TEMEPHOS
B-41

Gas Chromatographic Determination of ABATE and ABATE Sulfoxide Residues in Cottonseed

A. Principle

ABATE and ABATE sulfoxide are extracted from cottonseed with hexane. After filtration and centrifugation the hexane is evaporated and the oily residue extracted with methanol. The oils are precipitated by freezing and the supernatant methanol evaporated. The residue is dissolved in hexane and percolated through a Florisil column. The ABATE is eluted from the column with methylene chloride and the effluent evaporated. The resulting residue is dissolved in a known volume of mehtanol for the measurement of ABATE content by gas chromatography using a thermionic detector.

The ABATE sulfoxide is eluted from the Florisil with 20% acetone in methylene chloride. The effluent is evaporated to dryness and the residue dissolved in glacial acetic acid. The solution is reacted with titanium trichloride which reduces the ABATE sulfoxide to ABATE. The solution is diluted with water and the ABATE is partitioned into carbon tetrachloride. The solvent is evaporated and the residue dissolved in a known volume of methanol. Gas chromatographic measurement of this solution yields the concentration of ABATE sulfoxide.

B. Reagents

1. ABATE: Material of known purity. Obtainable from: Agricultural Division, American Cyanamid Company, Post Office Box 400, Princeton, New Jersey 08540.

2. Hexane: Reagent grade.

3. Methyl Alcohol: Reagent grade.

4. Methylene Chloride: Reagent grade.

5. 20% Acetone in Methylene Chloride: Measure, by graduated cylinder, 200 ml of acetone and dransfer to a 1000 ml volumetric flask. Dilute to the mark with methylene chloride. Mix well.

6. Florisil (60-100 mesh): Fisher, F-100.

7. Glacial Acetic Acid: Reagent grade.

8. Titanium Trichloride: 20% solution, Fisher Scientific SO-T-43.

9. OV-22: Cat. No. 1229, Supelco, Inc.

10. Gas-Chrom Q: 60/80 mesh, Cat. No. 02001, Applied Science Labs Inc.

C. Apparatus

1. Gas Liquid Chromatograph: F & M Model 402 or an equivalent instrument, equipped with a thermionic detector consisting of a flame ionization electrode system fitted with a cesium bromide salt tip. The tip is available from Varian Aerograph, Walnut Creek, California.

2. Gas Chromatographic Column: 79 cm, 4 mm I.D., Pyrex column packed with 1.0% OV-22 on Gas Chrom Q (60/80 mesh). The column packing is prepared by dissolving, in chloroform, a quantity of OV-22 equivalent to 1.0% of the weight of the support. The OV-22 solution and solid support are added to an evaporation flask and the solvent removed by using a rotary evaporator. The resulting coated support is transferred to a flat pyrex disk and the residual chloroform removed by heating on a hot plate.

3. Micro Syringe: Hamilton #701 microliter type, 0-10 µl range.

4. Analytical Balance and Triple-Beam Balance.

5. Bottles: 1) 32 ounce, narrow mouth, with polyseal caps.
2) 6 ounce, narrow mouth, with polyseal caps.

6. Reciprocating Shaker.

7. Cheese Cloth: 5 x 5 inch squares.

8. Chromatographic Columns: 1) 15 x 300 mm, with 250 ml reservoir.
2) 19 x 300 mm, with 250 reservoir, K-42-280, Kontes Glass Company, Vineland, New Jersey.

9. Centrifuge.

10. Glass Fiber Filter: (12.5 cm) Reeves Angel 934AH.

11. Planimeter: Ott Model No. 31L or equivalent.

12. Rotary Film Evaporators: Rinco or equivalent and constant temperature bath set at 30 - 40°.

D. Preparation of Standard ABATE Solution

Accurately weigh 100 mg to the nearest mg of Standard ABATE of known purity on an analytical balance. Quantitatively transfer to a 100 ml volumetric flask and add a small amount of methanol to dissolve the ABATE. Dilute to the mark with methanol, mix well, and label as Standard Solution A. The concentration of this solution is 1,000 nanograms ABATE per microliter.

