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PESTICIDE RESIDUES

Colorimetric Determination of Ethyl 4,4′-Dichlorobenzilate (Chlorobenzilate) as a Spray Residue

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An analytical method is presented for the microdetermination of the acaricide, ethyl 4,4′-dichlorobenzilate (Chlorobenzilate), as a spray residue. Essentially, the method involves nitration of the compound, followed by the interaction of the nitrated product with sodium methylate to produce a red color which is measured spectrophotometrically at 538 mμ. The method has been further developed to determine the compound in the presence of DDT. This involves saponification of the Chlorobenzilate to 4,4′-dichlorobenzilic acid, followed by its separation by extraction from the dehydrochlorinated DDT, and its subsequent nitration. The nitrated 4,4′-dichlorobenzilic acid gives the same colored complex with sodium methylate as the ester, with maximum absorption at 538 mμ.

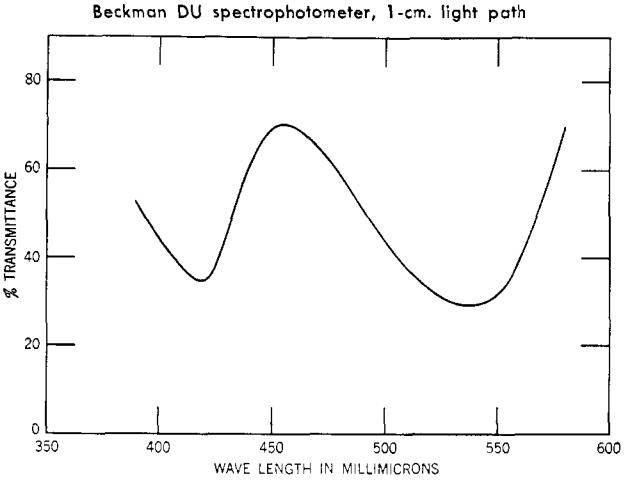
CHLOROBENZILATE has been thoroughly tested in the field by experimental workers and found to be a potent weapon in the control of various species of mites. Already accepted for certain agricultural crops, the compound should find wide-spread uses as an acaricide. This necessitates the development of an accurate microanalytical method, in order to check on the magnitude of the residues remaining on crops at harvest time.

The structural similarity of Chlorobenzilate to DDT suggested the use of the Schechter-Haller (*4*) procedure for the determination of the compound. Investigation showed that the method is suitable for Chlorobenzilate. When the compound is nitrated, and the nitrated product made to react with sodium methylate, a red colored complex is formed which shows maximum absorption at 418 and 538 mμ. The absorption curve for this complex is reproduced in Figure 1.

This adaptation of the Schechter-Haller procedure was tested at the University of California Citrus Experiment Station for the microdetermination of Chlorobenzilate in the presence of citrus extractives, and it was reported (*1*) that extensive isolative procedures are required for its successful use. Two satisfactory alternative methods for citrus have been developed by Blinn, Gunther, and Kolbezen (*1*) based on the hydrolysis of Chlorobenzilate to 4,4′-dichlorobenzilic acid, which is then selectively oxidized to 4,4′-dichlorobenzophenone. The latter is determined either by its absorption at 264 mμ or by the absorption of its 2,4-dinitrophenylhydrazone derivative at 510 mμ. Gunther and Blinn (*2*) have also suggested an adaptation of a total chlorine method, although this procedure is nonspecific for Chlorobenzilate.

The modified Schechter-Haller procedure described in this paper has proved to be satisfactory for the determination of Chlorobenzilate on a variety of crops. Because of the widespread use of DDT in pest control, some way of eliminating this interfering substance prior to nitration was desirable. A satisfactory procedure is based on a method for determining DDT in biological materials by Prickett, Kunze, and Laug (*3*), in which the DDT is dehydrochlorinated with alcoholic potassium hydroxide and extracted with petroleum ether. Under these conditions, Chlorobenzilate is saponified to 4,4′-dichlorobenzilic acid and remains in the aqueous phase as the potassium salt. Upon acidification, the salt is converted to the acid, which is then removed by extraction with ethyl ether. The 4,4′-dichlorobenzilic acid is then determined colorimetrically using the modified Schechter-Haller procedure. The acid gives the same red colored complex as the ester with maximum absorption at 418 and 538 mμ.

Figure 1. Transmittance curve for complex resulting from interaction of nitrated Chlorobenzilate and sodium methylate



Reagents

All chemicals are analytical reagent grade. Where solvents were distilled, it was done in an all-glass apparatus. Distilled solvents are

preferred and were used in most cases in development of the method reported. Preparation of dry benzene is time-consuming and is not necessary except as a medium for the development of the colored complex.

