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DETERMINATION OF BENEFIN RESIDUES IN AGRICULTURAL CROPS

GENERAL PROCEDURE
5801230

Reagents:

1. Methanol A.R.
2. Sodium Chloride Solution - 5% Aqueous
3. Sodium Sulfate Anhydrous
4. n-Hexane - Redistilled
5. Methylene Chloride - Redistilled
6. Benzene A.R.
7. Florisil - Deactivated and Standardized

Column Preparation: Insert a glass wool pledget into a 22 mm. I.D. column fitted with a stopcock and add Florisil while vibrating the column until a 7.5 cm. column is formed. Then add 2.5 cm. of anhydrous sodium sulfate to the top of the Florisil. Wash the column with 100 ml. of n-hexane, keeping a layer of hexane on the column.

Due to the variation in activity of activated Florisil, it is necessary to standardize it as follows: Using a control with 100 mcg. of benefin added, the procedure is followed. This will give a visual column check of the amount of forerun to discard and the amount of eluant to collect. Under normal conditions, a forerun of 70 ml. is discarded and the next 100 ml. retained. (It is to be noted that at least 50 ml. must be collected after the visual band is removed in order to assure complete elution.)

If the benefin fraction is not well defined or is very slow in elution, the Florisil can be deactivated by allowing it to equilibrate with atmospheric moisture overnight.

This treatment has been satisfactory for lots of Florisil used in this laboratory. An alternate procedure is as follows: It has been found that Florisil with a moisture content of 1.5% (measured by Karl Fischer reagent) is satisfactory for this procedure. Determine the moisture content of approximtely 500 g. of Florisil and then add the amount of water to bring the moisutre content to exactly 1.5%. This is done by coating the walls of a large jar with the required moisture, capping and mixing thoroughly. Keep the jar tightly sealed between usage.

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Apparatus:

1. Rinco Evaporators
2. Omni Mixers
3. Glass Columns--27 x 450 mm. fitted with stopcocks
4. Gas Chromatograph equipped with electron capture cell

Gas Chromatograph Operating Conditions:

Jarrell-Ash Model 700 with electron capture cell
Columns--6 ft. x 1/4 in. O.D. Borosilicate glass packed
with XE-60 - 3% on 60/80 mesh Chromosorb W or
with SE-30 - 5% on Chromosorb W
Flash Heater--280°C.
Column--178°C.
Cell--190°C.
Cell Voltage--18.0 V.
Gas--Prepurified Nitrogen at 90 ml/min
Electrometer--10^{-9} Amperes
Retention Time of Benefin--Approximately 3-4 minutes

Preparation of Sample: (Remove extraneous material) from the sample of R.A.C. Grind and blend the samples using appropriate equipment. For analysis use 25 gram samples of control, control plus standard and treated tissue.

Procedure:

Weigh a representative finely ground 25.0 g. sample and transfer to a quart mason jar. Add 200 ml. of methanol and blend for 5 minutes using an Omni mixer. Filter through Whatman #1 filter paper using vacuum. Rinse the mason jar with 2-25 ml. portions of methanol and wash the residue with these rinses. Transfer the combined extract and washes to a 1 liter separatory funnel. Add 500 ml. of sodium chloride solution to the separatory funnel and mix. Rinse the suction flask with 50 ml. of methylene chloride and add to the separatory funnel. Extract for approximately 1 minute. Allow the layers to separate. Drain the methylene chloride layer through sodium sulfate into a 300 ml. round bottom flask. Repeat the extraction with 2-50 ml. portions of methylene chloride collecting all extractions in the 300 ml. round bottom flask. Wash the sodium sulfate with 25 ml. methylene chloride.

Evaporate the methylene chloride using a Rinco evaporator and a 50°C. water bath. (NOTE: THE FLASK MUST BE REMOVED AS SOON AS THE METHYLENE CHLORIDE IS EVAPORATED TO PREVENT LOSS OF BENEFIN.) Add 5 ml. of n-hexane to flask, swirl to dissolve contents and transfer to the Florisil column. Rinse the flask with 4-5 ml. portions of n-hexane, allowing each portion to go into the column before the next addition. Start collecting the eluate immediately after introducing the extract. Collect the fraction as determined in the standardization of Florisil. (Normally 70 ml. is discarded and the next 100 ml. saved.) Transfer this fraction to a 200 ml. round bottom flask and evaporate just to dryness as before. Add 2.0 ml. benzene to the flask. Swirl and transfer to a small screw capped vial which has an aluminum foil liner. (NOTE: Avoid exposure of benzene solution to direct sunlight.)

Measurement of Benefin Content: Inject a 1.5 - 1.8 µl. sample into a gas chromatograph operated under the conditions described. Measure observed peak height in centimeters at benefin's retention time. Compare to the peak height of the standard recovery.

Calculations:

$$\frac{\text{pk. height sample}}{\text{pk. height std. recovery}} \times 0.01 = \text{ppm Benefin}$$

Standard Recovery: Add 0.25 mcg. of benefin standard to a 25 g. sample (0.01 ppm) of control material in a quart mason jar. Then add 200 ml. of methanol and proceed as described above. This standard recovery is compared to a standard solution containing 0.125 mcg/ml injected directly into the instrument to obtain the percentage recovery (or the percentage loss through the procedure).

NOTE 1: All glassware must be rinsed with the solvent to be used immediately before use to eliminate the possibility of contamination.

NOTE 2: All reagents must be checked to make sure no contaminant is present that will interfere with determination of benefin.

NOTE 3: If pesticides, Zineb, BHC, and Ethion are present, use procedure No. 5801110.

NOTE 4: Tissues that have a high oil content, e.g., nut crops, change the retention time of benefin on the Florisil column. To avoid this problem, the methylene chloride extract is evaporated just to dryness, the residue dissolved in 25 ml of n-hexane and partitioned with 2-25 ml volumes of acetonitrile. The acetonitrile extract is evaporated just to dryness, the residue dissolved in 5 ml of n-hexane, and transferred to the Florisil column following procedure previously described.

NOTE 5: This procedure is applicable to a wide range of vegetables, plant tissue, soil and water samples including oily crops. If a large peak is observed, such as might be encountered when running a soil sample, dilute the benzene solution so that observed peak height is within linear response of instrument.

NOTE 6: Under the conditions described, a test sensitivity of 0.005 - 0.010 ppm can be attained with an expected recovery greater than 80%.