Determination of Residual *p*-Chlorophenyl *p*-Chlorobenzenesulfonate in Orange Pulp

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A new method for the determination of the acaricide, Ovex, in orange pulp involves hydrolysis of Ovex to *p*-chlorophenol and sodium benzene sulfonate. The *p*-chlorophenol, recovered by steam distillation, is nitrosated, chromatographed, and measured colorimetrically. This procedure eliminates the phenolic-like impurities, making it possible to determine less than 5 γ of Ovex with a recovery of at least 90%.

OVOTRAN (trade-mark of the Dow Chemical Co. for the mixture containing Ovex) has found extensive use as an acaricide on citrus fruits. The active toxicant is *p*-chlorophenyl *p*-chlorobenzenesulfonate, known also as K6451 and now sold under the trade name of Ovex. Since orange pulp has been used for cattle roughage, a quantitative method was needed for detecting trace amounts of this compound in both the wet and dry orange pulp.

The method of Kutchinski and Luce (*5*) was satisfactory for determining the minute amounts of *p*-chlorophenyl *p*-chlorobenzenesulfonate in the edible portion and for the analysis of the surface residues, but was not satisfactory for the pulp analysis because of interfering materials. The interferences are thought to be flavanones such as naringenin, or other pigments which could not be removed by distillation procedures. The method of Gunther and Jeppson (*4*), published after the work on this paper was completed, would also be acceptable for the surface residue analysis. It, however, would not offer a satisfactory solution for the pulp analysis, because of the same interferences mentioned above. Other methods tried and found unsatisfactory were the Millon test as refined by Chapin (*2*), the nitration method of Deichman and Schafer (*3*), and the azo formation procedure as described by Averell and Norris (*1*).

The *p*-chlorophenol resulting from the hydrolysis of *p*-chlorophenyl *p*-chlorobenzenesulfonate could be nitrosated under carefully controlled conditions and then separated from these interfering materials by chromatography. This method will detect less than 5 γ of Ovex with a recovery of more than 90%.

Apparatus

Steam Distillation Apparatus. The apparatus used by Kutchinski and Luce (*5*) is reproduced in Figure 1. The water reservoir should have a capacity

