Application of a Systematic Identification and Determination of Pesticides to Multiresidue Analysis*

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The method of the systematic identification and determination of pesticides was attempted by a combination of column, thin-layer and gas chromatography. Many kinds of pesticides were classified into six divisions and twenty two groups by thin-layer chromatography (*tlc*), and further divided into six fractions corresponding to divisions by column chromatography. In this paper, in order to apply this systematic method to the determination of pesticide residue in crops, food products or soils, an extract procedure, solvent partitioning and Florisil column cleanup procedures were decided with feed stuff-corns, which had some difficulty in analysis because of being rich in oils and coloring materials. Furthermore, recovery experiments at the level of residue analysis were performed to evaluate the efficiency of this systematic method. Fifteen pesticides in the First and Second Divisions containing tetrasul, heptachlor, quintozone, CNP, chlorpyrifos and nitrogen, etc. were mostly eluted into Fractions I and II from a silica column, respectively, and recovered approximately 100%. Twelve pesticides in the Second Division containing trifluralin, leptophos, dichlofenthion, dichlobenil and chlorfenson, etc. were mostly eluted into Fraction II and recovered more than 90%, except for chlorothalonil.

INTRODUCTION

The systematic identification and determination of individual pesticides from a mixture of various pesticides are very useful for the determination of these residues in crops, of phytotoxic ingredients in technical products, and of toxicological ingredients in mammalia. Although numerous analytical methods have been established, most of them are individual procedures for specific chemicals. For systematic identification and determination, pesticides in extracts from crops or soils must be separated into several fractions by column chromatography prior to *tlc* treatment, because a large amount of extract is necessary for a single analysis.

However, column chromatography is time-

consuming as regards finding suitable conditions of development to separate pesticides in the extract into several fractions. Therefore, in a preliminary trial, about two hundred pesticides were classified into six divisions by *tlc*^{2,3)}. Organochlorine pesticides were in the First and Second Divisions, diphenyl ether herbicides in the Second and the Third, carbamates and triazine herbicides in the Fourth and the Fifth, and phenoxy herbicides in the Sixth. Organophosphorus pesticides were in all divisions except in the First. It also was recognized that these pesticides were classified into six divisions, depending on such their physical and chemical properties as solubilities, the organic or inorganic properties,³⁾ and *p*-values⁴⁾; that is, partition coefficient of pesticides between hydrophilic and hydrophobic solvents. Moreover, about one hundred pesticides were classified into six fractions corresponding to these divisions by column chromatography instead of *tlc*, and the pesticides in

* Studies on Systematic Identification and Determination of Pesticides (Part XII). See reference.¹⁾

six fractions were separated from each other by a combination of *tlc* and *gc*. Thus, the authors established the systematic identification and determination of individual pesticides from a mixture of various pesticides. Furthermore, the application of this systematic method to such samples having the unknown or incomplete history of treatment as imported crops or foods, was investigated by employing corns which involved some difficulties in the analysis because of being rich in oils and coloring matters. That is, an extract procedure, solvent partitioning and Florisil column cleanup procedures for this systematic method were studied, and then about one hundred pesticides containing organochlorine pesticides, organophosphorus pesticides, carbamates, triazine and urea herbicides, etc. were added into corns at a level of pesticide residues for recovery experiments to evaluate the efficiency of systematic method. About twenty seven pesticides in the First and the Second—mainly organochlorine pesticides—were examined in this paper.

MATERIALS AND METHODS

1. Compounds

The purified compounds listed in Tables 1, 2 and 3 by common and the other names*, were supplied by manufacturers.

2. Organic solvents

Solvents for pesticide residue analysis were used.

3. Silica Gel for Column Chromatography

As in the previous paper¹⁾.

4. Florisil

Florisil was 60/100 PR grade and available from Floridin Co. It was activated at 110°C for over 24 hr, and stored for 30 min in a desiccator before use.

