

Thin Layer Chromatography에 의한 유기 카바메이트系 농약의 스크리닝 테스트에 관한 연구(1)

朴 聲 雨 · 李 完 求

國立科學搜查研究所

Studies on screening test for carbamate insecticides by thin layer chromatography

Sung Woo Park, Wan Koo Lee

The National Institute of Scientific Investigation

요 약

한국에서 사용하고 있는 carbamate系 살충제의 使用率(%)은 1968年頃¹⁰⁾에는 2.1%인데 비해서 現在는急增되고 있는 실정에 있다. 따라서 이로 인한 자타살 사건, 환경오염, 살포시 급만성중독 등의 사고가 빈번히 발생하고 있고 동시에 法化學의 分析에도 많은 問題點이 대두되고 있으므로 1차적으로 시판되고 있는 carbamate系 살충제를 thin layer chromatography에 의해서 성분의 分離 확인 시험을 실시한 결과

- 1) Silica Gel G-60 및 Silica Gel F-254에서 적합하였고
- 2) 전개용매는 chloroform: benzene: carbontetra

chloride (60:30:25), cyclohexane: acetone (80:20), cyclohexane: acetone(70:30), ether: hexane (80:20)에서 9종의 살충제가 잘 분리되었으며

3) UV light (2537 Å) 및 2,6-dibromoquinone-4-chlorimide는 전개는 Rf치를 육안으로 구분하는데 가장 양호하였고

4) Carbamate계 살충제 중 Bux-2와 Sevin은 Silica Gel G-60에서 UV light (2537 Å)에서 특이한 정보라 및 형색 형광을 관찰할 수 있었다.

5) 이상의 결과를 보아 法化學的 시료중에 함유된 카바메이트계 농약의 분석은 가능할 것이며 또한 이 방법의 적용이 現在의 실정으로서는 적합한 것으로 사료되는 바이다.

Introduction

The use of carbamate (carbamic acid ester) insecticides are on the increase as compared with organophosphorus insecticides (TEPP; synthesis in Germany, 1942) and organic chlorinated pesticides (BHC; synthesis in Great Britain, 1825)¹⁰⁾

Therefore, the incidence of suicide and homicide due to these compounds, environmental pollution and intoxication when they are dusted or sprayed in the season of cultivation are serious problems.

At the same time, the carbamate insecticides pose some unique problems relative to forensic analysis.

Most of these compounds are unstable under conditions normally used for gas chromatographic analysis and require special attention if this technique is to apply to the carbamates. Moreover, the carbamates are commonly metabolized to products which are toxicologically significant and which must be included in

any analytical considerations.

Various analytical methods for carbamate insecticides have been reported as follows. 1) Thin Layer Chromatography, 2) Enzymatic Techniques, 3) High Pressure Liquid Chromatography, 4) Detection by in situ Fluorometry, 5) Spectrophotometric Methods, 6) NMR and IR and 7) Gas Chromatography. ^{1,2,3,4,5,6,7,8,9).}

It is purpose of this paper to establish a rapid method for screening of carbamate insecticides by Thin layer chromatography so as to determine the component from the samples of forensic chemistry (gastric juice and blood from corpse).

The comparative observation on Rf values, colorations and fluorescence and spots under ultraviolet light (2536Å) were carried out for 9 kinds of market products.

The more advanced reports on analytical method from human tissue, blood, grains, soils and others would be presented later.