Nitrating acid, 1 to 1 mixture of fuming nitric acid and concentrated sulfuric acid.

Potassium hydroxide, solution containing 5 grams of potassium hydroxide per 100 ml. of water.

Diethyl ether. When used for extracting the nitrated derivatives, distill just before use. Otherwise, use as received.

Sodium chloride, saturated solution of sodium chloride in water.

Methanol, absolute, distilled.

Benzene, distilled.

Benzene, dry. Distill until no more water comes over, replace with a dry condenser, and continue distillation.

Sodium methylate, 10.0 ± 0.1% solution, prepared as directed in the original Schechter-Haller method.

Stearic acid, a solution of 0.5 gram in 100 ml. of benzene, and a solution of 0.5 gram in 100 ml. of acetone.

Petroleum ether, 30° to 60° C. boiling point range.

Dilute hydrochloric acid, *ca.* 3*N*.

Acetone, distilled.

Sodium sulfate, anhydrous.

Methanolic potassium hydroxide, 20 grams of potassium hydroxide per liter of methanol.

Determination of Chlorobenzilate in Strip Solutions

Place a measured aliquot of the benzene strip solution in a nitrating tube (22 × 175 mm.), add a small glass bead and immerse the tube about one-third its length in a steam bath. Boil down the benzene solution to about 10 ml. To the tube add 2 ml. of the stearic acid in benzene solution and continue the boiling until about 5 ml. remain. Then place the tube in a water bath at a temperature of approximately 50° C. and remove the remaining benzene with the aid of a gentle current of air. Add a few milliliters of methanol and again remove the solvent, using the water bath and current of air.

Place the tube in an ice-water bath and introduce 5 ml. of cold nitrating acid with a pipet taking care to wet down the sides of the tube. Place the bath on a hot plate and bring its temperature to 85° C. in 30 to 35 minutes. Then remove the tube, immerse about one-third its length in an active steam bath, and heat for exactly 1 hour, with intermittent swirling of the tube.

Chill the tube in a beaker of cold water and add 25 ml. of ice-cold distilled water to stop the nitration. Mix the contents of the tube by gentle swirling. Rinse the contents of the tube quantitatively through a small funnel into a separatory funnel (250- or 500-ml. capacity) with about 25 ml. of water from a wash bottle and 75 ml. of freshly distilled ether. Shake vigorously for about 1 minute. After complete separation of layers, draw off and discard the lower layer. Add 10 ml. of 5% potassium hydroxide and shake vigorously for exactly 0.5 minute. Draw off lower layer and discard. Repeat extraction twice more, using 10-ml. portions of potassium hydroxide and shaking for exactly 0.5 minute each time.

Next, wash the ether with three 15-ml. portions of saturated salt solution. Draw off the final salt wash as completely as possible. Pack a 0.75-inch plug of fine glass wool tightly in a glass Gooch crucible holder, moisten with ether, and allow the ether solution from the separatory funnel to filter slowly into a 125-ml. Erlenmeyer flask. Rinse the separatory funnel with 15 ml. of ether and add this to the flask through the crucible holder. Repeat with two 10-ml. portions of ether.

Place a small glass bead in the Erlenmeyer flask and remove the ether on a steam bath. Remove the last traces of ether with the aid of a gentle current of air, while the flask is still being heated. After cooling, add 25 ml. of dry benzene with a pipet, and swirl the flask to dissolve the residue. Pipet a 5-ml. aliquot into a 125-ml. Erlenmeyer flask. With a pipet add 10 ml. of sodium methylate reagent to the flask and swirl gently until the solution is homogeneous. After allowing 15 minutes for maximum development of color, pour the solution into a 1-cm. cell and take a reading immediately at 538 mμ in a Beckman DU spectrophotometer, using a slit opening of 0.04 to 0.05. Readings will remain unchanged for a reasonable time.

Preparation of Standards Pipet suitable quantities of a benzene solution containing from 0.1 to 0.5 mg. of Chlorobenzilate into nitrating tubes. Add 2 ml. of the stearic acid in benzene solution to each tube. (If the volume of the solution is much greater than 5 ml., add a glass bead and boil the solution down to about 5 ml. on a steam bath.) Place the tubes in a water bath kept at approximately 50° C. and remove the solvent with the aid of a gentle current of air. (As a precaution against volatilization of the compound, the tubes should not be heated any longer than necessary.) Add about 3 ml. of methanol and again evaporate to dryness, using the water bath and gentle current of air. Process the residues as described for the strip solution. Plot the relationship between transmittance or density and concentration.