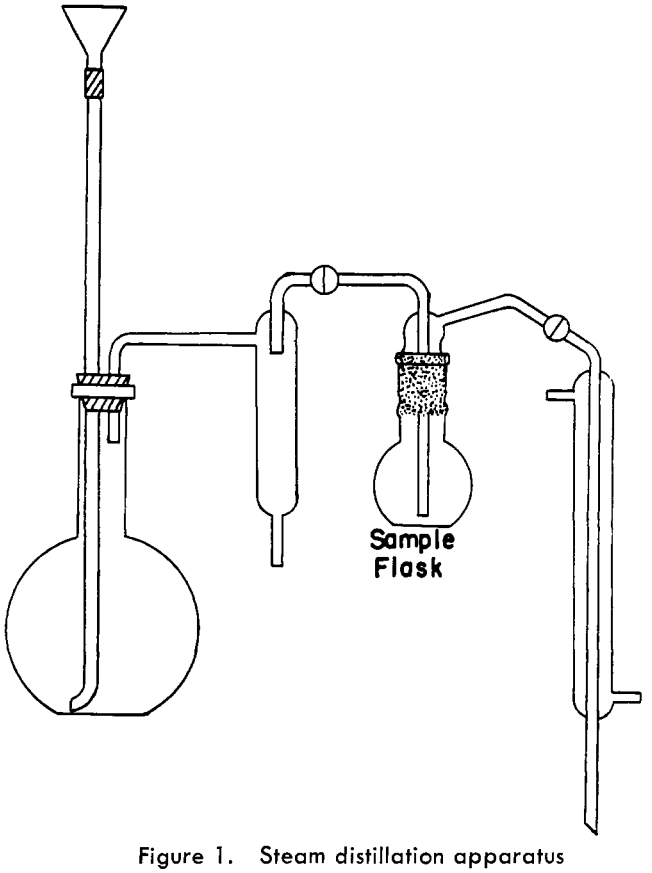

Figure 1. Steam distillation apparatus

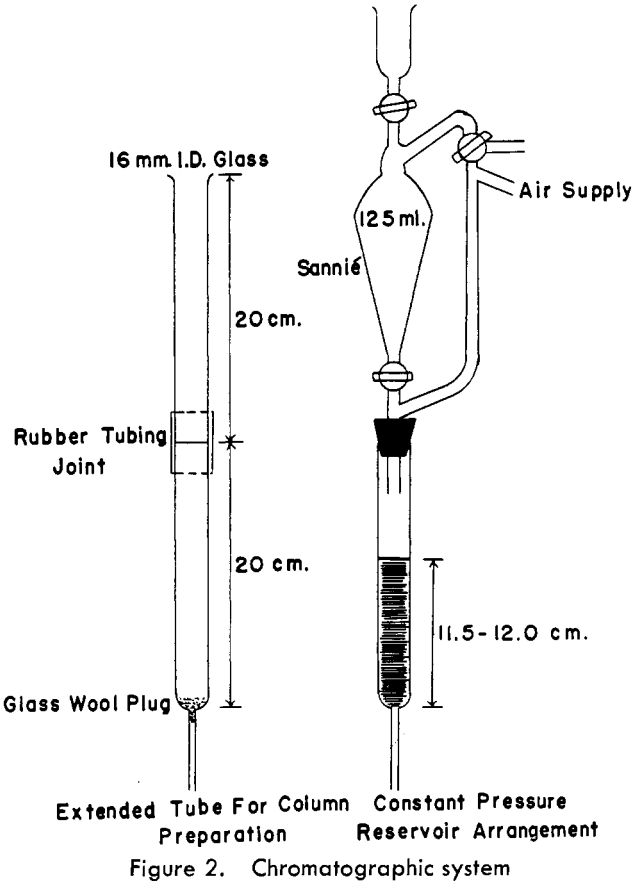

Figure 2. Chromatographic system

of at least 1 liter. The sample container is a 250-ml. Florence flask.

Chromatographic System. Figure 2 indicates the constant pressure reservoir system devised by Sannie (*6*).

To force-pack the chromatographic adsorbent, the following plunger is recommended: a perforated brass disk 3 mm. thick and 15.5 mm. in diameter attached to a brass rod 15 cm. long.

A Coleman Spectrophotometer, Model 14, equipped with 13.0-mm. cuvettes was used in this investigation. Any photometer measuring light transmittance at 430 mμ equipped with 1.0- to 1.5-cm. cuvettes should be suitable.

Reagents

Benzene, redistilled; potassium hydroxide, approximately 0.5*N* in 95% ethyl alcohol; orthophosphoric acid, 85%; sodium hydroxide, aqueous, approximately 1*N*.

Sulfuric acid, diluted 1 to 2 with water; sulfuric acid, concentrated; acetic acid, glacial; nitric acid, concentrated; sodium nitrite, approximately 1% aqueous solution prepared fresh daily; carbon tetrachloride, technical grade.

Magnesium carbonate, heavy powder; Celite No. 545, a diatomaceous silica prepared by Johns-Manville.

n-Butyl ether, practical grade; *n*-valeric acid 0.075% in *n*-butyl ether; sodium sulfate, anhydrous; sodium hydroxide, approximately 1.0%.

Chromatographic Adsorbent. A 1 to 1 mixture (by weight) of magnesium carbonate heavy powder and Celite No. 545 is dried at 130° C. for 24 hours.

Eluting Solvent. Wash 1500 ml. of *n*-butyl ether four times with 100-ml. portions of 1.0% sodium hydroxide followed by seven 100-ml. portions of water. Dry and store the ether in an amber bottle using anhydrous sodium sulfate as the desiccant. To prepare the 0.075% eluting solvent, add 0.41 ml. of *n*-valeric acid to 700 ml. of the washed *n*-butyl ether. This is sufficient eluent for four chromatograms.

Procedure

Place 100 grams of the fresh pulp, previously ground in a food chopper, or 100 grams of the dried pulp powdered by a burr mill, in a suitable glass-stoppered container with 200 ml. of benzene. Allow a soaking period of 2 or more days with intermittent shaking. Recover the benzene by filtration through a 1-cm. filter pad of Celite No. 545. Rinse both the filter pad and container with two 50-ml. portions of benzene. Wash the combined benzene filtrates with one 25-ml. portion of water and then transfer to the sample flask of the steam distillation apparatus.

Evaporate the benzene slowly on a steam bath to approximately 25 ml. In three portions, add 10 ml. of 0.5*N* alcoholic potassium hydroxide, adding the last portion just as dryness occurs. Allow the sample to remain on the steam bath an additional 10 minutes after the last portion of alcohol has been evaporated.

