination of the sample extracts with diquat from the standard analyses.

7. This procedure will separate diquat from paraquat. The reduction product of diquat has a retention time of 3.7 minutes (Figure 1) and the paraquat reduction product has retention time of 4.8 minutes (Figure 2).

8. Reduction of diquat results in the formation of four products. Quantitation is conducted on the major product which has the longest retention time. Preliminary experiments have demonstrated that the reproducibility of the reduction reaction is sufficient for estimation of low levels of diquat in soil.

REFERENCES

(1) Ukai, S., Hirose, K., Kawase, S., Eisei Kagaku (J. Hyg. Chem.), 19, 281, (1973)

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