Determination of Phosphoric Acid, Dimethyl p-Methylthiophenyl Ester in Cottonseed

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The insecticide GC-6506[1] (phosphoric acid, dimethyl p-methylthiophenyl ester) is a potential acaricide and insecticide for use on cotton. A sensitive and reasonably specific method for determining residues of the compound in cottonseed was required to establish the levels that might be found following its use. A gas chromatographic procedure was developed, utilizing a phosphorus-sensitive detector, that provided the degree of minimum detectability (0.05 ppm) and specificity required for the study.

Experimental

Sample Preparation. A sample of seed (not delinted) weighing 26-30 g was found in a Wiley mill and 25.0 g of the ground product was transferred to the Omni-Mixer container. The seed was stored in a freezer and was ground without thawing, a procedure that minimized the problem of cleaning the mill, and that also gave a more uniform grind. One-hundred-fifty ml of hexane was added and the mixture was blended for 5 minutes at 85 v. The macerate was filtered through a 7-cm Whatman No. 2 filter paper in a Buchner funnel; the cake was transferred back to the Omni-Mixer and again blended with 100 ml of hexane and filtered as before. This procedure was repeated twice more (a total of 4) and the Omni-Mixer container and filter were rinsed with an additional 100 ml of hexane. (Note: all solvents used in this and succeeding steps were redistilled in glass.) The extracts (a total of 550 ml less holdup) were combined and stored in a cold room in tightly sealed bottles.

Cleanup. The hexane was removed from the total sample in a rotary vacuum evaporator at 60°C (water bath) and the oil transferred to a 250-ml separatory funnel with 50 ml of a 65:35 acetonitrile-water mixture. A few grains of sodium chloride were added and the funnel was shaken for 1 minute. The lower phase was drained into a 500-ml separatory funnel and the upper phase was extracted a second time in the same manner, adding the new lower phase to that from the first extraction. (The addition of the small amount of sodium chloride aids in separation of the phases.)

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[1]Allied Chemical Corporation, Agricultural Division, Hopewell, Virginia.

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 1, 1970. published by Springer-Verlag New York, Inc.

To the combined lower phase (acetonitrile-water) in the 500-ml separatory funnel was added 100 ml of 2% sodium chloride solution and 40 ml of emthylene chloride. The funnel was shaken for 1 minute and the clear methylene chloride was drawn off into a 250-ml separatory funnel. The aqueous acetonitrile solution was extracted twice more with 20-ml portions of methylene chloride, which were added to the first along with 40 ml of distilled water. The funnel was shaken vigorously for about 5 seconds, and the separated methylene chloride was drawn off into a 250-ml round-bottom flask through a funnel containing a cotton plug on which was placed about 10 g of anhydrous sodium sulfate. Funnel and sodium sulfate were washed with 25 ml of fresh methylene chloride and the combined extract and washings were then evaporated to near dryness in a rotary vacuum evaporator at 60°C (water bath). The residual solvent was removed at room temperature with a jet of air, and the residue dissolved in 10 ml of 3:1 hexane-ether.

Ten grams of Florisil (activated byheating at 130°C for 4 hours or more) were poured into a Shell-type chromatographic column and settled by applying vacuum and tapping; about 1 cm of anhydrous sodium sulfate was placed on top of the Florisil. The column was wet with 20 ml of hexane and the sample was transferred from the flask to the column as soon as the hexane had entered the adsorbent. The column must never be allowed to run dry. The flask was rinsed into the column 3 times with 10-ml portions of the hexane-ether mixture and after the last rinsing from the flask had just entered the adsorbent, 170 ml of hexane-ether mixture was added to the reservoir. The column was allowed to elute until the last of the solvent had just entered the column, then 200 ml of acetone was placed in the reservoir, the receiver was changed, and elution was continued to completion. The elution rate was about 1 drop per second; slight vacuum was necessary to maintain this rate.

The acetone eluate, containing the pesticide, was reduced to about 2 ml in a Kuderna-Danish evaporative concentrator and diluted to 10.0 ml (or less, as required) with acetone.

Gas Chromatography. The acetone solutions were analyzed with either a thermionic phosphorus detector or a flame photometric detector equipped with the 526 mμ filter for phosphorus. Both types of detectors are equally satisfactory and the choice is a matter of availability and/or personal preference. Operating parameters were as follows:

Thermionic detector:

Column: stainless steel, 18" x 1/8", 3% Carbowax 20 M on Gas Chrom Q, 60/80 mesh.

Temperatures: injector 210°C, column 175°C, and detector 220°C.

Carrier gas: nitrogen, prepurified, 30 ml/min.

Flame photometric detector:

Column: stainless steel, 18" x 1/4", 3% Carbowax 20 M on Gas Chrom Q, 60/80 mesh.

Temperatures: injector 210°C, column 175°C, and detector 160°C.

Gas flow rates: nitrogen, prepurified, 100 ml/min; oxygen 25 ml/min; and hydrogen 160 ml/min.

The short Carbowax columns listed above will separate GC-6506 from all pesticides registered for use on cotton except malathion. If the latter is present or suspected, it may be separated from GC-6506 (1) with the column and operating parameters given below. GC-6506 can be measured and distinguished from all other pesticides registered for use on cotton by using this column, thus providing the required specificity for the method.

Column: stainless steel, 6' x 1/8", 7% QF-1 and 9% OV-17, each coated on Gas Chrom Q, 80/100 mesh, and mixed 1:1.

Temperatures: injector 210°C, column 210°C, and detector 160°C.

Gas flow rates: as required for satisfactory response.

Discussion

Volumes injected into the gas chromatograph were 1-5 µl. Retention time for both GC-6506 and malathion on the 18-inch columns was near 3.5 minutes, but on the 6-foot column the elution times were about 7.3 minutes for GC-6506 and 9.4 minutes for malathion. The response for both detectors was linear from 0.5-6 ng of GC-6506.

Recoveries of GC-6506 added to extracts from control samples were 100 $\pm$ 5% at 1 ppm, 0.1 ppm and 0.05 ppm levels.

Acknowledgment

Technical GC-6506, 92%, was furnished as a reference standard by Allied Chemical Corp., Hopewell, Virginia.

The research was supported in part by a grant-in-aid from this company.

Reference

1. Hazleton Laboratories, Inc., private communication, May 21, 1968.