UDC 632.951:543.43

108. Takashi Mitsui, Hiroshi Ozaki, Hiroaki Kumano, and Hajime Sano : Insecticide Determination. I. Colorimetric Determination of Dimethyl 2,2-Dichlorovinyl Phosphate (DDVP).

(*Research Laboratory, Chugai Pharmaceutical Co., Ltd.*[*1])

Several methods for the determination of dimethyl 2,2-dichlorovinyl phosphate (DDVP) are proposed. Organic chlorine or phosphorus method,[1~3] by which total chlorine or phosphorus in the compound is measured titrimetrically or colorimetrically, is often adapted to determine the compounds because of its simple procedure. But it is doubtful whether the value obtained by this determination shows the true purity of the compound or not. Generally, organic phosphorus compounds are determined by measuring the inhibition rate of acetylcholineesterase activity. Giang and Hall[4] further developed the enzymatic method for determining organic phosphorus insecticides. This method was modified to adapt to the determination of DDVP by Giang, Smith and Hall.[5] It is sensitive but non-specific and complicated in procedure and time consuming for determination.

The infrared absorption spectral method has been most reliable and often adapted but it is not general because it requires special apparatus. The other method, the colorimetric method has been reported by Geiger and Furer.[6] During an investigation of the determination for DDVP, we found that orange to red colors were produced upon the addition of DDVP to ketones in the presence of alkali. This coloration is applied to the determination of DDVP.

Experimental and Results

Colors produced by Mixture of DDVP and Ketones with Ethanolic KOH——Eleven ketones were tested. 10 ml. of each ketone in which 10 mg. of DDVP are dissolved, were added to 5 ml. of 0.5*N* ethanolic

Table I. Colors produced by Mixture of DDVP and Ketones with Ethanolic KOH

Ketone	Color
Acetone	orange-pink
Methyl ethyl ketone	yellow
Methyl propyl ketone	yellow-brown
Diethyl ketone	yellow
Methyl *sec.* butyl ketone	yellow-brown
Dipropyl ketone	faint yellow-brown
Diisobutyl ketone	yellow
Methyl isobutyl ketone	red-brown
4-Hydroxy-4-methyl-2-pentanone	red-pink
Cyclohexanone	faint yellow
4-Methyl-3-penten-2-one	dark red-brown

*1 Takadaminami-cho, Toshima-ku, Tokyo (満井 喬, 尾崎 博, 熊野博昭, 佐野 肇).

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KOH to produce orange to red colors. These solutions become yellow or pale yellow on acidification with mineral acid. The data obtained in this experiments are summarized in Table I. Among these ketones, Me_2CO produced deeper color, so it is most suitable for the determination of DDVP.

Absorption Spectrum of Addition Product——In order to determine the optimum wave length for colorimetric analysis of the colored addition product, conditions permitting a sufficient degree of color stability over a reasonable period of time were ascertained by preliminary trials and incorporated in the following procedure. Two test tubes with glass stopper were placed on the bench. Into one of the tubes, 10 mg. of DDVP dissolved in 10 ml. of Me_2CO and 5 ml. of 0.5N ethanolic KOH were transferred. Into the other tube (blank), 10 ml. of Me_2CO and 5 ml. of 0.5N ethanolic KOH were transferred. After reserving the tubes for 2 hr. at 25°, 1 ml. from each tube was diluted with 20 ml. of EtOH.

Absorbance readings were taken at various wave lengths, beginning at 320 mμ and ending at 430 mμ (Fig. 1). Absorbance reading reveals a maximum at 370 mμ.

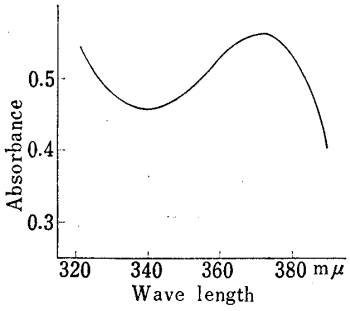

Fig. 1. Absorption Spectrum of DDVP-Acetone Complex

Rate of Color Development and Optimum Conditions for Determination of DDVP——As a reasonable color stability is required over a sufficient period, the variation of absorbance at 370 mμ with respect to time was studied, employing various concentrations of ethanolic KOH (A normality), volume of it (B ml.) and Me_2CO (C ml.) for fixed amount of DDVP (10 mg.). The levels of A, B and C were decided as following and arranged according to latin square method.

Table II. Various Conditions for Coloration

A_i : Concentration of ethanolic KOH

$A_1 = 1N$ $A_2 = 0.5N$ $A_3 = 0.25N$

B_j : Volume of ethanolic KOH

$B_1 = 15$ ml. $B_2 = 10$ ml. $B_3 = 5$ ml.

C_k : Volume of acetone

$C_1 = 10$ ml. $C_2 = 5$ ml. $C_3 = 1$ ml.