Dissolve the hydrolysis residue in 35 ml. of water and acidify with 5 ml. of 85% orthophosphoric acid. Attach the flask to the steam distillation apparatus and collect 125 to 135 ml. of distillate. Filter the distillate directly from the condenser through a medium-fluted paper into a tall 200-ml. beaker containing 2 ml. of 1*N* sodium hydroxide. Evaporate the distillate slowly to 15 or 20 ml. and transfer to a 125-ml. Erlenmeyer flask. Continue the evaporation to approximately 1 ml.

The *p*-chlorophenol is nitrosated by a modification of the procedure described by Wetlaufer, Van Natta, and Quattlebaum (*7*). Add 10 ml. of water to the residue in the Erlenmeyer flask, then successively 0.2 ml. of 1 to 2 sulfuric acid, 12.5 ml. of glacial acetic acid, 3 drops of concentrated sulfuric acid, 3 drops of concentrated nitric acid, and 0.5 ml. of 1.0% sodium nitrite solution. Cover with a watch glass and place the flask in a 60° C. water bath for ten minutes. Cool in an ice bath for 5 minutes and transfer with water to a 60-ml. separatory funnel. Extract the nitrosated *p*-chlorophenol with 5-, 5-, 3-ml. portions of carbon tetrachloride.

Wash the combined carbon tetrachloride extracts with one 25-ml. portion of water. Then extract the water wash with 2- and 1-ml. portions of fresh carbon tetrachloride. Transfer the combined extracts to another 60-ml. separatory funnel and dry by shaking with 5 grams of anhydrous sodium sulfate. Place a small plug of cotton in the stem of the separatory funnel and filter the dried extract into a 30-ml. beaker. Rinse the separatory funnel with several small portions of carbon tetrachloride and evaporate the combined solvent to approximately 2 ml. on a steam bath, using a fine air stream directly over the surface. When the desired volume is obtained, transfer to a freshly prepared chromatographic column.

Chromatography Add 1.5 ml. of water dropwise to 10 grams of the adsorbent in a mortar while stirring with a pestle. The mixture must be homogeneous. Add carbon tetrachloride slowly with grinding until a smooth paste is obtained. Transfer the mixture to the extended column as shown in Figure 2 with carbon tetrachloride. Agitate the slurry with a long glass rod to remove any trapped air.

Apply air pressure at 5 pounds per square inch until the adsorbent layer recedes into the lower portion of the extended column. After rinsing with carbon tetrachloride, remove the extended tube and rubber joint, and force the solvent surface down to the adsorbent surface with the 5 pounds per square inch of air pressure. Place a 17-mm. diameter fiber glass filter disk on top of the adsorbent and with the aid of the perforated brass plunger, compact the adsorbent to a height of 11.5 to 12.0 cm. as shown in Figure 2.

Table I. Recovery from Fortified Pulp

Added, γ	Recovered, γ	Recovery, %
	Fresh Pulp	
20	21.0	105
30	29.5	98
40	40.0	100
50	50.7	101
100	95.0	95
150	137.0	93
200	189.8	95
	Average	98
	Dried Pulp	
100	94	94
100	94	94
100	92	92
	Average	93

After rinsing and removal of the plunger pour off the excess carbon tetrachloride and add the sample immediately without rinsing. Apply air pressure at 2 pounds per square inch until the carbon tetrachloride surface just reaches the adsorbent surface, collecting the percolate in a graduated cylinder. Rinse the beaker and sides of the column with a small portion of carbon tetrachloride and force it into the adsorbent with the 2 pounds per square inch air pressure. Repeat the rinsing procedure until 10 ml. of percolate have been collected. Then add 3 ml. of the eluting solvent and attach the Sannie reservoir, filled with the eluting solvent.

With the air pressure set at 2 pounds per square inch, force the eluting solvent into the adsorbent. Without removing the pressure add another small portion of the eluting solvent to the column. Repeat the rinsing procedure until the *n*-butyl ether appears in the percolate. Its presence there can be detected visually by the abrupt change in the refractive index. At this point open the reservoir, permitting a continuous flow of eluting solvent. Increase the air pressure to 3.5 to 4.0 pounds per square inch, depending on the rate of percolation. This rate should be maintained at 4 ml. per each 5 minutes.

The zones of the chromatogram appear as soon as the sample has entered the column. On addition of the eluent, the interfering materials appear in a wide-yellow leading zone, while the desired nitrosated *p*-chlorophenol, moving at a lower rate, forms a distinct narrow yellow zone. Concentrations as low as 10 γ of *p*-chlorophenol are easily visible on the column.

