Determination of Terbacil Residues Using Microcoulometric Gas Chromatography

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A programmed temperature microcoulometric gas chromatographic method for determining terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) residues in soil, plant, and animal tissues is based on the measurement of the intact molecule after it has been extracted from the sample with an alkaline solution and subsequently partitioned into an organic solvent. Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) can also be determined by the same procedure. The method has a sensitivity of about 0.04 p.p.m. based on a 25-gram sample, and an average recovery of better than 90% has been demonstrated on a variety of substrates.

Terbacil (3-*tert*-butyl-5-chloro-6-methyluracil), one of several substituted uracil herbicides, has recently been introduced for selective weed control in certain crops. Residue tolerances of 0.1 p.p.m. have been established covering current registered uses in sugarcane, citrus fruits, peaches, apples, and peppermint. It also has potential for other crops, including alfalfa. A sensitive, highly selective gas chromatographic method has been developed for determining residues of terbacil in soils, plants, and animal tissues. Residues of bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) can also be determined by this procedure with only minor modifications in the chromatographic conditions. The earlier method for determining bromacil residues (Pease, 1966) is not suitable for terbacil.

The method is based on the gas chromatographic measurement of the intact uracil after it has been extracted from the sample with an alkaline solution and the extract purified by solvent partitioning and Florisil column cleanup. The use of programmed temperature gas chromatography permits determination of both terbacil and bromacil simultaneously. A selective microcoulometric detector is used to minimize the possible interferences. Satisfactory recoveries at the 0.04-p.p.m. terbacil level have been demonstrated on a variety of samples. Average recoveries of about 94% have been obtained on soil, animal tissue, and many crops.

APPARATUS AND REAGENTS

The MT-220 gas chromatograph (Micro-Tek Instruments, Inc., Baton Rouge, La.) was equipped with Dohrmann microcoulometric titrating system, consisting of C-200 microcoulometer, T-300S titration cell, and S-100 sample inlet/combustion unit.

The chromatographic column was 5% General Electric XE-60 silicone gum plus 0.2% Epon Resin 1001 on 60- to 80-mesh Gas Chrom Q (Applied Science Lab., Inc., State College, Pa.), 4 feet, glass, $^1/_4$-inch O.D., $^3/_{16}$-inch I.D.

The Florisil column was a chromatographic tube 10 × 150 mm. with a coarse fritted disk. Fill the chromatographic tube, with gentle tapping, to a depth of 70 mm. with Florisil that has been activated at 130° C. for 4 hours. A glass wool plug may be used at the base of the column to prevent clogging of the fritted disk. Add about 10 mm. of anhydrous sodium sulfate to the top of the column. Wash the column with 25 ml. of *n*-hexane just prior to use.

The terbacil was standard reference material available from E. I. du Pont de Nemours & Co., Wilmington, Del.

The *n*-hexane, ethyl acetate, and chloroform were Distilled-in-Glass solvents, Burdick & Jackson Laboratories, Inc., Muskegon, Mich.

EXPERIMENTAL

Gas Chromatographic Calibration. Equilibrate the gas chromatograph as follows: vaporizer temperature, 230° C.; transfer temperature, 250° C.; furnace temperature, 850° C.; column temperature, 225° C.; carrier flow, helium, 100 cc. per minute; purge flow, helium, 50 cc. per minute; oxygen flow, 50 cc. per minute.

Condition the column by maintaining its temperature at 225° C. for at least 48 hours. Prepare a calibration curve by chromatographing appropriate aliquots of standard solutions containing 1, 5, 25, 50, and 100 μg. per ml. of terbacil in ethyl acetate and plotting peak heights *vs.* micrograms of terbacil injected. Peak height is used because of the base line elevation obtained at the high chromatographic temperatures of this method. This base line shift nullifies the advantages of an electromechanical integrator for determining peak area. Set the column temperature to 100° C. and the coulometer sensitivity at 90 ohms. Inject the sample, 100 μl. of the 1 and 5 μg. per ml. standards, and the appropriate volumes of the 25, 50, and 100 μg. per ml. standards to deliver 0.5 to 0.8 μg. of terbacil. Two minutes after completion of the injection, begin programming the column temperature at 10° C. per minute. Program to 225° C. and hold at this temperature for about 10 minutes to recondition the column. The retention time for terbacil from start of programming is about 13 minutes. A typical gas chromatographic scan of a standard solution is shown in Figure 1. The response to terbacil is linear throughout the range studied, and it is chromatographed without change. By utilizing the absolute measurement characteristics of the microcoulometer, terbacil is shown to be chromatographed quantitatively.

