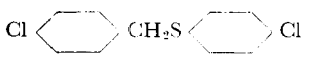
Determination of *p*-Chlorobenzyl *p*-Chlorophenyl Sulfide (Chlorobenside) and *p*-Chlorobenzyl *p*-Chlorophenyl Sulfoxide (Chlorobenside Sulfoxide) Residues on Apples

C. C. WATSON

Dominion Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand

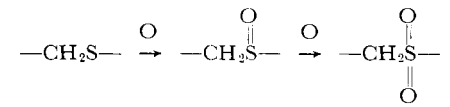
A method is described for the determination of Chlorbenside and Chlorbenside sulfoxide residues on apples. These acaricides can be determined alone, in the presence of each other, or in the presence of the insecticides, Ovotran (p-chlorophenyl p-chlorobenzenesulfonate), DDT, and Aramite [2-(p-*tert*-butylphenoxy)-1-methylethyl-2-chloroethyl sulfite]. Analysis of both substances is based on the ultraviolet absorption of Chlorbenside at 262 mμ after the removal of most interfering materials by a simple chromatographic procedure. Chlorbenside sulfoxide is readily reduced with tin and hydrochloric acid. Good recoveries of acaricides from apple extracts and mixtures are obtained.

CHLORBENSIDE, *p*-chlorobenzyl *p*-chlorophenyl sulfide,



has proved valuable for the control of Tetranychus mites on crops (*2*). A method for the determination of its residues on plant materials, which is specific for Chlorbenside in the presence of most commonly used insecticides except DDT, has been published (*5*). Chlorbenside and its sulfoxide can be determined within the range 4 to 17 p.p.m. as residues on apples in the presence of DDT and other insecticides by the present method. By increasing the aliquot taken to 10 ml., the quantity found (100γ) represents 2 p.p.m. As the mammalian toxicity (LD_{50}) is low, more than 3 grams per kilogram (*6*), acaricides in the 0- to 2-p.p.m. region are not of great importance.

Residues of Chlorbenside oxidize on leaf surfaces (*2*) to the corresponding sulfoxide or even to the sulfone. Both these oxides have acaricide properties similar to those of Chlorbenside.



The method adopted by Higgons and Kilbey (*5*) for the determination of Chlorbenside is similar to the Schechter-Haller method (*7*) for DDT. Chlorbenside sulfone, when nitrated with sodium methylate reagent, develops an intense purple color similar to the blue color given by DDT, whereas both Chlorbenside and its sulfoxide gave a weak orange color. Residues of both were oxidized to the sulfone with glacial acetic acid and hydrogen peroxide prior to nitration and color development.

As reported in a previous note (*11*), Chlorbenside absorbs strongly in the ultraviolet region of the spectrum at 262 mμ (ϵ = 9200) (Figure 1) (*4*). The intensity of this peak makes it suitable for the analysis of solutions containing 50 γ of Chlorbenside in 10 ml. of 95% ethyl alcohol.

Oxidation to the sulfoxide causes a change in the wave length of maximum absorption to 253 mμ (ϵ = 9200) (Figure 1). Further oxidation to the sulfone removes the absorption peak entirely. The sulfoxide, however, may be readily reduced to Chlorbenside with tin and hydrochloric acid; the sulfone is unaffected by this reducing agent and cannot be determined by this method. Measurement of Chlorbenside and the reduced sulfoxide was therefore made at the absorption peak at 262 mμ. Mixtures of Chlorbenside and sulfoxide can be separated by chromatography.

Reagents

Alumina, British Drug Houses, Ltd., reagent grade aluminum oxide for chromatographic adsorption.

Acids, analytical grade concentrated hydrochloric and glacial acetic acids.

Chlorbenside was extracted from a sample of 20% (by weight) commercial dust with acetone in a Soxhlet extractor. Recrystallization from methanol-water mixture gave fawn-colored crystals which, after being decolorized with carbon and recrystallized twice from methanol, gave a melting point of 72° C. (All melting points are uncorrected.)