5. Florisil Column

A cotton-wool plug was placed in the bottom of a chromatographic tube (1.5×30 cm) to support a column. After 40 ml of hexane was

poured to the tube, twelve grams of Florisil was added to the tube and tapped gently with a hand (Florisil column: 10 cm).

6. *Tlc* and *Gc*

As in the previous paper.¹⁾

7. Cotton and Glass Wool

As in the previous paper.¹⁾

8. Procedure

Pesticide residues in corns were extracted and divided from a silica column into Fraction I and II according to Figs. 2, 3 and 4. The eluates were evaporated to dryness as fast as possible and dissolved in an appropriate volume of hexane, followed by injection into a gas chromatograph. They, if necessary, had to be cleaned up according to Figs. 5 and 6, prior to *gc*.

RESULTS AND DISCUSSION

In preliminary examination, losses of pesticides through column chromatography and *tlc* procedures were studied according to the schematic diagram of the systematic separation and determination of pesticides as described in the previous paper¹⁾ (Table 1). The recoveries of propoxur, chlorothalonil and diuron were low to a slight degree, but most of pesticides were recovered approximately 100%.

Table 1 Losses of pesticides through column chromatography and *tlc* procedures*.

Pesticide	Added (μg)	Recovery (%)
<i>p, p'</i> -DDT	1	100
	5	100
Aldrin	1	100
	5	100
EPN	5	100
Fenitrothion	5	100
Diazinon	5	100
Propoxur	10	80
Chlorothalonil	5	72
Simazine	5	98
Diuron	5	83
	10	100

* Pesticides were submitted to the procedure according to the schematic diagram of the systematic separation and determination of pesticides as described in the previous paper.¹⁾

* CNP: *p*-nitrophenyl 2,4,6-trichlorophenyl ether

1. Procedure

1.1 The whole procedure

The summary of the whole procedure was presented in the previous paper.¹⁾ However, since this was a procedure for the systematic identification and determination of pesticides assuming that dozens of pesticides might exist in a sample, it took much time to perform the whole procedure. Figure 1 showed the simplified whole procedure which was practically adequate for determination of several pesticides in a sample.

1.2 Extraction

This procedure was the modification of the official method⁵⁾ in Japan developed for organochlorine and organophosphorus pesticides (Fig. 2). The sample was extracted with acetone and benzene, in addition, with methanol by considering the slight decrease in solubility of pesticides in the Fourth to the Sixth, for example, triazine herbicides⁶⁾.

1.3 Solvent partitioning cleanup

This procedure removed hydrophilic and hydrophobic substances interfering with the determination of pesticides from the above

extract (Fig. 3). When the acetonitrile phase in a separatory funnel was separated from the hexane phase, and transferred into a flask, care must be taken not to add the particles suspended between the two phases to prevent bumping during evaporation of acetonitrile. Triazines were considerably lost during evaporation when acetonitrile was concentrated to less than ten milliliters. Dichloromethane was dried over anhydrous sodium sulfate and concentrated to a small volume under reduced pressure, followed by evaporating completely through a nitrogen jet. Aldrin, heptachlor, α -BHC, trifluralin, chlorpyrifos, and dieldrin, were all recovered approximately 100% in this cleanup procedure.

1.4 Fractionation by column chromatography

A cotton-wool plug was placed in the bottom of a chromatographic tube (1.5×30 cm) to support a column. Fifteen grams of silica gel was added to the tube and the column was tapped gently with a hand (the column: 17.0–17.5 cm).

The cleaned up extract was mixed thoroughly with 2.0 g of inactivated silica gel and transfer-

Fig. 1 Simplified whole procedure of the systematic identification and determination of pesticides.

Fig. 2 Extraction.

Fig. 3 Solvent partitioning cleanup.

red quantitatively to the tube, and then a glass-wool plug was pressed down firmly on the surface of the extract with the aid of a glass rod. The column was developed successively with 150 ml of hexane-benzene (9 : 1) and an equal amount of hexane-benzene (1 : 3) at a flow rate of 1.5 ml per minute.

