# Determination of Kelthane and Its Dichlorobenzophenone Degradation Product by Thin-Layer Chromatography and Oscillopolarography

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In a search (1) for an analytical method to distinguish quantitatively between the o,p'- and p,p'-isomers of Kelthane, an unsuccessful attempt was made to do so with thin-layer chromatography (TLC) and oscillopolarography. However, it has now been found that this combination of analytical techniques will separate and quantitate p,p'-Kelthane and a gas chromatographic degradation product (2,3), p,p'-dichlorobenzophenone.[1/]

## Method

### Reagents

(a) TLC adsorbent.--Silica gel $GF_{254}$ (manufactured by E. Merck, Darmstadt, West Germany; distributed by Brinkmann Instruments, Inc., Westbury, N. Y.).

(b) Electrolyte solution.--Prepare a 0.2 M aqueous solution of tetramethyl ammonium bromide (Eastman Organic Chemicals, Rochester, N.Y.); this solution is stable under normal laboratory

1/ The use of Kelthane and of dichlorobenzophenone hereafter applies to the p,p'-isomers. Kelthane is 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethanol. NOTE: No appreciable residue metabolism or other degradation of Kelthane on field treated citrus was found by GUNTHER et al. (4).



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conditions.

(c) TLC chromogenic reagent.--Prepare a solution of 100 mg. of N,N-dimethyl-p-phenylazoaniline (methyl yellow) in 100 ml. of 95% ethanol. This solution retains its effectiveness for more than one month without special storage.

(d) Standard solutions.--Prepare a 100 μg./ml. solution of Kelthane; weigh 5 mg. of the purified compound into a 50-ml. glass-stoppered volumetric flask, dissolve in, and dilute to the mark with, redistilled n-hexane. Similarly prepare a 100 μg./ml. solution of dichlorobenzophenone in methylene chloride.

Special Apparatus

Davis Southern Analytical Differential Cathode Ray Polarotrace Model A.166CA and Gajan oscillopolarographic cells (Western Scientific Associates, San Ramon, Calif.). Operate with an amalgamated #22 gauge silver wire as reference electrode (5).

Procedure

Prepare 0.5-mm. thick plates in the usual manner from a 1:2 slurry of silica gel $GF_{254}$ and distilled water. Air dry the plates about 15 minutes before activating them at 110° C. for 30 minutes. Spot samples of Kelthane or of dichlorobenzophenone or mixtures of the two in the range 0.5 to 20 μg. by use of a microliter syringe. Shorten spotting time with a current of cool air blowing across the plate. Develop the plate in 10% methyl alcohol in n-pentane, in a closed TLC development tank lined with filter paper, to a line previously scored 10 cm. above the applied spots.

Examine the developed and cool air-dried plate under an ultraviolet lamp at 2537 Å and mark the visible spots.

For additional qualitative information spray half of a developed TLC plate with the chromogenic reagent and save unsprayed the mirror-image half for oscillopolarographic analysis as described below.

Scrape uniform areas of the plate which encompass each spot into separate 12-ml. centrifuge tubes. Add 1.0 ml. of 95% ethyl alcohol to each and mix thoroughly with a stirring rod for 30 seconds. Add 1 ml. of electrolyte solution, mix briefly by agitation, and centrifuge. Decant the supernatant solution into a Gajan oscillopolarographic cell. Analyze the solution oscillopolarographically after deoxygenation by bubbling prepurified water-pumped nitrogen through it for 3 minutes; scan from -0.5 to -1.0 v. to observe the two peaks from Kelthane and from -1.0 to -1.5 v. for the dichlorobenzophenone peak.

## Results and Discussion

$R_f$ values for 5 µg. each of Kelthane and dichlorobenzophenone chromatographed in adjacent channels are 0.29 to 0.36 and 0.77 to 0.86, respectively, when the leading and trailing edges of each spot are measured. $R_f$ values are essentially the same when the two compounds are chromatographed in admixture. Variations in $R_f$ values are no more than ± 0.03 for either compound in the range 0.5 to 10 µg. whether chromatographed singly or in admixture. It should be noted, however, the higher $R_f$ values are

typically obtained when TLC plates of decreased thickness are used or when the ambient temperature is increased above 25° C. Applications of 5 µg. quantities of Kelthane and dichlorobenzophenone to TLC plates 0.25 mm. thick show $R_f$ values of 0.36 to 0.43 and 0.82 to 0.89, respectively.

In addition to detection of Kelthane and dichlorobenzophenone by the use of fluorescent quenching of fluorescent TLC plates under ultraviolet light, a sensitive chromogenic reagent, N,N-dimethyl-p-phenylazoaniline (methyl yellow), may also be used. Use of this spray reagent for the detection of Kelthane and other chlorinated hydrocarbons on paper chromatograms has been reported by KRZEMINSKI and LANDMANN (6). We have found that exposure to strong ultraviolet light as recommended (6) is not essential to obtain good detection on TLC plates of both Kelthane and dichlorobenzophenone. Best detection of the purified compounds at levels of 0.5 and 1.0 µg. is obtained approximately 10 minutes after application of the chromogenic reagent. As the plates are air dried under a draft of cool air, the adsorbed compounds become visible as deep yellow spots against a lighter yellow background, but the color distinction between background and spots is short lived (about 15 minutes) because of increasing intensity of background color upon continued exposure to ordinary light. For this reason, $R_f$ areas should be marked as soon as spots become visible. When plates are exposed to strong ultraviolet radiation immediately after spraying, background color intensifies and obscures $R_f$ areas of dichlorobenzo-

phenone at any level and for Kelthane at 1.0 μg. or less. At higher μg. levels, the Kelthane complex becomes a deep orange color which is relatively stable. When Kelthane and dichlorobenzophenone are chromatographed in the presence of orange peel extractives at a fortification level of 1 p.p.m. or less, detection of the test compounds by the use of methyl yellow indicator is no longer possible.

Oscillopolarography of purified Kelthane at 2.5 μg./ml. in the analytical solution reveals two reduction potentials: -0.65 and -0.85 v. These reduction potentials become slightly more negative with increasing concentration from 1.3 to 10.0 μg./ml. in the final analytical solution. The single reduction potential for purified dichlorobenzophenone was typically -1.26 $\pm$ 0.01 v. over the range 2.5 to 10 μg./ml. Both compounds in these concentration ranges exhibited straight-line relationships between relative polarographic units (relative to maximum instrumental sensitivity) and concentration (μg./ml.) in the final analytical solution. Reduction potentials for compounds from standard solutions compared to those following TLC showed no significant variation.

It should be pointed out that the reduction potentials of the two compounds of interest are sufficiently separated that it should be possible to estimate them in admixture without the need for TLC separation and isolation. However, in unknowns from formulations or crop samples the TLC procedure would be needed as the cleanup (or part of the cleanup) method. In this situation, for

quantitative purposes unknowns must be related to standards chromatographed on the same plate at approximately the same amounts to account for recovery losses in the TLC procedure. These losses may vary from plate to plate, but are adequately consistent for any given plate; see the report by HEARTH et al. (5) for a detailed discussion of the same problem with the acaricide Morestan under TLC conditions.

Polarography of Kelthane has been briefly mentioned earlier (7). To our knowledge, however, the present report is the first mention of the polarographic analysis of p,p'-dichlorobenzophenone even though the polarography of ketones in general is well established.

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