Chlorbenside sulfoxide was prepared by dissolving 3.75 grams of Chlorbenside in 25 ml. of acetone, adding 1.5 grams of 30% hydrogen peroxide, and allowing the solution to stand at room temperature for 2 days (*9*). Evaporation of the acetone left a crystalline residue which on recrystallization twice from petroleum ether, gave pure Chlorbenside sulfoxide, melting point 124–125° C. This substance gave the orange color reaction (*5*) and an infrared absorption peak at 1057 cm.$^{-1}$ in carbon disulfide, which is within the 1040 to 1060 cm.$^{-1}$ region reported for sulfoxides (*1*, *3*).

Chlorbenside sulfone was prepared by dissolving 3.1 grams of Chlorbenside in warm glacial acetic acid and adding excess 3% potassium permanganate until a persistent purple color was obtained. The solution was decolorized with sodium bisulfite and 2 to 3 volumes of crushed ice were added (*10*). The precipitated Chlorbenside sulfone was filtered off and recrystallized twice from methanol-water mixture and gave a melting point of 152° C. It was also identified by nitration and color reaction with sodium methylate, and by infrared absorption bands at 1156 and 1332 cm.$^{-1}$ in carbon disulfide. Reported range for sulfone bands is between 1120 and 1160 cm.$^{-1}$ and also between 1300 and 1350 cm.$^{-1}$ (*1*, *8*).

Cotton wool, extracted with benzene or petroleum ether.

Ethyl alcohol, reagent grade 95%.

Magnesium sulfate anhydrous powder, analytical grade.

Sodium sulfate, anhydrous powder, reagent grade.

Solvents, British Drug Houses, Ltd. petroleum ether, boiling range 40° to 60° C., and benzene, both analytical grade.

Standard solutions were made by dissolving a weighed quantity of the substance in petroleum ether in a standard flask fitted with a polyethylene stopper. Chlorbenside sulfoxide was dissolved in a few milliliters of benzene and made up to volume with petroleum ether.

Tin, analytical grade, powdered tin.

Apparatus

Spectrophotometer, Beckman Model DU with 1-cm. silica cells.

Apparatus for directing a jet of air into the flask to assist in evaporating solvents.

Plain tubes of internal diameter 15 mm., constricted at one end to 5 mm., were used for chromatography.

Erlenmeyer flasks, 100- to 250-ml. capacity.

Procedure

The results in this paper were obtained by adding known amounts of Chlorbenside and its sulfoxide to petroleum ether extracts of unsprayed apples.

These extracts were obtained by me-

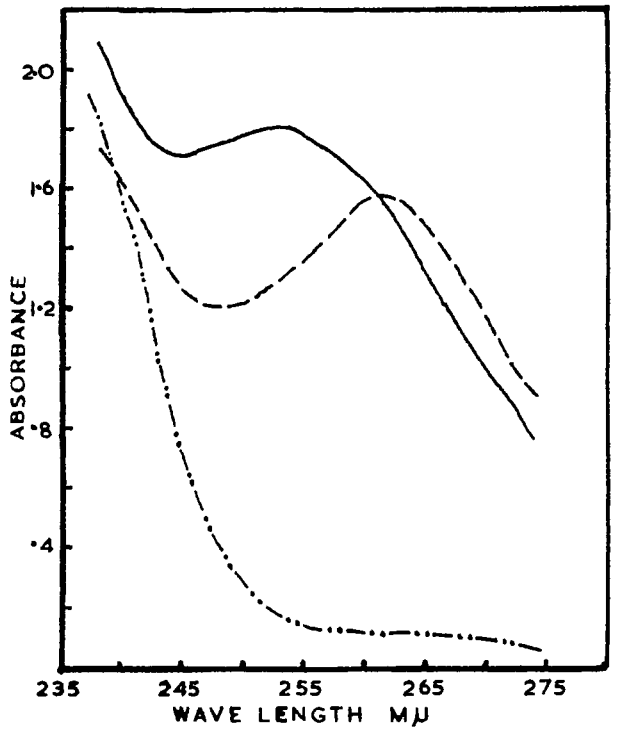


Figure 1. Ultraviolet absorption in 95% ethyl alcohol

-- Chlorbenside; 48 γ/ml.
— Chlorbenside sulfoxide; 56 γ/ml.
-·- Chlorbenside sulfone, 44 γ/ml.