Isolation. For analysis of crops, place 25 grams of a representative sample into a Waring Blendor jar, add 100 ml. of 1% NaOH solution, cover, and blend at high speed for 3 to 5 minutes. When analyzing soil, weigh 25 grams

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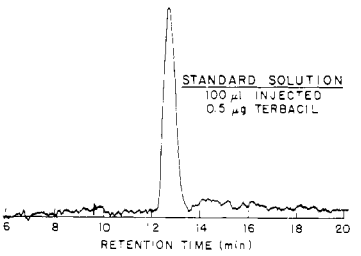

Figure 1. Gas chromatogram of terbacil standard solution

into a 250-ml. glass-stoppered Erlenmeyer flask, add 100 ml. of 1% NaOH solution, stopper, and shake on a wrist-action shaker for 15 minutes.

Transfer the blended sample quantitatively to a 250-ml. centrifuge bottle using several small portions of the 1% NaOH solution as wash. Centrifuge at 2000 r.p.m. for 5 to 10 minutes, and carefully decant the aqueous phase through glass wool into a 500-ml. separatory funnel. Add 100 ml. of the NaOH solution to the substrate remaining in the centrifuge bottle, stopper with a cork plug, and shake vigorously for 2 to 3 minutes. Centrifuge as before and combine the aqueous phase with the contents of the separatory funnel. Acidify the combined extracts with 10 ml. of 10*N* H_2SO_4, and add 50 ml. of chloroform. Shake for 2 minutes and allow the phases to separate. (Centrifuge to get a clean separation.) Collect the chloroform in a 150-ml. beaker. Repeat the extraction two more times, each time using 50 ml. of chloroform and combining the solvent extracts in the 150-ml. beaker. Discard the aqueous phase.

Evaporate the solvent to dryness in a hood at room temperature. Take up the residue with 50 ml. of 1*N* NaOH solution and transfer to a 125-ml. separatory funnel. Continue the transfer using 50 ml. of *n*-hexane. Shake for 2 minutes and allow the phases to separate. Discard the hexane layer. Repeat the hexane wash using a second 50-ml. portion of *n*-hexane.

Add 50 ml. of ethyl acetate to the separatory funnel and shake for 2 minutes. After a clear separation of the layers, collect the ethyl acetate in a 100-ml. beaker. Repeat the extraction using a second 50-ml. portion of ethyl acetate. Because of the very weak acidity of terbacil, it can be extracted from an alkaline solution with polar solvents. Combine the solvent extracts and evaporate to about 0.5 ml. Transfer the concentrated extract to a Florisil column, prepared as described under Apparatus and Reagents, using several small washings of ethyl acetate. Allow the solvent to absorb on the column and wash with 25 ml. of *n*-hexane. Discard the *n*-hexane. Elute the terbacil residue using 25 ml. of ethyl acetate, collecting the solvent in a 30-ml. beaker. Evaporate to about 0.5 ml., and transfer the concentrated extract to a 1-ml. volumetric flask using a finely drawn out dropper. Wash with several small portions of ethyl acetate, dilute to volume, and mix thoroughly.

Gas Chromatographic Analyses. Equilibrate the gas chromatograph and inject an aliquot of the sample as described under Calibration. Determine the micrograms of terbacil from the calibration curve prepared as described under Calibration. Calculate the residue of terbacil in p.p.m. by dividing the micrograms found, corrected for aliquot factor and recovery factor by the sample weight in grams.

RESULTS AND DISCUSSION

The gas chromatographic method described is sensitive to about 1 µg. of terbacil or 0.04 p.p.m. based on a 25-gram sample. Its applicability to a variety of substrates has been demonstrated by analyzing untreated controls to which known quantities of terbacil were added. Results of these recovery studies are summarized in Table I. Essentially quantitative recoveries were obtained for all substrates over a range of 0.04 to 5 p.p.m. Nearly all background was eliminated, and no interference was encountered from untreated controls. Typical gas chromatograms of extracts from a control sample and one fortified with terbacil are illustrated in Figure 2. The upper curve was obtained in the extract of oranges fortified with terbacil at 0.08 p.p.m.; the lower curve represents the control oranges.