Pipet 10 ml of Standard Solution A into a 100 ml volumetric flask. Dilute to mark with methanol, mix well, and label as Standard Solution B. The concentration of this solution is 100 nanograms ABATE per microliter.

For determination of linearity of response pipet 1, 3, 5, 7 and 9 ml aliquots of Standard Solution B into 50 ml volumetric flasks. Dilute to the mark with methanol and mix well. Inject 5 microliter aliquots of these solutions into the gas chromatograph. This procedure need not be run each time samples are assayed but only at the initial application of the procedure.

Prepare a working standard solution by diluting 2 ml of Standard Solution B to 100 ml with methanol and mixing well. This solution contains 2 micrograms of Abate per milliliter.

E. Gas Chromatographic Conditions*

Condition the prepared gas chromatographic column for two hours at 300° with the exit end unconnected to the detector. After conditioning, connect the column to the detector and set the controls to provide the conditions listed below:

Column Temperature	260°
Flash heater temperature	300°
Detector temperature	265°
Hydrogen flow rate	20 ml/minute (5 psig,tank)
Helium flow rate	100 ml/minute (50 psig,tank)
Air flow rate	190 ml/minute (25 psig,tank)
Range	10
Attenuation	256
Recorder chart speed	1 inch per minute

NOTE: The gas flow settings are approximate. The flow rates should always be set so that the ratio of ABATE response to detector noise is greatest.

*Gas chromatographic conditions as described are for an F & M Model 402 equipped as described in Section "E". Conditions may need modification to achieve equivalent operation on other equipment.

Ignite the flame and turn on the recorder. Reduce the hydrogen flow slowly until the chart pen moves from off scale to detector zero. Turn on the chart drive and inject 1 microliter of Standard Solution B. After the Abate peak has completely eluted minor changes in the gas flow rates may be necessary to effect optimum detector response. After final conditions have been determined, 1 microliter injections of Standard Solution B should be continued until a constant response is obtained.

F. Determination of Abate in Cottonseed Samples

1. Weigh 50.0 grams of ground cottonseed on triple-beam balance and transfer to a 32-ounce narrow-mouth glass bottle.

2. Add 500 ml hexane to the bottle, seal with a polyseal cap and extract overnight (16 hours) on the reciprocating shaker.

3. Filter the resulting slurry ghrough 4 layers of cheesecloth placed in a 4 inch funnel, into a 500 ml graduated cylinder. Transfer the solution to a centrifuge bottle and centrifuge at moderate speed for 5 minutes.

4. Transfer 400 ml of the clear hexane solution to a 1000 ml round bottom flask and evaporate on a rotary film evaporator (water bath 40°) to a volume of 25 ml.

5. Transfer the solution to a 6-ounce, narrow-mouth bottle and wash the flask with 10-20 ml hexane.

6. Attach the bottle to rotary film evaporator with solvent-resistant rubber tubing (4 cm O.D., 2 cm I.D.) and evaporate the hexane.

7. Add 50 ml of absolute methanol to the oily residue, seal the bottle with a polyseal cap and extract on a reciprocating shaker for 5 minutes.

8. Place the bottle in a dry-ice isopropanol bath for 5 minutes.

9. Immediately pour the methanolic layer through a glass fiber filter into a 250 ml pear-shap evaporation flask.

10. Warm the frozen oil in the bottle to room temperature and extract with an additional 50 ml methanol as in Step 7.

11. Repeat steps 8-9, combining the methanolic extracts. Evaporate on a rotary film evaporator (40°) until only an oily residue remains.

12. Dissolve the residue in 50 ml hexane.

13. Prepare a Florisil column by placing a glass wool plug in the bottom of a chromatographic column and fill the barrel of the column with hexane. Open the stopcock to allow a moderate flow of hexane (2-3 drops per second) and slowly add 15 gm Florisil. When the hexane has reached the level of the Florisil bed stop the column flow.

14. Transfer the hexane solution to the column and allow it to percolate through the column at a flow rate of 2 drops per second. When the solution has reached the level of the Florisil, wash the column with an additional 50 ml of hexane. When the hexane has reached the level of the Florisil close the stopcock and discard the effluent.

