Quantitative Determination of Hexachlorophene in Ointments Containing Salicylic Acid and Methyl *p*-Hydroxybenzoate

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Hexachlorophene in ointments is determined spectrophotometrically by the "difference" method after selective removal of all interfering components.

THE PRESENTLY available colorimetric (1, 6–10, 14, 15) and spectrophotometric (2–5, 11, 12) methods for quantitative estimation of hexachlorophene [2,2′-methylenebis(3,4,6-trichlorophenol)] are unsuitable for the determination of hexachlorophene in ointment preparations containing both salicylic acid and methyl *p*-hydroxybenzoate.

The colorimetric method using 4-aminoantipyrine requires the color complex to be in aqueous solution (6). In the case of sulfur-containing ointments, the sulfur remains suspended[1] in the aqueous solution and causes considerable interference (*cf. Reference 13*).

Attempts to remove salicylic acid from both ether and chloroform extracts of the ointments with sodium bicarbonate resulted in emulsion formation. The emulsions were extremely stable and permitted only a 50% recovery of hexachlorophene.

To measure the absorbance of hexachlorophene at 257.5 mμ, where the absorbance of salicylic acid is independent of pH change (5), would result in interference due to methyl *p*-hydroxybenzoate, which absorbs (5) at 257 mμ in pH 7.5 buffer.

A procedure for hexachlorophene assay which overcomes the above difficulties is reported here. The organic components of the ointments are extracted into ether from a methanol–aqueous sodium chloride suspension which suppresses emulsion formation. The salicylic acid is removed selectively into pH 8 buffer, and the methyl *p*-hydroxybenzoate saponified. The hexachlorophene may then be measured spectrophotometrically by the "difference" method (2, 5), with no interference from other components.

EXPERIMENTAL

Apparatus.—A Cary model 14M was used in this work.

Reagents.—All solutions are aqueous unless otherwise specified. The hexachlorophene standard solution is prepared by dissolving 100 mg. of hexachlorophene U.S.P. in 100 ml. of methanol.

Procedure.—A quantity of ointment containing 2 mg. of hexachlorophene was weighed into a 125-ml. separator and shaken with 15 ml. of methanol. Fifteen milliliters of 7% sodium chloride was added to the solution which, after cooling to room temperature, was extracted with 25 ml. of ether. The aqueous-methanol layer was re-extracted with 25 ml. of ether in a second 125-ml. separator. Each ether extract was washed by allowing 1 ml. of 7% sodium chloride to pass through the ether layer without shaking. After draining and discarding the sodium chloride layer, 1 ml. of 35% sodium chloride and 10 ml. of pH 8 Clark and Lubs buffer were added to each ether extract. Each separator was shaken gently, and the aqueous layers were combined in a single 125-ml. separator. After extracting the aqueous layer with 25 ml. of ether, the aqueous portion was discarded. The three ether extracts obtained were combined into a 100-ml. volumetric flask and evaporated to dryness. The residue was dissolved in 5 ml. of methanol and 10 ml. of 1 *N* sodium hydroxide and the solution heated at 75° for 30 min. The solution was cooled, diluted to volume with methanol, and filtered. Exactly 20 ml. of the solution was placed in a 25-ml. volumetric flask and diluted to volume with methanol. This solution was placed in the sample cell of the spectrophotometer. Another 20 ml. of the solution was placed in a second 25-ml. volumetric flask and diluted to volume with 1 *N* methanolic hydrochloric acid. This solution was placed in the reference cell of the spectrophotometer and the maximum differential ultraviolet absorbance of hexachlorophene measured at 320 mμ. The concentration of hexachlorophene was determined by comparison with a standard hexachlorophene solution which had been carried through the above procedure beginning with the methanol–1 *N* sodium hydroxide treatment.

DISCUSSION

The presence of fairly large quantities of surfactant in ointments resulted in incomplete extraction of hexachlorophene due to formation of stable emulsions. When the extractions were preceded by treatment with sodium chloride–methanol, a quantitative separation of the hexachlorophene was obtained. Attempts to separate the salicylic acid using sodium carbonate or sodium bicarbonate were unsuccessful due to emulsion formation and concomitant loss of hexachlorophene.