$A_1B_1C_3$	$A_2B_1C_1$	$A_3B_1C_2$
$A_1B_2C_2$	$A_2B_2C_3$	$A_3B_2C_1$
$A_1B_3C_1$	$A_2B_3C_2$	$A_3B_3C_3$

The curves in Fig. 2 show the two instances of this conditions, and reasonably constant absorbance were obtained from 80 to 120 min. after treatment. Other conditions which are not described in this paper also showed the same results. Here, the three factors A, B and C were arranged according to three-way layout, and the absorbance at 120 min. after treatment were measured in each combination A_i, B_j and C_k. As the result, we draw the following conclusions from the significance tests.

(a) The differences among A_i, B_j and C_k are significant at 1 % level.

(b) B×C interaction effect is significant at 5 % level.

The estimates of population mean of each factor were plotted as a function of each factor in Fig. 3.

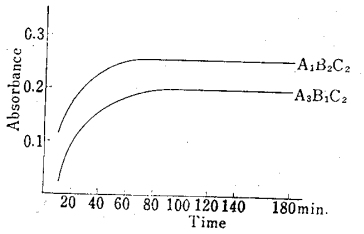

Fig. 2. Rate of Color Development

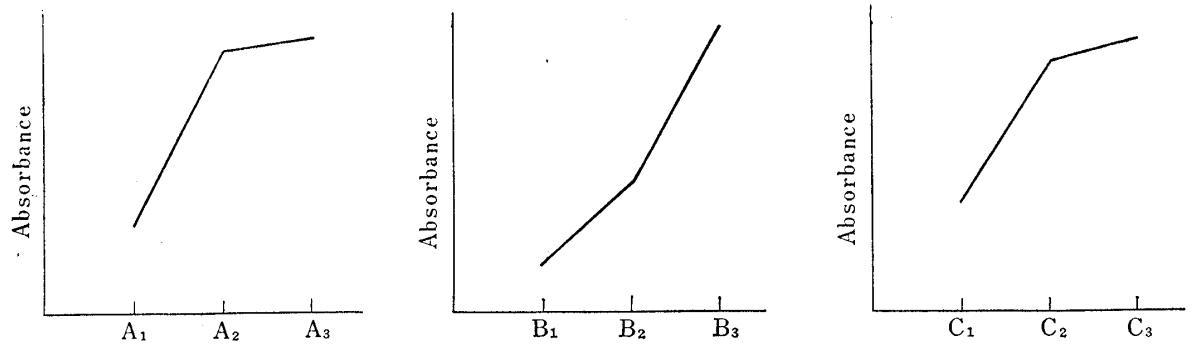

Fig. 3. Relationship between Absorbance and Each Factor

It is apparent that when lower concentration of ethanolic KOH are used with a given amount of DDVP, considerably higher absorbances are obtained, and the condition $A_3B_3C_3$ (5 ml. of ethanolic KOH (0.25N) and 1 ml. of Me_2CO) gives the highest absorbance. When a small amount of Me_2CO is used, the precipitate is inconveniently pipetted when 1 ml. from the solution is diluted to 20 ml. of EtOH.

We found that the conditions which B plus C were 15 ml. were the best in procedure. Therefore three factors A, B and C were decided as following.

Table III. Various Conditions for Coloration

A_i : Concentration of ethanolic KOH
$A_1=0.4N$ $A_2=0.5N$ $A_3=0.6N$

B_j : Volume of ethanolic KOH
$B_1=3$ ml. $B_2=4$ ml. $B_3=5$ ml. $B_4=6$ ml. $B_5=7$ ml.

C_j : Volume of acetone
$C_j=15-B_j$ ml.

Absorbances at 120 min. after treatment were measured in each combination A_i, B_j and C_j. We found that the differences between A_i and B_j were significant at 5 % level but A×B interaction effect was not significant at 5 % level. The estimates of the population mean of each factor were plotted in the same way as before in Fig. 4.

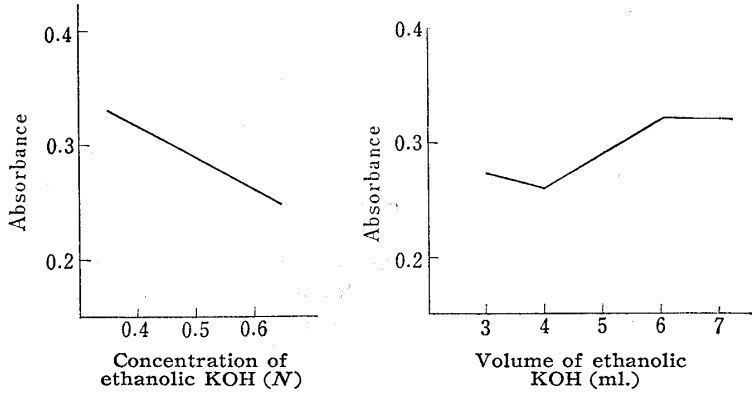

Fig. 4. Relationship between Absorbance and Concentration or Volume of Ethanolic KOH

Any condition, above mentioned, may be available for the following experiment because of no A×B interaction effect.