Experimental

A) Materials

Name of market products of carbamate insecticides in Korea ¹¹⁾

Trade name	Chemical name
Farbam	ferri dimethyl dithio carbamate
Mipcin	2-iso propyl phenyl-n-methyl carbamate
Bassa	2-sec-butyl phenyl-n-methyl carbamate
Sevin	1-naphthyl-n-methyl carbamate
Bux-2	m-(1-methyl butyl)-phenyl methyl carbamate
Dithane stenless	diammonium ethylene bis dithio carbamate
Dithane M-45	zinc complex manganese ethylene bis dithio carbamate
Dithane M-22	manganese ethylene bis dithio carbamate
Zineb Z-78	zinc ethylene bis dithio carbamate

B) Solvent systems used for development ²⁾

- 1) ether: hexane (90 : 10)
- 2) ethyl acetate: chloroform (60 : 40)
- 3) chloroform: benzene: carbon-tetrachloride (60 : 30 : 25)
- 4) cyclohexane: acetone (80 : 20)
- 5) hexane: acetone (70 : 30)
- 6) cyclohexane: acetone (70 : 30)
- 7) ether: hexane (80 : 20)
- 8) ethyl acetate: toluene (50 : 50)
- 9) chloroform: acetonitril (80 : 20)
- 10) benzene: tetrahydrofuran (80 : 20)

C) Chromogenic agent (Spray reagent)

- 1) Rhodamin B; 0.05% aqueous solution
- 2) 2,6-dibromoquinon-4-chlorimide; freshly prepared 0.5% cyclohexane solution.
- 3) Dragendorff's reagent;

Solution a) : 0.85g basic bismuth nitrate is dissolved in a mixture of 10 ml acetic acid and 40 ml water.

- Solution b) : A solution is made of 8g potassium iodide in 20 ml water
 Stock solution : Equal volumes of a and b are mixed (can be stored for a long time in dark glass vessels)
 Spray reagent : 10 ml stock solution is mixed with 5 ml acetic acid and 25 ml water before use.

4) 0.5 N-sodium hydroxide; sodium hydroxide in methanol solution.

D) Apparatus

- 1) Thin layer chromatographic tank
- 2) Glass plate (20×20cm)
- 3) Applicator (Shandon Scientific Co., Ltd.)
- 4) Chromato-VUE (Mfd. by Ultraviolet Products Inc.)
- 5) Silica gel G-60 (E. Merck)
- 6) Aluminum oxide 150 F-254 (E. Merck)
- 7) Silica gel F-254 (E. Merck)

E) Thin layer chromatography

Migrational characteristics of carbamate insecticides and structurally related bases were examined in 10 kinds of solvents on silica gel G-60, glass plate (a) of 250-micron thickness and two kinds of commercially prepared silica gel F-254, plastic roll (b) of 200-micron thickness and aluminum oxide 150 F-254, glass plate (c) of 250 microns thick.

The former were prepared by mixing 25 grams of silica gel G were evenly mixed with 50 ml distilled water by grinding in a mortar and coating 20-cm², glass plates with slurry using the Shandon adjustable spreader.

Thus Thin-layer chromatography was carried out for 9 kinds of market products on each plate. a), b), c). The spray reagents used in each plate were as follows;

plate a): 1) Ultraviolet light (2536Å)

2) 0.5N-sodium hydroxide in methanol + 2, 6-dibromoquinon-4-chlorimide

3) Dragendorff's reagent

plate b): 1) Ultraviolet light (2536Å)

2) 0.5N-sodium hydroxide in methanol + 2, 6-dibromoquinon-4-chlorimide

3) Rhodamin B

4) 0.5N-sodium hydroxide in methanol + Rhodamin B

plate c): 1) Ultraviolet light (2536Å)

2) 0.5N-sodium hydroxide in methanol + 2, 6-dibromoquinone-4-chlorimide

3) Rhodamin B

After development, each plate was dried for five minutes in a stream of warm air. Spots were detected by examination under ultraviolet light. Also, the above reagents were used as a spray reagent. And observed comparatively on R_f value, coloration and fluorescence for each plate.

The result of observation in each solvent systems are shown in Table 1, 2 and 3. Figure 1, 2 and 3 illustrate the chromatograms of 9 kinds of the carbamate insecticides on each plate.

