原　著

Thin-Layer Chromatographic Separation and Colorimetric Analysis of Dimethoate Residue. Takashi Mitsui, Shigeyuki Suzuki (Research Laboratories of Chugai Pharmaceutical Co., Ltd.) and Noriaki Kotaka (Developmental Research Laboratories of Chugai Pharmaceutical Co., Ltd., Toshimaku, Tokyo) Received May 23, 1966. *Botyu-Kagaku* 31, 103, 1966.

14. 薄層クロマトグラフィーによるジメトエートの分析

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動植物組織およびバター，脂肪に添加されたジメトエートを抽出し，シリカゲル G による薄層クロマトグラフィーで分離後，比色定量して，添加されたジメトエートの 90% 以上を回収することができた．次にこの方法を応用し，動植物体中のジメトエート残留量を測定した．

(1) ジメトエートを含む液中に植物を20時間水耕した場合生葉 1g 当たり 6.59μg が検出され，10日以内に 0.1μg 以下に消失する．　(2) 粒剤として施用した場合，施用後10日で生葉中の含量は最大に達し生葉 1g 当たり 6.0μg であった．以後次第に減少し，25日以内に消失する．　(3) ラットに経口投与した場合，投与後2時間で，各組織中に，最大量が検出される．その後は急速に減少し，1日後ではそのうちの約20～30% が，7日後では約5%が検出される．

Dimethoate (*O*, *O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate) has systemic activity as well as contact activity against certain insects and mites on a variety of fruits and vegetables. The increasing usage of this systemic insecticide made it necessary to analyse easily dimethoate residues on agricultural crops and animals. Gas chromatography equipped with an electron capture detector was often adopted for the analysis of residues on agricultural crops because of its high sensitivity. A pesticide residue method is, however, not complete unless it concludes with satisfactory extraction and cleanup procedures. Column chromatography has been used in many cases for cleanup, but it is time-consuming. It is necessary for cleanup procedure to develop the more rapid and sensitive method. For this purpose, thin-layer chromatography offers promise as a cleanup tool, because of its high capacity enough to use for determination of pesticides, great speed and ease of operation.

Walker and Beroza[1] discussed the use of this technique as a method for the cleanup of insecticide residues. Morley[2] and other investigators[3,4] applied this technique to various insecticides and miticides residues. Steller[5] also reported this technique for measurement of residues of dimethoate and its oxygen analogue. Colorimetric methods[6,7], total phosphorus method[5] and gas chromatographic method[8] were reported for determination of dimethoate. In this experiment, the colorimetric method by Giang[6] was employed.

Methods

a) Extraction of dimethoate

Method I—fruits and vegetables—Homogenize 100g sample of plant material with 100cc distilled water. Add 100cc of 10% trichloroacetic acid (TCA) solution, blend at high speed for 3 min. and let the layers separate. Centrifuge at 1,500 r. p. m. for 10 min. and decant the supernatant through a filter paper into a 500cc separatory funnel. Repeat the extraction twice with 100cc portion of 5% TCA solution, centrifuge, and decant the supernatant into another separatory funnel. Add 150cc chloroform into it, shake vigorously and let the layers separate. Transfer the chloroform layer into another separatory funnel and extract with chloroform. Repeat this procedure twice with 100cc chloroform. Concentrate the combined chloroform layer by evaporation under reduced pressure to 1～2cc. Dilute to 15cc with chloroform in a 15cc volumetric flask. Apply the solution to thin-layer chromatographic cleanup.

Method II—milk and animal tissue—Homogenize a sample with a convenient volume of distilled

water. Add the same volume of 10% TCA solution as that of the used water, and 100cc chloroform and blend at high speed for 3 min. Centrifuge at 2,000r. p. m. for 10min. and collect the chloroform layer through a filter paper. Re-extract the residue twice with 100cc chloroform. Concentrate the combined chloroform phases to 15cc as mentioned above.

Method III—butter fat—Dissolve 50g butter fat in *n*-hexane, add the same volume of acetonitrile as *n*-hexane, and blend at high speed for 5min. Centrifuge at 1,500r. p. m. for 10min. and collect the acetonitrile layer into a 500cc separatory funnel. Rinse the extracts with small volume of *n*-hexane and transfer the acetonitrile layer to a flask.

Evaporate acetonitrile under reduced pressure. Add chloroform to the flask and transfer into another 50cc flask through a filter paper. Concentrate chloroform by evaporation to 15cc as mentioned above.

b) Thin-layer chromatography for cleanup

Prepare the plates for thin-layer chromatography according to the procedure of Walker *et al*[1] with only minor variations.