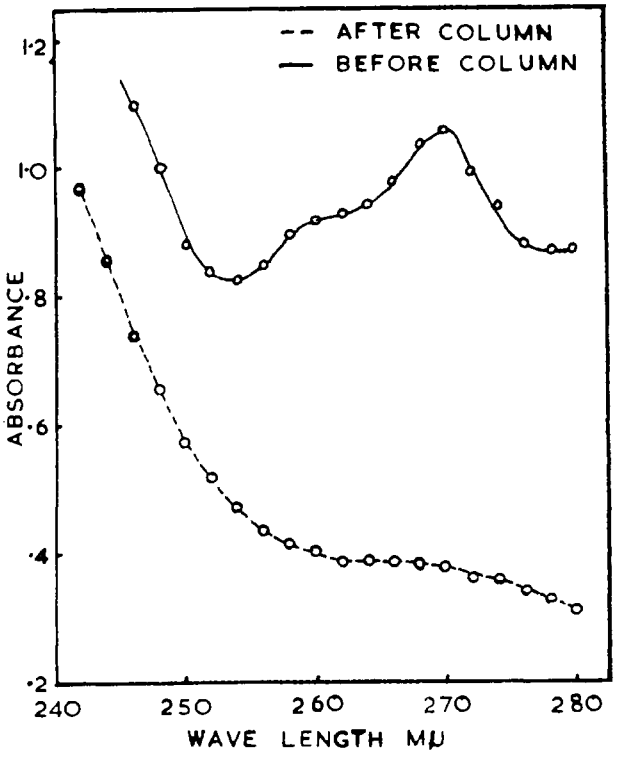


Figure 2. Ultraviolet absorption of untreated apple extracts before and after chromatography

chanically shaking the peelings from about 1 kg. of apples with 200 ml. of petroleum ether in a flask for 10 minutes, drying the extract with anhydrous sodium sulfate, and filtering it into a stoppered cylinder.

A chromatographic tube was fitted with a small plug of cotton wool and the dry alumina added and consolidated with gentle tapping to give a column 1 inch in height.

An aliquot, usually 5 ml., of the petroleum ether extract was pipetted on to the column followed by the standard quantity of Chlorbenside and the column was rinsed with a few milliliters of petroleum ether. The size of the aliquot used is limited only by the absorbance of the solution to be measured. A low blank value is desirable as less variation is found between readings.

Thirty-five milliliters of mixed solvent containing 25% of benzene by volume in petroleum ether was then added to elute the Chlorbenside; any sulfoxide present was retained by the alumina. Evaporation of the solvent on a water bath at 75° to 80° C. with an air jet left a residue of acaricide and wax from the apple skin. The flask which contained the residue was removed from the bath just before reaching dryness and the last trace of solvent was removed, by aeration with the jet for 30 seconds. Ten milliliters of 95% ethyl alcohol were added by pipet; the flask was warmed to dissolve the residue and then chilled in ice.

Precipitated wax was removed by filtration and the absorbance of the solution measured against a blank of alcohol at 262 mμ. An extract from untreated fruit was processed, concurrently.

Comparison of the absorbance of the extract from treated apples, after deduction of that from untreated apples, with a concentration–absorbance graph, prepared from pure Chlorbenside in ethyl alcohol gave the concentration present in the solution. These solutions obey Beer's law within the concentration range 0 to 500 γ per 10 ml. of ethyl alcohol.

In practice, the sulfoxide is found with Chlorbenside as a residue on fruit (*2*) and, to determine the sulfoxide in the mixture, a reduction process is necessary.

