

properties necessary for biliary excretion and is not able to form a conjugate directly, the results indicate that it is very poorly absorbed after oral administration and is, thus, toxicologically less important than techlofthalam.

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A Simple Colorimetric Method for the Determination of Endrin in Emulsifiable Concentrates

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A simple colorimetric method for the determination of endrin from emulsifiable concentrates is described. The method is based on the reaction of formaldehyde-sulfuric acid with endrin in the presence of sodium perborate. The method is sensitive and obeys Beer's law for the concentration range 4-40 $\mu\text{g mL}^{-1}$ with $99 \pm 2\%$ recovery.

Quantitative analysis of endrin contents by determining total organic chlorine and infrared spectrophotometric methods are recommended by AOAC (1975) in the case of endrin formulations. Bann et al. (1958) have reported a colorimetric method based on determination by dechlorination of endrin and subsequent coupling with diazotized sulfanilic acid. There are some other colorimetric methods reported in the literature (O'Donnell, 1954, 1955; Beckman, 1954). On thin-layer chromatograms many reagents such as zinc chloride in hydrochloric acid (Wienke and Burke, 1969), sulfuric acid (Chou and Cochrane, 1969), ethanolic silver nitrate followed by UV irradiation (Abbott et al., 1964), silver nitrate-formaldehyde-potassium hydroxide-nitric acid-hydrogen peroxide in succession (Salo et al., 1963), *O*-toluidine (Salo et al., 1963), dianisidine (Kawashiro and Hosogai, 1964), etc. are used for the quantitative estimation. Bioassays using vinegar flies (*Drosophila melanogaster*) (Phillips et al., 1962), water fleas (*Daphnia magna*), and goldfish (*Carassius auratus*) (Davidow and Schwartzman, 1955) are also reported for endrin quantitation. Excellent reviews of instrumental methods like GLC, mass spectrometry, etc. are also reported (Beynon and Elgar, 1966; Williams and Cook, 1967; Zweig, 1978).

There is a shift in the peak at the higher concentration of endrin by the infrared method (Indian Standards Institute, 1959), while in the sulfanilic acid method the excess sulfanilic acid and sodium nitrite give high background values. We observed that endrin reacts with formaldehyde-sulfuric acid in the presence of sodium perborate, producing a yellow-colored compound. This yellow-colored compound has an absorption maximum at 405 nm, and it can be used in the quantitative determination of endrin (Figure 1).

EXPERIMENTAL SECTION

Materials. The following chemicals were used: Carbon disulfide, methyl alcohol, acetone, sodium perborate ($\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$), formaldehyde (40% v/v), sulfuric acid

(concentrated), acetic acid (glacial), sodium hydroxide (10% m/v, aqueous), and endrin standard (Shell Chemical Corp). All the chemicals were analytical grade unless specified otherwise.

Preparation of Sodium Perborate Solution. To a mixture of 150 mL of methyl alcohol and 150 mL of glacial acetic acid 100 g of sodium perborate is added. The mixture is allowed to stand overnight to ensure saturation.

Preparation of Endrin Standard Solution. Accurately weighed pure endrin (500 mg) is dissolved in ~20 mL of the solvent acetone and diluted to 50 mL in a volumetric flask by using methyl alcohol. The concentration of endrin in this solution is 10 mg mL^{-1} . One milliliter of this solution is transferred to a 10-mL volumetric flask and diluted to the 10-mL mark by using methyl alcohol. The concentration of endrin in this diluted solution is 1 mg mL^{-1} .

Analytical Procedure. One milliliter of the sample under test was pipetted in a 25-mL volumetric flask. One milliliter of the sodium perborate solution and 1 mL of formaldehyde were then added. The contents were properly mixed by gently shaking the flask. Two milliliters of sulfuric acid was added very slowly with constant swirling. The flask was allowed to stand for 30 min at room temperature after which the volume was made up to the 25-mL mark by using methyl alcohol. Absorbance of the color developed was measured at 405 nm, against a reagent blank. The reagent blank was prepared by treating 1 mL of methanol in place of the sample.

Standard Curve. A series of endrin standard solutions having concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 μg were taken in 10 different 25-mL volumetric flasks and treated as per the analytical procedure described earlier. The graph of endrin concentration against optical density was a straight line passing through the origin and was found to obey Beer's law for the concentration range of 4-10 $\mu\text{g mL}^{-1}$.

Application of the Method to Emulsifiable Concentrates. The method was tried for five emulsifiable concentrates from the market. The amount of endrin determined and the percent of declared are shown in Table I.

