Gas Chromatographic Determination of Residues of *S,S,S*-Tributyl Phosphorotrithioate in Cottonseed and Cottonseed Oil

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An electron-capture gas chromatographic method has been developed for the determination of *S,S,S*-tributyl phosphorotrithioate (DEF) residues in cottonseed and cottonseed oil. A carbon-Florisil column is used in the cleanup procedure. The oil is washed through the column with petroleum ether while the DEF is retained. Acetone is then used to elute the DEF. The eluate is evaporated and made to volume in hexane and an aliquot injected into the chromatograph. Recovery generally exceeds 95% at the 0.1-p.p.m. level.

THE use of *S,S,S*-tributyl phosphorotrithioate (DEF, trade-mark of Chemagro Corp.) as an agricultural defoliant necessitated the development of a method for determining submicrogram amounts of the compound in plant material. Although a fluorometric method does exist for determining DEF (*1*), it is lengthy and subject to atmospheric contamination with mercaptans. With the advent of the electron-capture detector, it was thought possible to develop a gas chromatographic method that would eliminate these difficulties. The electron-capture detector was reasonably sensitive to DEF and a good symmetrical peak was obtained using a 4-foot column of SE-30 coated on silanized Chromosorb W.

Recently, a paper discussed use of the microcoulometric gas chromatograph for the determination of DEF (*3*). On the premise that DEF would not penetrate into the seed, a surface extraction of the whole cottonseed was used. Since under these conditions only a small amount of oil was extracted, it was possible to compensate for the relative insensitivity of the instrument by injecting large amounts of the concentrated extract.

In this laboratory, an exhaustive extraction of ground cottonseed and a method to analyze the refined oil were needed. Analysis of such samples by microcoulometric gas chromatography is difficult because of the large amounts of oil that must be injected to achieve a sensitivity of 0.1 p.p.m. The oil fouls the system rapidly. Electron-capture detection is much better because equivalent sensitivities can be achieved with much smaller sample injections.

In the procedure described, ground cottonseed is extracted with Skellysolve B in a Soxhlet apparatus to remove DEF along with the oil and pigments. Then, to separate the DEF from the oil the extract is passed through a carbon-Florisil column. The oil is washed through the column with Skellysolve B, while the DEF and more polar pigments are retained on the column. DEF is then eluted with acetone. The acetone is evaporated on a steam bath and the residue is transferred to a 25-ml. volumetric flask with hexane. An aliquot is then injected into the chromatograph and the DEF measured by electron-capture gas chromatography.

Apparatus

Chromatographic tube, borosilicate glass, 20 × 400 mm., with integral 300-ml. reservoir and Ultramax stopcock.

F & M Model 700 gas chromatograph equipped with pulsed typed electron-capture detector, or equivalent.

Hamilton 701N microliter syringe.

Soxhlet extractors, extra large, 1-liter capacity.

Wiley mill.

Reagents

Acetone, ACS reagent grade.

Activated carbon, Darco 20- to 40-mesh (refining grade).

DEF standard solution. Weigh 0.1 gram of DEF into a clean 100-ml. volumetric flask. Make to volume with reagent grade *n*-hexane and shake to mix. Transfer 50 μl. (with microliter syringe) of the 1000 μg. per ml. standard solution to a clean 250-ml. volumetric flask. Make to volume with hexane and shake to mix. This flask contains 0.2 μg. per ml. of DEF.

Florisil, 60- to 100-mesh. (Place in oven overnight at 130° C. to activate.)

n-Hexane, analytical reagent grade.

Petroleum ether, Skellysolve B, boiling range 60–65° C.

Superbrite beads, 100- to 140-mesh, Minnesota Mining and Manufacturing Co.

All solvents should be redistilled in an all-glass apparatus.

Preparation of Sample

Cottonseed. Grind the entire sample in a Wiley mill and mix thoroughly. Weigh a 50-gram portion of the sample into a large Soxhlet extraction thimble and cover with a plug of glass wool. Extract the sample overnight in a Soxhlet extractor (1-liter capacity), using 800 ml. of Skellysolve B as the solvent. Evaporate the extract to approximately 100 ml. on a stream bath under an air jet.

Prepare a chromatographic column as follows: Tamp a plug of glass wool into the bottom of a 20 × 400 mm. glass chromatographic tube with 300-ml. integral reservoir. Pour in a 1-inch layer of Superbrite glass beads. Slurry 20 grams of activated Florisil in Skellysolve B and wash into the chromatographic tube. Slurry 5 grams of carbon in Skellysolve B and pour off the solvent two or three times to eliminate the fines and then wash into the chromatographic tube. Drain the solvent down to the level of the carbon.

Pour the concentrated extract (or dissolved oil) onto the chromatographic column. Allow the extract to percolate through the chromatographic tube slowly at the rate of approximately 1 to 2 drops per second. Wash the column with two 15-ml. portions of Skellysolve B, rinsing down the sides of the reservoir and allowing the solvent to drain down to the level of the carbon each time. Wash with 70 ml. additional of Skellysolve B, keeping the flow rate at about 1 to 2 drops per second. Discard the eluate. Elute the DEF with 100 ml. of acetone added in several portions and collect the eluate in a 250-ml. beaker. Evaporate the acetone on a steam bath under an air jet. About 1 ml. of oil should remain. Transfer the residue from the beaker to a 25-ml. volumetric flask with small portions of *n*-hexane. Make up to volume and shake to mix.

Cottonseed Oil. Dissolve 50 grams of oil in 100 ml. of Skellysolve B and proceed with the chromatographic column step above.