The reduction was effected by adding to the 5 ml. of apple extract containing the acaricides, 8 ml. of glacial acetic acid as solvent, 5 ml. of concentrated hydrochloric acid, and excess tin powder. After the mixture was heated for 40 minutes on a boiling water bath, 3 ml. of concentrated hydrochloric acid was added and the heating was continued for 20 minutes. The solution was then allowed to cool and was diluted with about 100 ml. of distilled water. This solution was extracted with 25 ml. of petroleum ether and the solvent was washed twice with water. The extracted solution and the first washings were again extracted with 25 ml. of petroleum ether and the solvent was washed as before. Anhydrous magnesium sulfate was used to dry the combined petroleum ether extracts, which were then evaporated to a small volume in preparation for the chromatographic column.

A sample of extract from untreated apples was reduced and processed along with the treated sample.

Discussion and Results

Chromatography. To select the most suitable adsorbent for maximum retention of apple extractives, equal volumes of the same apple extract were passed through silicic acid, alumina, gas carbon, and Nuchar (Eastman Kodak Co.) powdered charcoal. The ultraviolet absorption in ethyl alcohol of the eluted material, after removal of the wax, showed that Nuchar and alumina were the most adsorbent. Alumina was chosen for this work as the percolation rate of solvents was more rapid.

Wet-packed alumina columns showed no advantage in adsorption over dry-packed columns, which allowed easier manipulation and greater control of the height of adsorbent.

Elution of the columns with benzene gave high blanks from the apple extracts, and petroleum ether as eluting solvent caused maximum retention of

Table I. Variation of Blank Absorbance with Evaporation Temperature

Temp. of Bath, °C.	Absorbance at 262 Mμ
45	0.730
50	0.500
60	0.450
70	0.375
80	0.315

Chlorbenside. A series of mixtures of benzene and petroleum ether (total volume, 35 ml.) was used as the eluting solvent with apple extract and with Chlorbenside separately. Benzene (25% by volume) in petroleum ether gave complete elution of Chlorbenside and minimum elution of apple extractives from the column.

The sulfoxide is strongly retained by the column under these conditions and 0.51 mg. of this substance was completely adsorbed on the alumina. Attempts at elution of the sulfoxide also cause the elution of the apple extractives, which results in high blank values.

Blanks. Variations in the absorbance of untreated apple extracts were caused by variations in the temperature of the water bath used for the evaporation of the eluates from the columns (Table I).

Apple extracts, therefore, contain a volatile substance which is removed with the solvent during evaporation. The temperature selected for the bath in this work was 75 ± 2° C.

Chlorbenside, although volatile at low temperatures in the dry state, does not volatilize until the last traces of solvent have evaporated. If the flask is removed from the bath just before this occurs and the last trace of solvent is removed by a cold-air jet for 30 seconds, there is no loss of acaricide.

As the absorbance curve for the apple extract after chromatography was almost horizontal over the region of the Chlorbenside peak (Figure 2), the absorbance due to Chlorbenside was obtained by the difference of absorbance at 262 mμ between solutions from treated and untreated apples, both measured against alcohol.

The absorbance of 5-ml. aliquots from extracts of untreated apples was determined in duplicate and averaged. The values thus obtained from five different samples ranged between 0.230 and 0.385, with a mean of 0.320. Variations between samples are largely corrected by analyzing the untreated and treated samples concurrently. This is shown by the recoveries obtained (Table II) from adding known amounts of Chlorbenside to apple extracts. The quantities given are those in the 10 ml. of alcohol on which the absorbance was measured.

Table II. Recovery of Known Amounts of Chlorbenside Added to 5 Ml. of Apple Extract

(Concentration in 10 ml. of alcohol)

Added, γ	Recovered, γ	Recovered, %
100[a]	110, 110	110, 110
100[a]	100, 100	100, 100
100	95, 100	95, 100
110	110	100
220	210, 225	95, 102
225	225, 225	100, 100
330	335, 340	101, 103
440	450, 430	102, 98

[a] 10-ml. aliquot of apple extract used is equivalent to 50 γ in 5-ml. aliquot.