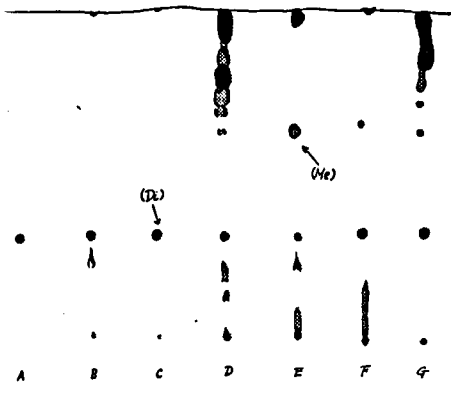
In our experiment, 30g silica gel G was mixed with 20cc distilled water and stirred for 30 seconds, and then it was added an additional 40cc distilled water and stirred for another 30~40 seconds. Apply the extracts to the plates with standard solution to the both sides of the plates, and allow to develop in the separating chamber using benzene and acetone mixture (7 : 3) as a solvent. After drying the plate, expose it to iodine vapor and mark the areas containing the yellow band for dimethoate.

c) Determination of dimethoate

About 10 minutes after removing the plates from iodine vapor, scrape the band for dimethoate into a 50cc flask. Add 30cc of chloroform, shake vigorously, allow to separate and then decant the chloroform layer into a 100cc flask through a filter paper. Repeat this procedure twice with 20cc chloroform. Concentrate the extract under reduced pressure and transfer it to a 40cc test tube. Evaporate chloroform and determine dimethoate content colorimetrically by the method of Giang[6].

Results and Discussion

The dimethoate residues in plants, milk and animal tissues were extracted by the methods as mentioned above and separated by thin-layer chromatographic technique. Dimethoate was clearly separated from extractives shown in Fig. 1. As shown in the figure, the extracts of liver and blood of rat contained another substance which seemed to be the oxygen analogue of dimethoate. On extraction of dimethoate from fruits and vegetables, method II (for milk and animal tissues) is also applicable. We found, however, that the extraction by method I gave an excellent background on thin-layer plates, and avoided troubles in color reaction for determination of dimethoate. At the thin-layer chromatographic cleanup, standards are also run on the both sides of the same plates, because iodine vapor is not sensitive enough for visual detection below approximately 500μg of dimethoate/100g sample.



Di : Dimethoate Me : One of the metabolites of dimethoate.
A : Standard solution, B : The extract from plant leaf, C : Plant stem, D : Butter fat, E : Liver, F : Blood, G : Intestine

Fig. 1. Thin-layer chromatogram of dimethoate

Acetonitrile is unsuitable as a solvent for applying the extract to the thin-layer plate because it spreads on the plate. On the extraction of dimethoate from butter fat, chloroform was used as a solvent to keep spot size to a minimum, after acetonitrile was evaporated.

The recovery values in Table 1 were obtained

by adding the acetone solution of dimethoate to the respective materials before homogenization. In this experiment, dimethoate was added with considerably higher concentration than that of Steller[5]. More than 90% were recovered in each material.

Table 1. Recovery of dimethoate from various materials.

Materials	Dimethoate μg/100g	Added p. p. m.	Recovery %
Chinese mustard	8,360	83.60	93.9
	1,988	19.88	92.6
	994	9.94	96.6
	400	4.00	93.0
Kidney bean	500	5.00	91.4
Cabbage	500	5.00	91.2
Orange	500	5.00	94.6
Apple	500	5.00	90.1
Milk	500	5.00	94.9
	250	2.50	89.1
Butter fat	1,000	10.00	92.1
Animal tissue	1,200	12.00	92.0

Application to Residue Analysis.

The Root Absorption Studies for Plant.

To evaluate the rate of root absorption, the well-established plants (kidney bean, 4 to 6 week-old) were placed in 3.13, 2.34, 1.56, 1.17 and 0.78 ppm dimethoate emulsion for 20 hours.