By these developing solvents, the extract was separated into Fractions I and II, respectively (Fig. 4). The solvent was added to the top of the tube through a reservoir which permitted the application of gas pressure from a nitrogen tank. Since chlorothalonil might be eluted from the column into Fraction II, the eluate had to be evaporated to dryness as fast as possible because of the reactivity of chlorothalonil with benzene in Fraction II.

1.5 Solvent partitioning cleanup for Fractions I and II

The AOAC method⁷⁾ developed for organochlorine or part of organophosphorus pesticides, was applied to the cleanup procedure in Fractions I and II, because the pesticides in these fractions were mainly organochlorine

Fig. 4 Fraction by column chromatography.

Fig. 5 Solvent partitioning cleanup for Fraction I and II.

Fig. 6 Florisil cleanup for Fraction I and II.

and organophosphorus pesticides (Fig. 5).

1.6 Florisil cleanup for Fractions I and II

As aldrin, DDT, heptachlor, isobenzan, quintozone, BHC, trifluralin in the First and the Second were eluted from a Florisil column

with 200 ml ethyl ether-hexane (6:100)^{7,8)}, this method was adopted as a cleanup procedure for the pesticides in Fraction I (Fig. 6). Dieldrin, endrin, diazinon and EPN in the Second to the Fourth were eluted from a

Florisil column with 200 ml of ethyl ether-hexane (15 : 100),⁸⁾ but dichlobenil and dicofol in group 4 of the Second were not completely eluted from the column. Therefore, 200 ml of ethyl ether-hexane (25 : 100) was used as a developing solvent in the cleanup procedure for the pesticides in Fraction II.

Chlorothalonil in group 4 of the Second also was completely eluted from the column under this condition.

1.7 Recovery tests

To evaluate performance of the proposed systematic method, ground corns were fortified with fifteen pesticides in the First and the Second, and submitted to the extraction and the solvent partitioning cleanup procedure. The cleaned up extract was separated into Fractions I and II by column chromatography. After evaporation of these eluats, the

Table 2 Recoveries of pesticides in the First and Second Divisions.

Division	Pesticides	Added (ppm)	Recovery(%)	
			F I	F II
I	Tetrasul	0.5	75	
I	Aldrin	0.05	75	
I	Heptachlor	0.025	80	
I	Quintozene	0.05	98	
I	Isobenzan	0.05	85	
I	<i>o, p'</i> -DDT	0.25	100	
I	<i>p, p'</i> -DDT	0.25	100	
II	α -BHC	0.025		100
II	Trifluralin	0.25		100
II	γ -BHC	0.025		100
II	α -endosulfan	0.1		100
II	CNP	0.25		100
II	Chlorpyrifos	0.25		100
II	Nitrofen	0.5		100
II	Dieldrin	0.05		100

Fig. 7 Chromatogram of pesticides in the First and the Second Divisions from corns fortified at the level of 0.025–0.5 ppm.

A, quintozene; B, heptachlor; C, aldrin; D, isobenzan, E, *o, p'*-DDT; F, tetrasul, G, *p, p'*-DDT. GC: Shimadzu 6 AM, Att. $\times 16$; Column, 2% OV-17, 1.5 m; Column temp. 200°C.

Fig. 8 Chromatogram of pesticides in the First and the Second Divisions from corns fortified at the level of 0.025–0.5 ppm.

A, α -BHC; B, γ -BHC; C, trifluralin; D, chlorpyrifos; E, α -endosulfan; F, dieldrin; G, nitrofen; H, CNP. GC: Shimadzu 6 AM, Att. $\times 32$; Column: 2% SP2401, 1.5 m; Column temp. 200°C.

Table 3 Recoveries of pesticides in the Second Division.