The colors are abbreviated as follows:

black; Bk

dark orange-yellow; DOY

blue; B

dark purple; DPu

blue-fluorescence; BF

dark reddish-brown; DRBr

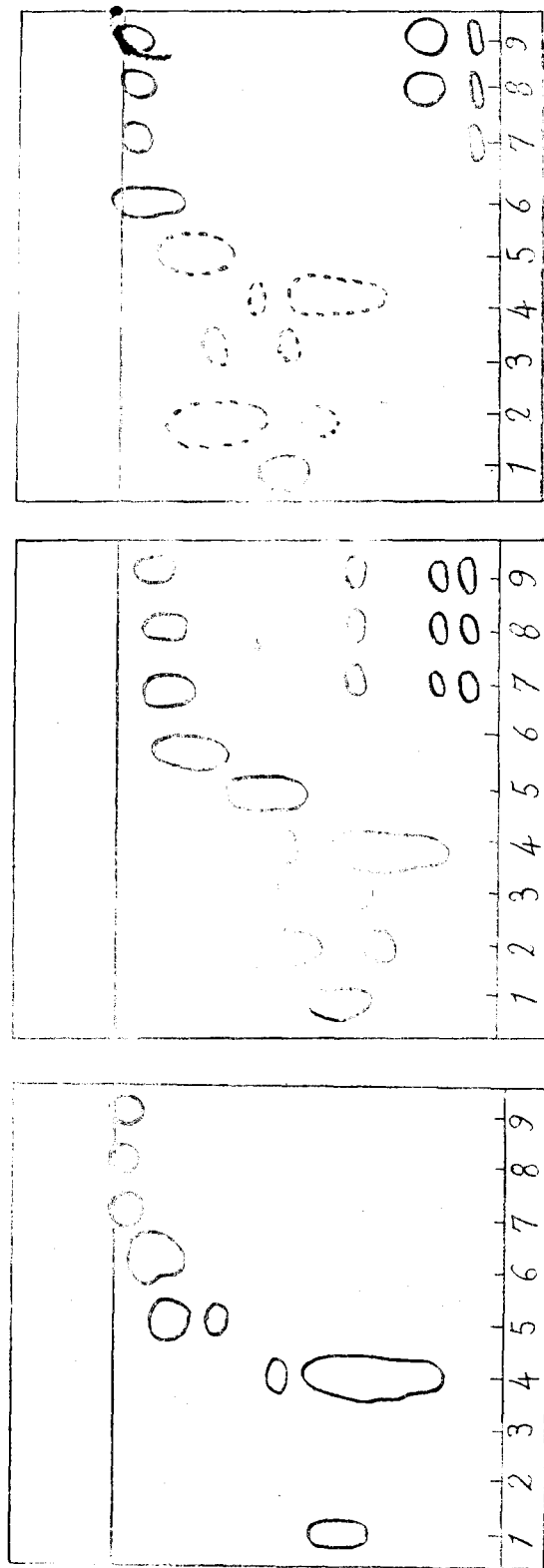
Table 1. Rf values and colorations of carbamate insecticides (silica gel G-60, glass plate)