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Table I. Quantitative Determinations of Endrin in Emulsifiable Concentrates (EC)

formulation, EC	declared concn of endrin, g % (w/w)	endrin determined ^a by proposed method, g % (w/w)	% ^a of declared	confidence limits	
				95%	99%
A ^b	20	19.88	99.4	99.4 ± 2.2	99.4 ± 3.2
B ^c	20	20.00	100.0	100.0 ± 2.2	100.0 ± 3.3
C ^b	50	49.38	98.8	98.8 ± 0.9	98.3 ± 1.3
D ^d	50	49.5	99.0	99.0 ± 0.9	99.0 ± 1.3
E ^e	20	20.06	100.3	100.3 ± 2.1	100.3 ± 3.1

^a Calculated as the mean of eight observations. ^b Bayer, India. ^c B. P. Mills, India. ^d Khandelwal Pesticides, India. ^e Sandoz, India.

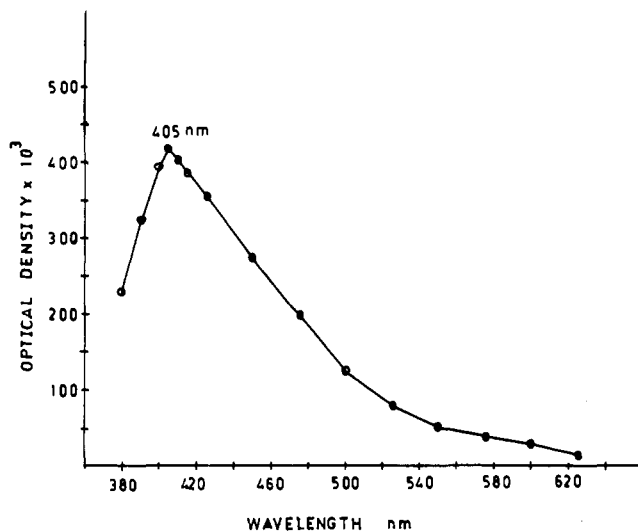


Figure 1. Absorption maximum of the reaction product of endrin with formaldehyde-sulfuric acid. $\lambda_{\max} = 405$ nm.

Preparation of the Sample. To an accurately weighed emulsifiable endrin concentrate (1 g), 50 mL of aqueous sodium hydroxide (10% w/v) was added. The mixture was transferred to a 250-mL separating funnel by using 50 mL of carbon disulfide. This was extracted 5 times by using 50 mL of carbon disulfide each time. All the extracts were collected together and the solvent was evaporated completely. The residue was dissolved in 10 mL of acetone and diluted to 100 mL in a volumetric flask by using methanol. One milliliter of this solution was pipetted in a 10-mL volumetric flask and made up to volume with methanol to give the working standard sample. One milliliter of the working sample was processed as per the analytical procedure described earlier. The concentration was determined by extrapolating the standard curve.

Calculations. If the amount determined (from a Beer's law plot) in 1 mL was X μ g, then the following apply: (1) in 10 mL the amount is $(X \times 10)$ μ g (this was in 1 mL of undiluted solution); (2) in the original 100 mL, the amount is $(X \times 10 \times 100)$ μ g (this was in 1 g of formulation); (3) in 100 g of formulation, the amount is $(X \times 10 \times 100 \times 100)$ μ g; (4) endrin per 100 g = $(X \times 10 \times 100 \times 100) / 1000000$ g % (w/w).

RESULTS AND DISCUSSION

At low concentration endrin shows an IR peak at 11.76 μ m (850 cm^{-1}) but at higher concentration there is a shift in the IR peak (White and McKinley, 1961) in addition to the background effect of carbon disulfide. In the colorimetric method reported by Bann et al., the excess sulfanilic acid reacts with diazotized sulfanilic acid. Though this background color was reduced by using ammonium sulfamate (Fahey and Schechter, 1961), it could

not be eliminated completely. The reagents used for TLC such as silver nitrate were not found useful for the colorimetric determination.

We have, therefore, suggested a method which is more simple, sensitive, and precise. The method is useful for the routine endrin determination as it does not require critical reaction conditions.

The effect of the volume of sodium perborate, formaldehyde and sulfuric acid on the reaction was critically studied. It was found that in the concentration range over which Beer's law is obeyed, 1 mL of sodium perborate solution, 1 mL of formaldehyde solution and 2 mL of sulfuric acid were found to be optimum for complete color development. The color developed was found to be stable for more than 1 hour after the complete development which required 30 min. The formaldehyde-sulfuric acid reagent known as Liberman's reagent is used for the identification of opium alkaloids, petroleum hydrocarbons, etc. on TLC. The proposed reaction was also responded to by other organochloro insecticides like aldrin, dieldrin, DDT, etc.

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