When suitable aliquots of a technical sample of Chlorobenzilate, containing 85% active ingredient, were carried through the procedure as outlined above, the calibration curve obtained was found to conform to Beer's law. To test the efficiency of the method in recovering Chlorobenzilate in strip solutions, known quantities of the compound were added to a strawberry extract and analyzed. The results are tabulated in Table I.

Table I. Recoveries of Chlorobenzilate from Strawberry Extract

Added[a], γ	Recovered, γ	% Recovery
32	30	93.7
63	62	98.4
95	94	98.9

[a] Added to 25-ml. aliquots of strawberry extract obtained by stripping approximately 450 grams of strawberries with 350 ml. of benzene.

Determination of Chlorobenzilate in Strip Solutions Containing DDT

Place a measured aliquot of the benzene strip solution in a 300-ml. ground-glass Erlenmeyer flask. (When much wax is present, it is desirable to keep the volume of the aliquot to a minimum, as the wax is saponified during the procedure and may give rise to troublesome emulsions.) Place the flask on a steam bath and reduce the volume of the solution to about 10 ml. Remove the flask from the bath and evaporate the remaining solvent at room temperature with the aid of a gentle current of air.

To the flask add 50 ml. of 2% methanolic potassium hydroxide and reflux the solution for 0.5 hour. After cooling, transfer the contents of the flask quantitatively to a 500-ml. separatory funnel, using 50 ml. of water to complete the transfer. To the separatory funnel add 100 ml. of petroleum ether, and shake the funnel vigorously for 1 minute. After allowing for complete separation of the layers, draw off the lower layer into a second separatory funnel containing 50 ml. more of petroleum ether and shake for 0.5 minute. (Any solid material floating between the layers should be retained in the first funnel.) After complete separation, draw off the lower water-alcohol layer into a 300-ml. Erlenmeyer flask. To the first separatory funnel add about 10 ml. of water and shake the funnel very gently for about 5 seconds. (Any emulsion that forms can usually be broken up by swirling the separatory funnel after shaking.) After separation, transfer this wash water to the second funnel, taking care to retain any solid or frothy looking material in the first funnel. Shake the second funnel with the water and, after separation of layers, also transfer the wash water to the Erlenmeyer flask. Repeat this wash procedure twice more with 10-ml. portions of water. Then place the flask on a hot plate or steam bath, where the alcohol is removed and the volume of the solution reduced to about 60 ml. (A small glass bead added to flask will facilitate removal of alcohol.)

After cooling, transfer the solution to a 500-ml. separatory funnel, using 25 ml. of water to complete the transfer. Make the solution acid to litmus by adding dilute hydrochloric acid. (Usually about 7 ml. of 3*N* acid are sufficient.) To the separatory funnel add 100 ml. of ethyl ether and shake the funnel vigorously for 1 minute. Transfer the aqueous layer to a second funnel and shake again with a 50-ml. portion of ether, then transfer to a third separatory funnel where it is shaken once more with 50 ml. of ether, and finally discarded.

Transfer the ether in the first separatory funnel into a 300-ml. Erlenmeyer flask, passing it through a Gooch funnel containing a small wad (0.5 inch) of fine glass wool and a layer (1.5 inches) of anhydrous sodium sulfate. Use the ether in the second separatory funnel to rinse the first separatory funnel and pass this too through the Gooch funnel into the flask. Use the ether in the third separatory to rinse the second, and this in turn to rinse the first, after which pass it too through the Gooch funnel into the flask. Add a small glass bead to the flask and evaporate the ether down on a steam bath to a small volume (about 5 ml.). Transfer the contents of the flask, including the glass bead, quantitatively to a nitrating tube (22 × 175 mm.) using 25 to 30 ml. of acetone. Evaporate the acetone to a volume of 10 ml. in a hot water bath or carefully on a steam bath, add 2 ml. of the stearic acid in acetone solution, and continue the evaporation until about 5 ml. of solvent remain, after which remove the rest of the acetone at room temperature using a gentle current of air. Then follow the usual spray residue technique for the Schechter-Haller procedure.

Prepare a standard curve by taking known quantities of Chlorobenzilate varying from 0.1 to 0.5 mg. and carrying them through the procedure outlined above. Plot relationship between transmittance or density and concentration.