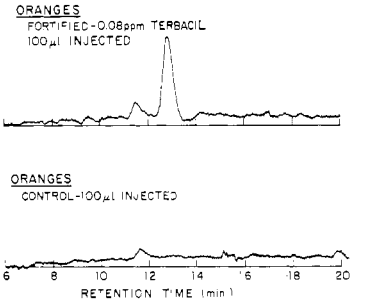

Figure 2. Gas chromatograms of extracts of control and fortified oranges

Table I. Summary of Terbacil Recovery Data

	Residue Level, P.P.M.	No. of Determinations	Recovery, % Av.	Recovery, % Range
Apples	0.04–0.40	6	95	90–100
Peaches	0.04–0.40	5	96	90–100
Pears	0.08–0.20	2	85	80–90
Oranges	0.04–0.40	8	94	83–100
Grapefruit	0.04–0.40	6	100	90–110
Lemons	0.04–0.20	3	91	88–95
Tangelos	0.08–0.20	2	88	86–90
Alfalfa	0.13–3.3	5	93	80–105
Sugar cane	0.04–1.0	6	98	92–110
Molasses	0.04–0.40	3	91	90–94
Bagasse	0.04–0.40	5	96	92–100
Peppermint oil[a]	0.04–1.0	6	100	86–110
Peppermint hay[a]	0.04–0.40	6	90	80–100
Soil	0.04–2.0	12	96	84–110
Urine	0.2–5.0	7	100	92–110
Feces	0.5–5.0	4	91	84–96
Kidney	0.12–1.6	4	87	75–97
Liver	0.08–2.0	5	92	75–100
Muscle	0.04–0.8	4	98	84–108
Fat	0.08–1.2	5	94	85–103
Blood	0.04–0.8	4	102	94–110

[a] When analyzing peppermint oil and hay, it is necessary to dilute the 25-gram sample with 10 ml. of acetone in order to assure quantitative extraction of terbacil with 1% NaOH.

The experimental procedure described herein is equally satisfactory for the determination of both terbacil and bromacil residues. To optimize response to bromacil, however, it is necessary to maintain a low furnace temperature (750° C.) and low oxygen supply (20 cc. per minute) as reported earlier (Pease, 1966). Simultaneous

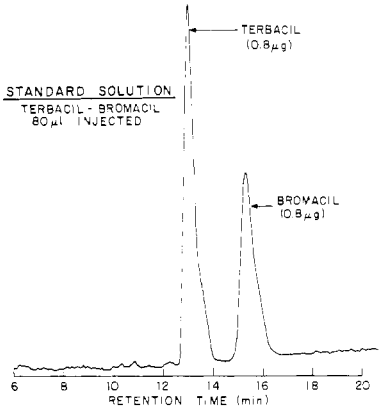

Figure 3. Gas chromatogram of standard solution containing both terbacil and bromacil

determination of terbacil and bromacil can be satisfactorily conducted by compromising on these two parameters. Figure 3 shows a gas chromatographic scan of a standard solution containing both of these compounds. The furnace temperature for this analysis was 800° C., and the oxygen flow was about 35 cc. per minute. With these conditions, yield of both compounds should exceed 80% of that obtained when the optimum conditions are adhered to.

The nitromethane-hexane solvent cleanup (Pease, 1966) can also be used successfully for terbacil. When analyzing for either terbacil or bromacil this cleanup procedure may be used in place of, or in addition to, the Florisil column, depending on the cleanup required for the analysis. Because of the selectivity and sensitivity of the improved C-200 microcoulometric detector, the large volume injection (500 μl.) is no longer required, and it is possible, on certain crops and soil, to eliminate both the nitromethane-hexane cleanup and the Florisil column. An example would be if high sensitivity is not desired or the residue is expected to be relatively high, such as in heavily treated soil samples. This, of course, will have to be determined in each laboratory.

High temperature electron-capture detector using nickel-63 as the radioactive source can be used in this procedure. For most crops, however, especially when high sensitivity work is required, the microcoulometric detection system for determining both terbacil and bromacil residues appears to have distinct advantages. Higher background arising from other electron-capturing species and, in many instances, interfering peaks having retention times similar to the compounds being analyzed, complicate the use of the electron-capture detector.

LITERATURE CITED

Pease, H. L., J. AGR. FOOD CHEM. **14**, 94 (1966).

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