Gas Chromatographic Procedure

Pack a 4-foot × 3-mm. i.d. borosilicate glass column with 3.8% SE-30 coated Chromosorb W, prepared as follows: Dissolve 2 grams of SE-30 in 77 ml. of hexane. Add 10 grams of 80- to 100-mesh silanized Chromosorb

Table I. Recovery of DEF from Cottonseed and Cottonseed Oil

Sample	Added, P.P.M.	Av. Recovery, %[a]	Std. Dev.
Cottonseed	0.1	98 (10)	2.0
Cottonseed oil	0.1	92 (10)	2.8

[a] Average recovery followed by number of determinations.

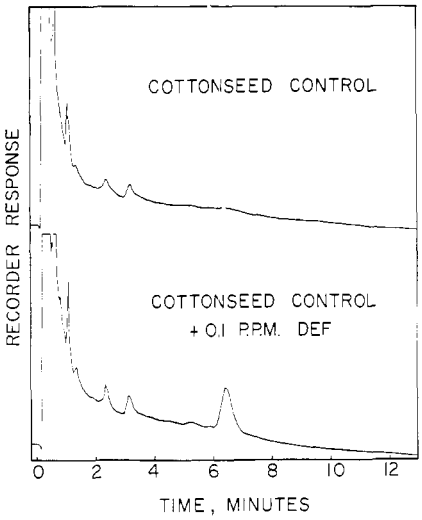

Figure 1. Chromatograms for recovery of DEF from cottonseed

W and allow to remain in contact overnight. Apply moderate vacuum to the solution and then release to allow the hexane solution to fill the interstitial spaces of the Chromosorb particles. Add the slurry to a fritted glass funnel and remove the liquid with suction. Spread the semidry material in an open pan and air-dry, shaking every 2 to 5 minutes, then oven-dry at 100° C. Pack the material into the glass column using a vibrator and light suction. Condition at 300° C. for 1 hour with no gas flow. Then condition overnight at 200° C. with 10 to 30 ml. per minute gas flow.

Use argon containing 5% methane for both the carrier and purge gas and set at 65 and 30 ml. per minute, respectively. Operate the column and detector cell at 200° C. and maintain the injection port at 240° C. Use an electrometer range setting of 100 with a detector pulse interval of 15 microseconds.

Using a microliter syringe, inject 5 μl. of the sample or standard solution into the chromatograph. Identify the DEF peak by its retention time and measure the area produced on the recorder strip chart with a polar planimeter. At the operating conditions employed, the retention time for DEF is 6.5 minutes.

Calculate the parts per million of DEF in a sample by comparing the response for an unknown directly to the response for a known standard of similar concentration. Under the above conditions, 1.0 nanogram of standard DEF injected corresponds to 0.1 p.p.m. in the unknown.

Discussion

The gas chromatographic method for DEF has definite advantages over the fluorometric method. It is shorter and interference due to mercaptans in the air is eliminated because DEF is measured directly. The low blanks lead to increased sensitivity.

With cottonseed samples, there are few problems. The column retains the major portion of the pigments and those which are eluted with acetone present no difficulty. With cottonseed oil samples, however, poor recoveries are obtained if the dissolved sample is allowed to percolate through the column too rapidly. Apparently, the large amount of oil present tends to swamp the Florisil, so that a longer contact time is required for adsorption of the DEF. Good recovery is obtained if the flow rate is no faster than 1 to 2 drops per second. In eluting with acetone, the DEF is usually off the column in the first 50 ml., so that good recovery in this step is assured by eluting with 100 ml. of solvent. After evaporation of the acetone, 1 or 2 ml. of oil remain in either case. This amount of oil remaining gives little difficulty.

An alternative method involving a Skellysolve B–acetonitrile partition system was tried as a means to separate the DEF from the bulk of the oil. However, the partition coefficient for DEF between these two solvents is approximately 1 and at least four extractions were required for acceptable recovery.

Response of the electron affinity detector is linear over at least a hundredfold range from 0.1 to 10 nanograms. Therefore, samples having residues in excess of about 2 p.p.m. should be diluted and re-injected in order to have the response fall on the linear portion of the curve.

Cottonseed oil samples should be gas chromatographed within 24 hours of preparation because control peaks which tend to obscure the DEF peak increase with time.

Very poor chromatographic response for DEF is obtained at the low levels employed in residue work unless the gas chromatographic column is prepared by the solution coating technique described above (*2*). By employing this technique, peak shape is improved and response is increased.

In the preparation of the samples for chromatography, the use of wash bottles with rubber stoppers or rubber connectors was discontinued because an extractive from the rubber caused a large interference peak with a retention time of about 20 minutes. This peak does not interfere directly but, if not eluted, masks the sample peak for subsequent injections.

Analyses were conducted on a number of samples of cottonseed and refined cottonseed oil to which known amounts of DEF had been added (Table I). In the case of cottonseed, the DEF was added to the dry material before extracting on the Soxhlet apparatus. For cottonseed, the recoveries are generally better than 95%. In general, recoveries from oil were slightly less. Chromatograms of control and spiked samples of cottonseed are shown in Figure 1.

Using a 50-gram sample containing 0.1 p.p.m. and a final volume of 25 ml. a 5-μl. aliquot injected into the gas chromatograph produces an area of about 0.2 square inch. This allows adequate precision for measuring with a polar planimeter. Because injections of more than 5 μl. tend to cause band spreading of the DEF peak due to increased amounts of oil injected, the sensitivity of the method is established at 0.1 p.p.m.

Literature Cited

(1) Loeffler, W. W., Anderson, C. A., MacDougall, D., Abstracts, p. 13A, 138th Meeting, ACS, New York, N. Y., September 1960.
(2) Parcher, J. F., Urone, P., *J. Gas Chromatog.* **2,** 184–5 (1964).
(3) Thomas, R. F., Harris, T. H., Abstracts, p. 23A, 148th Meeting, ACS, Chicago, Ill., September 1964.

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