Calibration Curve——DDVP was purified by redistillation, 200 mg. of this purified DDVP was accurately weighed, and dissolved in Me_2CO to make exactly 100 ml. Into each of the glass stoppered test tubes, about 2 cm. in diameter, 0, 1, 2, 3, 4, 5, 6, 7 and 8 ml. of this solution were introduced, and Me_2CO was added to make exactly 10 ml. To each of these solutions, 5 ml. of 0.5N ethanolic KOH was added. These solutions were mixed at once, and after reserving the tubes for 2 hr. at 25°, its 1 ml. was diluted with 20 ml. of EtOH. Absorbance of these solutions were read against that of blank (first solution) at 370 mμ. Adherence to the Lambert-Beer law is evident from the linearity of absorbance-concentration curve shown in Fig. 5.

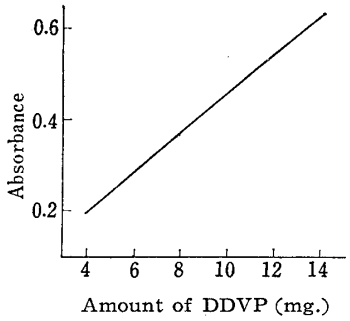

Fig. 5.
Calibration Curve

Determination of various DDVP Samples and Comparison with other Methods——One hundred milligrams of various kinds of DDVP sample weighed accurately was dissolved in Me_2CO, and brought exactly to 100 ml. Ten milliliters of this solution was placed in a glass-stoppered test tube and this was treated by the foregoing procedure. The absorbance of the solution was measured and purity of each sample was calculated from the calibration curve. Determinations were carried out on each of these samples and one allowed to stand at 100° for 100 hr.

These values were compared with the values obtained from the IR spectral method and total chlorine method and the result is shown in Table IV.

Table IV. Determination of Various DDVP Samples and Comparison with Other Method (%)

Sample	No.	Colorimetric method	Infrared spectral method	Total chlorine method
A	1	100	100	95.1
	2	87.7	86.9	95.7
B	1	92.5	93.7	92.2
	2	83.3	89.8	91.5
C	1	89.9	86.9	94.1
	2	87.8	87.4	93.6
D	1	99.1	98.8	98.4
	2	95.4	93.9	95.3
		$t_a=0.21148$		$t_b=1.385$

Each sample was allowed to stand at 100° for 100 hr.
No. 2 is the one allowed to stand at this condition.
t_a : pair comparison between the colorimetric and the infrared spectral method
t_b : pair comparison between the total chlorine and the infrared spectral method

Result of t-test of the difference in corresponding values between the colorimetric method and the IR method (t_a) showed good agreement, with $t_a=0.21148<t(7, 0.05)=2.365$, t_b showed no significance at 5% level, but the values obtained from the total chlorine method were apt to be larger than these obtained from other methods. The error of the colorimetric method did not exceed 0.5% when 4 to 16 mg. of DDVP were determined.

Interference Studies——In order to study for the interference of other insecticides (Lindane, DDT, Malathion, Diazinon and Dibrom), solvents and surfactants, the fixed amount of these materials shown in Table V were added to 10 mg. of DDVP and recovery rates were calculated. When Lindane or surfactant was added, the recovery was a little lower; on the other hand, when Diazinon, Malathion or DDT was added, the recovery was a little higher. Dibrom also gave orange to red color, so it is impossible to determine DDVP in the presence of Dibrom, and no examination has yet been made on a method for separatory determination.

Table V. Effect of other Insecticides, Surfactant and Solvent on Coloration

	g. (ml.)/0.2 g. of DDVP	Recovery (%)
Lindane	0.5 g.	96.9
DDT	1.0 g.	100.8
Malathion	1.0 g.	101.6
Diazinon	1.0 g.	99.5
Dibrom	0.5 g.	—
Kerosene	10.0 ml.	99.1
Surfactant+Solvent	2.0 g.	96.7

Discussion

Reaction mechanism of the foregoing colorimetric method is being examined and still remains obscure. Metcalf, *et al.*[7] reported that DDVP decomposed to form dimethylphosphoric acid and dichloroacetoaldehyde under alkaline condition. Therefore these two compounds were tested whether they gave a color or not. We found that dichloroacetoaldehyde reacts with acetone to form orange-red color in the presence of alkali. It is doubtful whether the value obtained by this colorimetric method shows the true purity of DDVP or not.

For this reason the values were compared with that from the infrared absorption spectral method and total chlorine method and these values were found to agree well, as shown in Table IV, indicating that this method can be used for determination. Dibrom and Dipterex, the analogues of DDVP, also gave colored products, and can be determined colorimetrically. These methods will be reported successively.

The authors wish to express their thanks to Goro Tatsui, Director of Research Laboratory of Chugai Pharmaceutical Co., Ltd. for permission to publish this work. They are indebted to many coworkers in the Laboratory for their earnest assistances.

Summary

A colorimetric method for the determination of dimethyl 2,2-dichlorovinyl phosphate (DDVP) is described, which is based on the color complex formed between DDVP and acetone in the presence of ethanolic KOH. The absorption at 370 mμ by the color reaction products follows Lambert-Beer's law. This method can be applied to Dibrom and Dipterex, the analogues of DDVP.

(Received September 11, 1962)

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