

PAPER AND PLATE CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF DICHLOROPHENE AND HEXACHLOROPHENE WHEN PRESENT TOGETHER

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The identification and determination of dichlorophene and hexachlorophene when present together has been attempted earlier by CLEMENTS AND NEWBURGER¹, who isolated and purified the compounds from a commercial product, by means of a series of extractions, and then identified and estimated them by means of their ultraviolet absorption.

The identification and assay of hexachlorophene are also described in the United States Pharmacopeia², and both compounds are described, and their methods of synthesis outlined, in the Merck Index³.

All the present methods of estimation have the drawback of being long and complicated, and the direct determination of chlorine as in the case of DDT and its derivatives⁴⁻⁶, can only be carried out with the pure products.

This paper deals with a proposed new method for the separation, identification and determination of dichlorophene and hexachlorophene by adsorption chromatography on plates and partition chromatography on paper, using *n*-heptane saturated with glacial acetic acid as a developer, as suggested by MOYNIHAN, cited by CRAMER⁷ and by BLOCK, DURRUM AND ZWEIG⁸, for separating the various isomers of some halogenated insecticides. The compounds are detected by spraying the chromatograms with a solution of potassium ferricyanide and ferric chloride⁹; both dichlorophene and hexachlorophene appear as blue spots on a lighter background.

For the quantitative determination of the compounds the ultraviolet absorption curves of the pure products are determined in *n*-heptane saturated with acetic acid, using perfectly dry 1 cm cells. Maximum absorption is found between 285 and 300 $m\mu$, and a wavelength of 290 $m\mu$ is chosen to obtain the standard curves.

Qualitatively, both the paper and the plate chromatograms are very satisfactory, well differentiated spots being produced in a very short time, especially on the chromatoplate (20 minutes).

EXPERIMENTAL

Reagents and materials

Dichlorophene (2,2'-methylene-bis-[4-chlorophenol]).

Hexachlorophene (2,2'-methylene-bis-[3,4,6-trichlorophenol]).

1 % aqueous solution of ferric chloride.

1 % aqueous solution of potassium ferricyanide.

n-Heptane (Hopkin and Williams, London).

Glacial acetic acid (B.D.H., "AnalaR").

Corn starch.

Silicic acid by wet process, dried (E. Merck, Darmstadt).

Whatman (chromatographic) paper No. 1.

Dichlorophene and hexachlorophene are purified by dissolving them in hot 95 % ethanol, adding water until a slight turbidity appears, boiling until the solution clears, and filtering. This process is repeated twice, until a melting point of 164° is reached for dichlorophene and 167° for hexachlorophene. The compounds are then used in *n*-heptane solution.

Paper chromatography

Ascending paper chromatograms are run on 6 × 30 cm strips of Whatman No. 1 paper soaked in glacial acetic acid, in a tank containing *n*-heptane saturated with acetic acid. Parallel chromatograms are run with the pure compounds and with a mixture of the two compounds, at room temperature (17–18°). After development, the chromatograms are dried and the compounds are detected by spraying with a freshly prepared mixture of equal parts of the solutions of ferric chloride and potassium ferricyanide. The following reproducible R_F values are obtained, both for the single compounds and for the mixture:

Dichlorophene 0.08

Hexachlorophene 0.73

Plate chromatography

Chromatoplates are prepared following the general technique described by DEMOLE¹⁰, using 6 × 15 cm glass plates. A mixture of 7 g silicic acid, 0.4 g corn starch, and 30 ml water is heated to boiling with constant stirring; 10 ml water are added, and the mixture is boiled for one more minute. After cooling, 0.91 ml of the suspension per 10 cm² of glass surface are spread evenly on the plate. This can be done conveniently by placing the required amount of suspension on the center of the plate, and then moving the plate to and fro horizontally so that the whole surface becomes covered with a uniform layer. The plate is then allowed to stand for not less than 3 hours and is dried at 105°. The origin, and a front 10 cm above it, are marked, and the plate is activated at 110° for 1 hour; it is then placed in a desiccator over calcium chloride.

Microdrops of the solutions of pure dichlorophene and hexachlorophene, and of the mixture of the two, are placed on the origin, and ascending chromatograms are run using *n*-heptane saturated with acetic acid as the developer. When the solvent front reaches the 10 cm mark (30–35 min) the plates are removed and dried, and the

spots are detected using the ferric chloride and potassium ferricyanide solution. In all cases the following R_F values were obtained:

Dichlorophene 0.32

Hexachlorophene 0.63

Estimation

Fig. 1 shows the ultraviolet absorption curves for both dichlorophene and hexachlorophene at a concentration of 1 mg per 100 ml of *n*-heptane saturated with acetic acid.

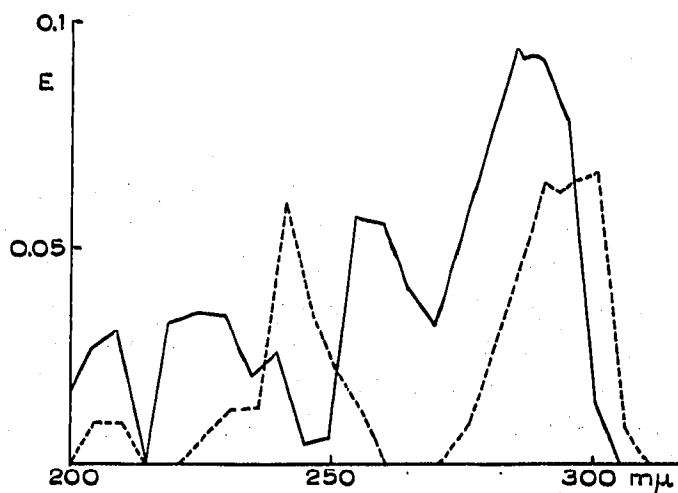


Fig. 1. Ultraviolet absorption curves of dichlorophene(—) and hexachlorophene(-----).

For the quantitative determination of the compounds, standard absorption curves at a wavelength of 290 $m\mu$ were obtained for concentrations ranging from 0.001 mg to 0.1 mg of each substance per ml.

To determine the compounds in a mixture, 0.25 ml of a solution containing 40 mg of each substance per 10 ml of solution (*i.e.* 1 mg of each substance) is spread along the origin of two chromatoplates over a length of 4.5 cm. Both plates are developed together, but only one of them is sprayed with the reagent; two clearly defined zones, corresponding to the R_F values of dichlorophene and hexachlorophene, are obtained, and these are used to determine the positions of the equivalent zones on the untreated plates. It is nevertheless convenient to examine this plate under ultraviolet light to ascertain the location of the two zones, which can be seen as separate absorption bands. Thus having determined the exact positions of the dichlorophene and the hexachlorophene, the corresponding zones are removed by scraping the silicic acid from the plates. These are transferred quantitatively to a small column, from which the compounds are eluted using *n*-heptane saturated with acetic acid, collecting a total volume of 50 ml. The ultraviolet absorption of each compound at 290 $m\mu$ is then determined, and the values obtained are interpolated in the standard curves. The results are quantitative, and are easily reproducible.

SUMMARY

A simple and rapid method is described for the separation of mixtures of dichlorophene and hexachlorophene. The compounds are resolved by ascending paper and plate chromatography, using *n*-heptane saturated with glacial acetic acid as the developer, and detecting the spots with a solution of ferric chloride and potassium ferricyanide. After elution from the plates, the isolated compounds are assayed by ultraviolet spectrophotometry.

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