Some of the plants were removed and divided into leaves and stems, which were separately homogenized in water and measured dimethoate residue as mentioned above. In the other experiment, plants in 3.13ppm dimethoate emulsion were removed from the insecticide source and transferred to culture water for more 10 days. At 0, 1, 2, 3, 4, 6, 7 and 9 days after transferring, mites (*Tetranychus telarius* L.) were placed on the downsides of the true leaves. Four replications of 40 mites each were allowed to feed on the treated plants for a period of 20 hours. At the end of this period, dead mites were counted and percentage mortality was calculated. Table 2 gives the results of analysis of dimethoate in leaves and stems. The percentage mortality is

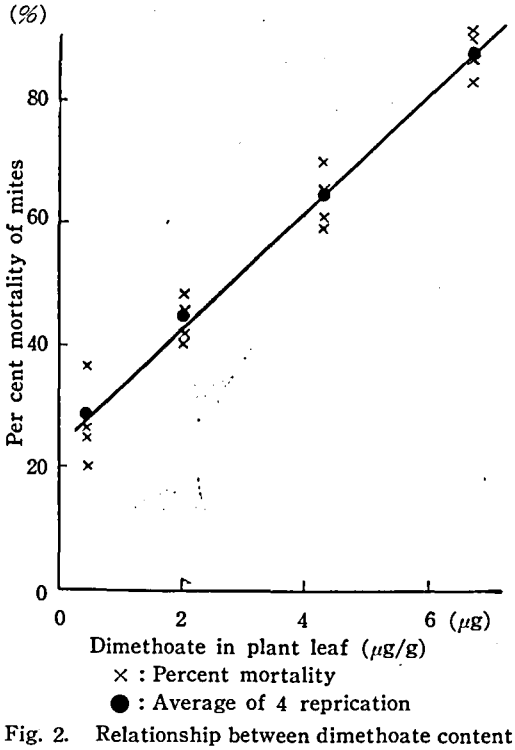


Fig. 2. Relationship between dimethoate content in plant leaf and per cent mortality of mites.

Table 2. Residues of dimethoate in plant (Kidney bean) cultured in dimethoate emulsion for 20 hours.

Rate of Application (p. p. m.)	Residues of dimethoate			Per cent mortality of mites
	Leaf (μg/g)	Stem (μg/g)	Plant (μg/g)	
3.13	6.59	2.79	5.33	87.8±2.8[a]
2.34	4.19	2.59	4.01	63.2±4.3
1.56	1.99	1.83	2.35	43.8±2.7
1.17	0.45	1.29	1.19	26.4±6.1
0.78	0.94	1.09	1.28	14.4±5.3

a : Standard deviation

also shown in this table.

As shown in Fig. 2, the linear correlation exists between the residue of dimethoate in leaf (x) and avarage of percentage mortality (y) in this experemental range. The relationship between the residue of dimethoate in leaf and the rate of application (ppm) is also given in Fig. 3. The persistence of dimethoate in plants was also evaluated in this root absorbing study. In this case, chemical analysis as above was not carried out, but content of dimethoate was read from the previously prepared regression line shown in Fig. 2. From the formula of the regression line, $y=9.85x+22.71$, limits of error can be calculated as $\pm\, t\sqrt{V_x}$. (V_x is a variance of x)

$V_x=0.022+0.0000085(y-55.3)^2$

max. $t\sqrt{V_x}=0.94$ at 0.95 probability level

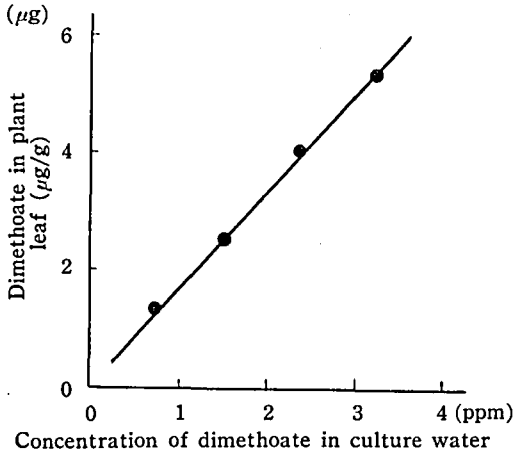


Fig. 3. Relationship between concentration of Dimethoate in culture water and μg dimethoate in plant leaf.

Therefore the limits of error confirm that the method as used to obtain these results is applicable for quantitative assay.

As shown in Fig. 4, dimethoate residues in plant leaves drop below 0.1 μg/g in less than 10 days after removing from the insecticide source. For testing effects of granulated formulation, 7 g/m² of granules (0.5% concentration) was applied as side-dress treatment to 6-week-old plants. Fig. 5 gives the results of analysis of plant leaves treated with dimethoate granules and sampled at intervals thereafter. In this study, dimethoate in plant reaches maximum about 10 days after treatment (6μg/g) and persists in less than 25 days.