The interfering materials will come off

in the first 20 ml. of *n*-butyl ether percolate following the change in refractive index. The next 40 to 50 ml. of percolate will not show a positive yellow color test when extracting with 1*N* sodium hydroxide.

When the desired zone (the only remaining zone) descends to within 3 to 5 mm. from the bottom of the adsorbent, collect the next 35-ml. cut and extract with exactly 5.0 ml. of 1*N* sodium hydroxide. Filter the sodium hydroxide extract through a plug of cotton directly into a 1-cm. cuvette and record the transmittance at 430 mμ with reference to water. The color is stable; however, it is recommended that the transmittance be recorded within an hour.

Standard Curve Prepare a standard curve from a benzene solution of Ovex containing 10 γ per ml. Dilute 2-, 5-, 10-, 15-, and 20-ml. aliquots to 300 ml. with benzene and follow the procedure as described for the pulp analysis including chromatography. The per cent transmittances obtained are plotted on semilogarithmic paper.

Experimental Results

Data obtained by the authors are shown in Table I.

This nitrosation and chromatographic procedure was developed to eliminate the interfering materials and produce a reasonable control sample blank. Nine determinations of 100 grams each of the control pulp yielded an average blank of 0.11 p.p.m. with a mean deviation of ±0.02.

As the amount of untreated dried pulp was limited, it was possible to run only three fortified recoveries and three control samples. However, the results are almost identical to the results obtained on the fresh pulp.

The three dried pulp controls produced identical blanks of 13.3 mg. or 0.13 p.p.m.

Discussion

Any variations in the nitrosation procedure as described will alter the chromatographic separation. For example, normal nitrosation procedures are usually performed by bringing the nitrosation mixture to a boil. The extract of such a mixture when placed on the adsorbent will produce three distinct zones, the usual yellow interference zone and two others, one of which will become fixed to the top of the column and remain there throughout the entire elution procedure for the second zone. The recoveries based on this second zone will vary between 40 and 60%.

Low recoveries will also result from varying the acetic acid concentration of the nitrosation mixture. The nitrosation method as described produces an acetic acid concentration of 50 to 55% by volume.

The single 25-ml. water wash on the carbon tetrachloride extract of the nitrosation mixture is necessary to reduce the acid content prior to the chromatographic separation. Neglecting this wash will cause the evolution of carbon dioxide and the subsequent eruption of the adsorbent.

To obtain a uniform chromatographic procedure an adequate supply of the magnesium carbonate and Celite No. 545 mixture should be set aside for subsequent use. To eliminate variations in the chromatographic separation of samples expected to contain less than 20 γ of Ovex, determinations should be carried out in groups of four.

1. Untreated control
2. Fortified untreated control (at least 20 γ of Ovex)
3. Treated sample
4. Treated sample (duplicate of No. 3)

The percolate data obtained from the fortified standard 2 can be used to cut the desired portion of the untreated control and treated sample chromatograms. The actual cut volume necessary containing the entire sample is about 15 ml. To ensure complete recovery and eliminate slight variations in columns even with the suggested grouping, a total cut of 35 ml. is recommended.

This procedure should be applicable to other citrus fruits and simple phenols and phenoxy compounds where similar phenolic-like materials interfere.

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PESTICIDE RESIDUES

Flavor of Selected Vegetables Grown in Pesticide-Contaminated Soils

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Undesirable off-flavors were detected in carrots, turnips, and green beans which were grown without insecticide treatment in soils contaminated with residues of technical benzene hexachloride (BHC) or lindane applied to preceding crops. Soil residues of the alpha, beta, and delta isomers of BHC also resulted in off-flavors in carrots. Heavy residues of aldrin (both technical and purified), dieldrin, heptachlor, Dilan, toxaphene, chlordan, endrin, isodrin, TDE, technical DDT, and methoxychlor did not cause significant flavor changes.

THE POSSIBILITY OF OFF-FLAVORS in food crops from use of insecticides, especially with respect to carryover of benzene hexachloride (BHC) in the soil, and conflicting reports concerning the effect on flavor of food crops of other insecticides carried over in the soil, point up the need for further study of the effect of growing various foods in soil exposed to contamination by residues of insecticides used in previous years.

Off-flavors were reported in potatoes grown in soil 2 (*11*) or 3 years (*8*) after the last application of benzene hexachloride (BHC). Off-flavors were also found in peanuts and peanut butter prepared from peanuts grown in soil treated with heavy applications of BHC