15. Add 100 ml methylene chloride to the reservoir and allow it to percolate through the column, collecting the effluent in a 250 ml evaporation flask at a flow rate of 2 drops per second. When the methylene chloride has reached the level of the Florisil, wash the column with an additional 50 ml of methylene chloride collecting the wash in the same evaporation flask. Close the stopcock when the methylene chloride level is about 5 mm above the Florisil bed. Evaporate the combined effluent and wash to dryness on a rotary film evaporator using a water bath set at 40°. Label the flask A.

16. Transfer 100 ml of the 20% acetone in methylene chloride to the column and allow it to percolate through the Florisil at a flow rate of 2 drops per second, collecting the eluate in a 250 ml evaporation flask. Evaporate the eluate to dryness on a rotary film evaporator using a water bath set at 40°. Label the flask B.

17. Dissolve the residue in flask B in 10 ml of glacial acetic acid. Add 0.5 ml of 20% titanium trichloride solution. Swirl the contents of the flask to throughly mix the reagents. Stopper the flask and allow to stand at room temperature for 10 minutes.

18. Transfer the mixture to a 60 ml separatory funnel using 20 ml of distilled water. Add 10 ml of carbon tetrachloride to the flask and shake vigorously for 30 seconds. Allow the layers to completely separate and draw off the carbon tetrachloride layer into a 100 ml pear-shaped flask. Repeat the extraction once more with 10 ml of carbon tetrachloride. Combine the carbon tetrachloride layers and evaporate to dryness on a Rinco rotary evaporator with the water bath set at 40°C.

19. Dissolve the resulting residue in 1 ml of methanol.

20. Inject 5 microliter aliquots of the working standard solution and the prepared sample solutions of fractions A and B into the gas chromatograph. The Abate peak heights should be between 20 and 90% full scale deflection. Solutions from each sample and the working standard solution are injected in duplicate. The standard solution is injected after every pair of duplicate sample solution injections and is used to calculate the samples that follow it.

21. If the sample injection yields an off-scale response, the solution should be diluted until the response is 30-90% of full scale deflection. Volume corrections are made by incorporating the additional dilution as a factor in the calculation.

G. Calculations

Calculate the Abate concentration in Fractions A and B as follows:

$$\text{ppm Abate} = \frac{(A1)}{(A3)} \times \frac{(V1)(V3)(C)(M1)}{(W)(V2)(V4)(M2)} \times V5$$

$$\text{ppm Abate sulfoxide} = \frac{(A2)}{(A3)} \times \frac{(V1)(V3)(C)(M1)}{(W)(V2)(V4)(M2)} \times V5$$

Where:

A1 = Average peak area for Fraction A.
A2 = Average peak area for Fraction B.
A3 = Average peak area for standard.
W = Weight in grams of sample taken for analysis (Step 1).
V1 = Volume in ml of extracting solvent added (Step 2).
V2 = Volume in ml of extract taken for analysis (Step 3).
V3 = Volume in ml of standard solution injected (Step 20).
V4 = Volume in ml of sample solution injected (Step 20).
C = Concentration in µg/ml of standard solution (Step 20).
V5 = Volume in ml of the minal methanol sample solution used for GLC analysis (Step 19, or Step 21 if futher dilution is required).
M1 = Molecualr weight of Abate sulfoxide.
M2 = Molecular weight of Abate.

If the procedure is carried out exactly as written, the calculation may be simplified as follows:

$$\text{ppm Abate} = \frac{A1}{A3} \times \frac{(500\ \text{ml})(5 \times 10^{-3}\ \text{ml})(2\ \text{ug/ml})}{(50\ \text{gms})(400\ \text{ml})(5 \times 10^{-3}\ \text{ml})} \times V5$$

$$= \frac{A1}{A3} \times V5 \times 0.050$$

$$\text{ppm Abate sulfoxide} = \frac{A2}{A3} \times \frac{(500\ \text{ml})(5 \times 10^{-3}\ \text{ml})(2\ \text{ug/ml})(482.2)}{(50\ \text{gms})(400\ \text{ml})(5 \times 10^{-3})(466.2)} \times V5$$

$$= \frac{A2}{A3} \times V5 \times 0.052$$