The saponification of methyl *p*-hydroxybenzoate is necessary for the quantitative estimation of the hexachlorophene. The maximum absorbance of methyl *p*-hydroxybenzoate both at pH 0.8 (the pH of the final methanolic acid solution) and at pH 11.5 (the pH of the final alkaline methanol solution) is 298 mμ, and the hexachlorophene absorption is seen only as a shoulder on the larger methyl *p*-hydroxybenzoate peak. Sodium *p*-hydroxybenzoate in methanolic solution at pH 11.5, when read using the "difference" method against the acid solution at pH 0.8, absorbs at 286 mμ. This hypsochromic shift is sufficient to separate completely the *p*-hydroxybenzoate absorption from that of the hexachlorophene, which can then readily be measured. The use of acidified 90% methanol suggested in U.S.P. XVII (16) was precluded for the following reason: it was necessary to hydrolyze the methyl *p*-hydroxybenzoate with strong alkali. Neutralization of the solution with acidified 90% methanol gave a final solution of pH 7.7. At this pH, the higher absorbance of the *p*-hydroxybenzoate inter-

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[1] The reviewer of the manuscript suggests the use of 2-oz. stainless steel syringes fitted with disposable membrane holders for the removal of sulfur by membrane filtration. This is an excellent suggestion, although it was not used in this case.

fered with the hexachlorophene absorbance. The wavelength of hexachlorophene absorbance is pH dependent. In the system reported here (pH 0.8/11.5), the absorbance was at 320 mμ. Using the U.S.P. XVII buffer–acid system, the absorbance is at 312 mμ, causing slight interference with the *p*-hydroxybenzoate absorbance.

RESULTS

The recovery of standard hexachlorophene carried through this method was 99 ± 2%. The accuracy and reproducibility of the spectrophotometric "difference" method have already been well documented (5).

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Identification of 1-, 2-, 3-, and 4-Chlorophenothiazine Isomers

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The infrared and ultraviolet spectral data for four monochloroisomers of phenothiazine are presented and discussed. From these data it is possible to make a positive and rapid identification of any of the isomers with a minimum amount of sample. The method can be used to identify the isomers alone or in combination.

THE PRESENCE of isomers in the preparation of chlorinated phenothiazines is always a possibility. The problem of determining the presence of an isomer and identifying the specific isomer present is a frequent analytical problem. A simple, rapid method for identifying the 1-, 2-, 3-, and 4-chlorophenothiazine isomers is presented.

EXPERIMENTAL

Reagents. — 1 - Chlorophenothiazine, 2-chlorophenothiazine, 3-chlorophenothiazine, and 4-chlorophenothiazine. All chemicals are of analytical grade as prepared at Smith Kline & French Laboratories.

Spectrophotometers.—The infrared spectra were recorded with a Perkin-Elmer model 21 double beam spectrophotometer with a sodium chloride prism. The phenothiazines studied were prepared as mineral oil mulls.

The ultraviolet spectra were recorded with a Cary model 14 recording spectrophotometer using matched fused silica cells with a 1-cm. light path.

RESULTS AND DISCUSSION

Infrared Spectra.—Figure 1 shows the infrared spectra obtained for the four chlorophenothiazine isomers. The area of greatest interest is the region between 1000 and 650 cm.$^{-1}$. This is the region which contains absorption bands due to C–H out-of-plane deformations in aromatic ring systems. Each of the isomers has its own unique pattern in this area owing to the particular position of the chlorine atom on the ring. The infrared pattern here is specific enough to distinguish one isomer from another, and the absorption bands are so located as to permit detection of one or more isomers in the presence of another.

Spectrum A, which is that of the 1-chloro isomer, shows strong absorption bands between 770 and 700 cm.$^{-1}$ which are assignable to 3 and 4 adjacent free hydrogen atoms in an aromatic ring (1). Spectrum B is that of the 2-chloro isomer with absorption

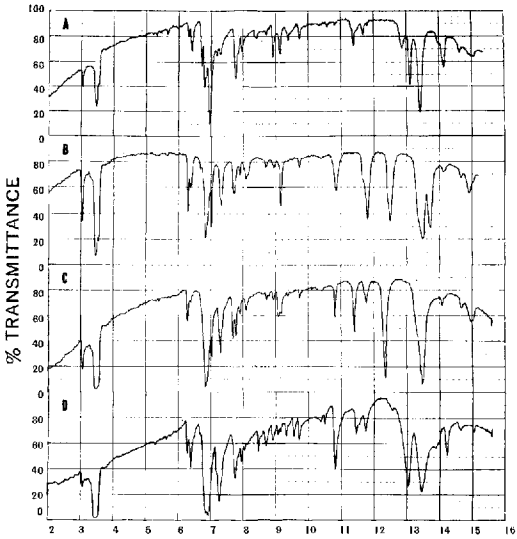

Fig. 1.—Key: A, 1-chlorophenothiazine; B, 2-chlorophenothiazine; C, 3-chlorophenothiazine; D, 4-chlorophenothiazine.

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