Division	Pesticide	Added (ppm)	Recovery(%) F II	
			Before <i>tlc</i>	After <i>tlc</i>
II	Trifluralin	0.5	100	
II	Leptophos	9.0		90
II	β -BHC	0.4	100	
II	Dichlofenthion	0.9	93	
II	Phenkapton	5.5	100	
II	δ -BHC	0.4	100	
II	Endrin	1.8		100
II	Dichlobenil	0.4	100	
II	Dicofol	4.0	95	
II	Chlorfenson	7.0		100
II	Chlorothalonil	1.4	(10)	
II	Tetradifon	7.0		100

residues were well dissolved in 10 ml of acetone and filled up to 100 ml with hexane in Fraction I, while to 40 ml in Fraction II. The result of the recovery test was shown in Table 2. Pesticides in the First and the Second were separated into Fractions I and II by column chromatography, respectively. Aldrin and tetrasul showed low recoveries, because they passed into hexane phase in the solvent partition cleanup procedure owing to the effect of oils in corns. Typical chromatograms were shown in Figs. 7 and 8 for fifteen pesticides in corns. The result of the recovery test for twelve pesticides in the Second was shown in Table 3. The sample was submitted to the Florisil cleanup procedure. After evaporation

Fig. 9 Chromatogram of pesticides in the Second Division from corns fortified at the level of 0.4-9.0 ppm.

A, dichlobenil; B, trifluralin; C, β -BHC; D, dichlofenthion; E, δ -BHC; F, chlorothalonil; G, dicofol; H, chlorfenson; I, endrin; J, phenkapton; K, tetradifon; L, leptophos, GC: Shimadzu 6 AM, Att. $\times 32$; Column: 2% OV-17, 1.5 m; Column temp. 200°C.

Fig. 10 Chromatogram of pesticides in the Second Division from fortified corns, after *tlc* treatment.

A, trifluralin; B, dichlofenthion; C, benzophenone (<dicofol); D, endrin; E, phenkapton; F, leptophos.

Condition: the same as in Fig. 9.

Fig. 11 Chromatograms of pesticides in the Second division from fortified corns, after *tlc* treatment.

A, β -BHC; B, chlorfenson; C, tetradifon; D, dichlobenil; E, δ -BHC; F, chlorothalonil; G, dicofol.

Condition: the same as in Fig. 9.

of the eluate, the residue was dissolved in 5 ml of acetone and filled up to 40 ml with hexane. The hexane solution was injected into a gas chromatograph before and after *tlc* separation into three groups, because leptophos, tetradifon, chlorfenson and endrin were not completely separated from each other, only by *gc*. As shown in Table 3, pesticides in the Second were eluted from the column into Fraction II.

Chlorothalonil showed a low recovery, presumably owing to adsorption on Florisil particles having the high adsorptive activity derived from some lots of Florisil. Typical chromatograms were shown in Figs. 9, 10 and 11. Trifluralin was observed at group 2 in Fig. 10, because it was partly introduced into group 2 on the *tlc* plate. As dicofol was partly degraded to benzophenone at group 2 of the Second through the procedure, benzophenone also was observed at group 2 in Fig. 10.

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要 約

農薬の系統分析法の多成分分析への応用*

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いままでに, *tlc* による分離状況の類似している農薬を一つの属, 亜属として6属, 22亜属を設定した。さらに, 各属に対応するようなCCによるフラクションを設定し, その中の農薬を亜属に分離した後, GCで相互分離・定量する方法を確立した。今回は, この方法を作物, 食品および土壌などの試料中の残留農薬の分析法に適用するために油脂, 色素の比較的多い飼料用トウモロコシを用いて抽出法, 抽出液に対する液・液分配によるクリーンアップ操作法, フラクションI, IIに対する液・液分配およびフロリジルカラムによるクリーンアップ操作法を設定した。これらの操作法によって第1, 2属の27種類の農薬の残留分析レベルでの添加試験を行なった。その結果, 第1, 2属の農薬はそれぞれフラクションI, IIに溶出し, 大部分の農薬の回収率は90%以上であった。

* 農薬の系統的定性・定量分析に関する研究 (第12報)