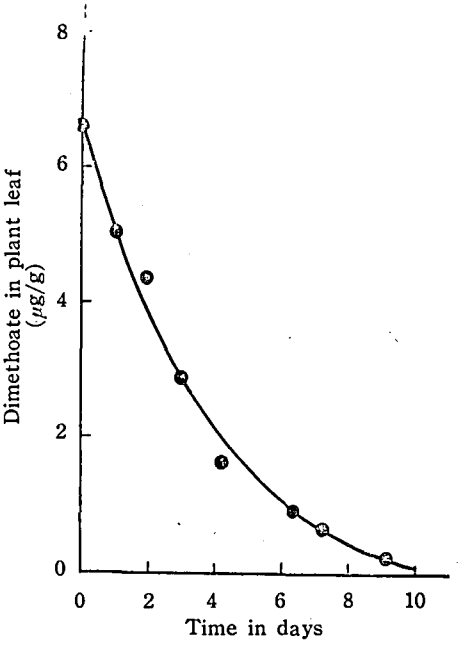


Fig. 4. Persistence of Dimethoate in plant leaf after removing from insecticide source.

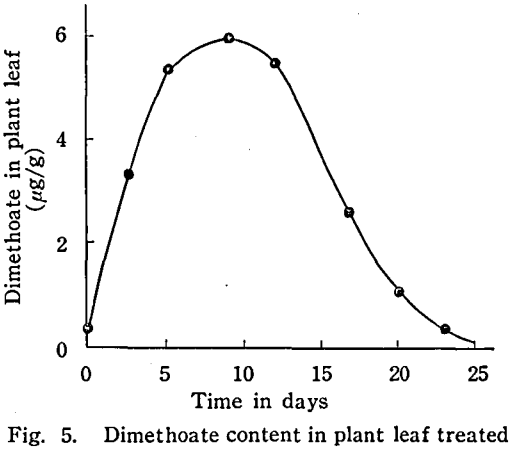


Fig. 5. Dimethoate content in plant leaf treated with granulated formulation.

Fate of Dimethoate in Rats.

Dimethoate was administered orally to rats 350 mg/kg in water suspended with acacia. Male rats weighing 280 to 320 g were used for this experiment. Dimethoate in each organ was analyzed following the methods as above, after

Table 3. Distribution of total dimethoate in male rats following 350mg/kg oral dose.

Tissue or Organ	Total dimethoate (μg/200g body weight) at time indicated					
	2 hrs	6 hrs	12hrs	1 day	3 days	7 days
Blood[b]	63.5	43.4	21.0	26.4	8.2	3.0
Heart	379.0	135.2	61.2	58.9	49.1	26.4
Liver	382.3	290.5	90.2	87.0	84.9	40.1
Kidney	288.3	204.6	107.0	94.5	38.2	22.4
Stomach	7596.3	7575.6	861.9	576.3	221.7	155.7
Intestine	1743.9	4456.2	1424.0	310.3	268.8	142.3
Fat	650.4	135.2	61.2	58.9	49.1	26.4

b : μg/g

sacrificing the rats at intervals thereafter (Table 3). Dimethoate is rapidly absorbed, distributed among the tissues and hydrolyzed.

The contents of dimethoate in each tissue are highest 2 hours after treatment. About 95% of dimethoate levels which were found 2 hours after treatment had been hydrolyzed in each organ in 7 days.

Summary

Dimethoate was extracted from plant materials, butter fat and animal tissues and cleaned up by thin-layer chromatography with silica gel G. Dimethoate content was estimated by a spectrophotometric measurement at 720mμ after scraping the band for dimethoate and extracting with chloroform. More than 90 per cent of dimethoate added to various materials was recovered with this procedure. This procedure was applied to residue analysis of dimethoate in plants and animals.

1) The plants were cultured in various concentration of dimethoate emulsion for 20 hours. In this case, maximum content of dimethoate was 6.59μg/g in a plant leaf and dimethoate residues dropped below 0.1μg/g in less than 10 days after removing from the insecticide source.

2) Dimethoate was applied to plants with granulated formulation. The content of dimethoate in a plant leaf reached maximum about 10 days after treatment and persisted in less than 25 days.

3) Dimethoate was administered orally to rats. The contents of Dimethoate in each organ were highest 2 hours after treatment. About 95% of dimethoate levels was hydrolyzed in 7 days.

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