When suitable aliquots of a technical sample of Chlorobenzilate, containing 85% active ingredient, were carried through the procedure as outlined above, the calibration curve obtained was found to conform to Beer's law. To test the efficiency of the method in recovering Chlorobenzilate in strip solutions, known quantities of the compound were added to an apple extract and analyzed. The results are tabulated in Table II.

Comments on Procedures

Stearic acid is added to strip solution aliquots as a precautionary measure to minimize any possible losses of Chlorobenzilate due to volatilization during the evaporation of solvents, or due to "burning up" during nitration.

In the extraction of nitrated Chlorobenzilate with aqueous potassium hydroxide, contact with the alkali should be no longer than necessary for the thorough extraction and separation of layers, in order to prevent any appreciable decomposition of the chromogenic compounds. This decomposition was evident when 2-minute extractions were carried out with a 10% potassium hydroxide solution. The fact that nitrated Chlorobenzilate is not removed by the alkaline wash would seem to indicate that the compound, in the form of the ester or its acid derivative, is converted during nitration to nitrated 4,4′-dichlorobenzophenone.

The procedure, as outlined, calls for the solution of the nitrated Chlorobenzilate residue in 25 ml. of dry benzene, and the subsequent interaction of a 5-ml. aliquot of this solution with sodium methylate. However, in analyzing agricultural crops, particularly at harvest time, where the concentration of residual Chlorobenzilate is low, the amount of dry benzene added to the nitrated Chlorobenzilate should be reduced in order to increase the intensity of the color produced with the methylate.

The colored complex actually shows two maximum absorption peaks, one at 538 and the other at 418 mμ. However, the higher wave length gives slightly higher absorption values and exhibits less interference when analyses are run on actual samples of strip solutions.

The effect of concentration of the sodium methylate on the intensity of the colored complex was investigated and found to be similar to that of DDT.

Table II. Recoveries of Chlorobenzilate from Apple Extract[a]

Added[b], γ	Recovered, γ	% Recovery
142	140	98.6
237	235	99.2
379	376	99.2
474	467	98.5
711	719	101.1
948	970	102.3

[a] Analyzed by method for Chlorobenzilate in presence of DDT.
[b] Added to 25-ml. aliquots of apple extract obtained by stripping approx. 1600 grams of apples with 500 ml. of benzene.

Table III. Chlorobenzilate Residues on Apples[a]

	One Application[b]		Two Applications[c]	
Sampling Date	Days after application	Residues, p.p.m.	Days after application	Residues, p.p.m.
7/19	1	3.3	..	...
7/26	8	2.3	1	5.8
8/1	14	2.0	7	4.8
8/11	24	2.0	17	3.7
8/29	32	1.3	25	2.4
9/17	51	0.9	44	1.8

[a] Apples sprayed with Chlorobenzilate 25% wettable powder at concentration of 1 lb. per 100 gallons.
[b] Date of application, 7/18.
[c] Dates of application, 7/18 and 7/25.

A concentration of sodium methylate of around 10% appeared to give a maximum color intensity after the usual 15-minute waiting period, and the colored complex showed more stability when developed with methylate of this concentration.

In the method for Chlorobenzilate in the presence of DDT, the efficiency of the extraction of the dehydrochlorinated DDT with petroleum ether was indicated by the very good recoveries reported by Prickett, Kunze, and Laug (*3*). This was further proved by an experiment in which 500 γ of DDT were added to a 25-ml. aliquot of apple extract and carried through the procedure as described. This amount of DDT is greater than the concentration that would normally be found on apples at harvest time. However, the nitrated residue, when treated with sodium methylate, gave no color, indicating no carry-through of DDT or its dehydrochlorinated derivative.

Discussion

This method of determining Chlorobenzilate has been used satisfactorily by chemists in agricultural experiment stations and commercial laboratories for the determination of residual Chlorobenzilate on such crops as apples, pears, peaches, cantaloupes, and strawberries.

The magnitude of the residues remaining on apples is indicated by the results of an experiment run in New Jersey during the summer of 1952, where apple trees were sprayed with Chlorobenzilate 25% wettable powder, at a concentration of 1 pound per 100 gallons. Application was made with a hydraulic sprayer at a rate of approximately 20 gallons per tree. Some of the trees received only one application on July 18; others received two applications, one on July 18 and the second on July 25. Samples of apples were taken at regular intervals and stripped with benzene; the strippings were analyzed for Chlorobenzilate. Results of this experiment, showing gradual drop in concentration over a 2-month period, are given in Table III.

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