Chlorbenside Sulfoxide. Of the reducing agents tried for the reduction of Chlorbenside sulfoxide—zinc and acetic acid, zinc and hydrochloric acid, and tin and hydrochloric acid with acetic acid as solvent—only the latter gave nearly quantitative reduction.

The optimum time for the reduction was 1 hour. When the reduction mixture was cooled and diluted with water, Chlorbenside was precipitated. Extraction of this dilute solution with two 25-ml. portions of petroleum ether was sufficient to give quantitative recovery of acaricide.

Allowance was made in this work for the small linear background absorption caused by the reducing reagents. This would not be necessary in samples when an apple blank is treated, concurrently.

The recovery of known amounts of the sulfoxide after reduction are given in Table III, and the recovery of Chlorbenside and the sulfoxide from mixtures in the ratios 1 to 1, 2 to 1, and 1 to 2 are given in Table IV.

Determination of Chlorbenside in Presence of Some Pesticides. No difficulty was found in estimating Chlorbenside in the presence of Aramite or Ovotran. Aramite, 1.15 mg., and Ovotran, 0.67 mg., were completely retained by the adsorption column using the conditions specified.

The determination of 110 γ of Chlorbenside in the presence of 330 γ of DDT was achieved with quantitative recovery. This quantity of DDT represents about 12 p.p.m. as a residue and is considerably in excess of the quantities yet found as a residue on fruit analyzed in this laboratory.

Table III. Recovery of Chlorbenside Sulfoxide[a] after Reduction to Chlorbenside

(Concentration in 10 ml. of alcohol)

Added, γ	Recovered, γ	Recovered, %
96.5	90	93
	75	78
193	172	89
	176	91
	160	83
290	285	98
354	320	91
	290	83
386	395	102
	360	93
	340	88
	340	88
	360	93
	320	83

[a] All weights calculated as equivalent amount of Chlorbenside.

Table IV. Recovery of Chlorbenside and Chlorbenside Sulfoxide from Mixtures

(Concentration in 10 ml. of alcohol)

Added		Recovered		
Total,[a] γ	Chlorbenside γ	Total, γ	Chlorbenside, γ	Total Recovery, %
203	100	185	95	91
		185		
406	200	360	160	89
			150	
406	200	345	170	85
		350	173	
306	198	235	160	78
		250	150	82
306[b]	198	260	200	85
		275	200	90
338	132	290	90	86
			90	
338[b]	132	250	110	74
		270	140	80

[a] Chlorbenside weight plus weight of sulfoxide calculated as Chlorbenside.
[b] In presence of 5 ml. of apple extract.

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Literature Cited

(1) Barnard, D., Fabian, J. M., Koch, H. P., *J. Chem. Soc.* **1949,** 2442.
(2) Cranham, J. E., Higgons, D. J., Stevenson, H. A., *Chemistry & Industry* **1953,** 1206.
(3) Cymerman, J., Willis, J. B., *J. Chem. Soc.* **1951,** 1332.
(4) Gunther, F. A., Blinn, R. C., "Chemical Analysis," vol. 6, p. 624, Interscience, New York, 1955.
(5) Higgons, D. J., Kilbey, D. W., *J. Sci. Food Agr.* **6,** 441 (1955).
(6) Metcalf, R. L., "Organic Insecticides," p. 199, Interscience, New York, 1955.
(7) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, A. L., *Ind. Eng. Chem., Anal. Ed.* **17,** 704 (1945).
(8) Schrieber, K. C., *Anal. Chem.* **21,** 1168 (1949).
(9) Shirley, D. A., "Preparation of Organic Intermediates," p. 33, Wiley, New York, 1951.
(10) Vogel, A. I., "Practical Organic Chemistry," 2nd ed., p. 485, Longmans, Green, New York, 1951.
(11) Watson, C. C., *Chemistry & Industry* **1956,** 349.

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