Solv	Item	CI											
		Farbam			Mipcin			Bassa			Sevin		
		UV	1	2	UV	1	2	UV	1	2	UV	1	2
1	Rf	89Ⓢ	90Ⓢ	90Ⓢ	84-77	91-75	91-75 (D)	—	92-88	92-88 (D)	89-74	95-74	95-74 (D)
	CL	Bk	Y. Br	L.O. Y	Bk	B	L.O. Y	—	B	O. R	B. V	DpBv	O. R
2	Rf	41Ⓢ	41Ⓢ	41Ⓢ	84-77	94-76	94-76 (D)	—	—	—	98-92	85-38	85-38 (D)
	CL	Bk	Y. Br	L.O. Y	Bk	B	L.O. Y	—	—	—	B. V	DpBv	O. R
3	Rf	5-0Ⓢ	21-0	21-0	—	33-0	33-0 (D)	—	18-13	18-13 (D)	11-2 2-0	20-13 10-0 (D)	20-13 10-0 (D)
	CL	Bk	Y. Br	L.O. Y	—	B	L.O. Y	—	B	O. R	B. V	DpBv	O. R
4	Rf	59-35	57-43	57-43	—	48-15	48-15 (D)	96-84	—	96-84 (D)	44-27	48-11	48-11 (D)
	CL	Bk	Y. Br	L.O. Y	—	B	L.O. Y	Bk	—	O. R	B. V	DpBv	O. R
5	Rf	38Ⓢ	35Ⓢ	35	—	55-29	55-29 (D)	—	47-41	47-41 (D)	38-30	38-29 27Ⓢ	38-29 27Ⓢ (D)
	CL	Bk	Y. Br	L.O. Y	—	B	L.O. Y	—	B	O. R	B. V	DpBv	O. R
6	Rf	42-36 32Ⓢ	52-36	52-36	—	75-48	75-48 (D)	—	62-5 41-38	62-5 41-38 (D)	45-17	54-49 45-15	54-49 45-15 (D)
	CL	Bk	Y. Br	L.O. Y	—	B	L.O. Y	—	B	O. R	B. V	DpBv	O. R
7	Rf	17Ⓢ	52-0	52-0	77-69	70-48	70-48 (D)	—	68	68 (D)	85-97 82-78 76-63 47Ⓢ	82Ⓢ	82Ⓢ (D)
	CL	Bk	Y. Br	O. R	Bk	B	L.O. Y	—	B	O. R	Bv	DpBv	O. R
8	Rf	88Ⓢ	91-82	91-82	77-69	98-66	98-66 (D)	—	82-76	82-76 (D)	83-78 72-61	85-58	85-58 (D)
	CL	Bk	Y. Br	O. R	Bk	B	L.O. Y	—	B	O. R	B. V	DpBv	O. R
9	Rf	98-77	94-75	94-75	82-76	90-69	90-69 (D)	—	90-82	90-82 (D)	87-71	90-68	90-68 (D)
	CL	Bk	Y. Br	O. R	Bk	B	L.O. Y	—	B	O. R	B. V	DpBv	O. R
10	Rf	95-77	95-85	95-85	80-73	85-68	85-68 (D)	84-76	84-76	84-76 (D)	74-57	77-56	77-56 (D)
	CL	Bk	Y. Br	O. R	Bk	DpB	L.O. Y	Bk	S. B	O. R	B. V	DpBv	O. R

CI; carbamate insecticides, CL; coloration
 SR; spray reagent 1) 2,6-dibromoquinone-4-chlorimide
 2) Dragendorff's reagent
 SOLV; solvent developed, (-); colorless, (D); disappeared, ⊕; tailing

Bux-2			Dithane stenless			Dithane M-45			Dithane M-22			Zineb Z-78		
UV	1	2	UV	1	2	UV	1	2	UV	1	2	UV	1	2
95-92 91-84	98⊕ (D)	98 (D)	97-81	99⊕ 68 56 22	99⊕ 68 56 22	96-93	85⊕ 75" 54" 23"	85⊕ 75" 54" 23"	96-93	85⊕ 73 54 23	85⊕ 73 54 23	97-93	73 50 19	73 50 19
B. F	DpB	O. R	Bk	Be	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	R. Br	O. R
100-9	100-83	100-83 (D)	98-94	72 63 20	72 63 20	99-98	97⊕ 81" 72" 58"	97⊕ 81" 72" 58"	99-98	97 81 72 58	97 81 72 58	99-98	97 33 14	97 33 14
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. B	Bk	R. Br	O. R
89-83 64-52	35-11	35-11 (D)	86-80	82-81 64 19	82-81 64 19	93-87	95-88 21	93-87 21	93-87	95-88 21	95-88 21	94-89	94-89 21	94-89 21
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	R. Br	O. R
86-78 69-65	53-19	53-19 (D)	90-80 36-21	88⊕ 84 54 35	88⊕ 84 54 35	90-83	90⊕ 21	90⊕ 21	93	90 35	90 35	96-92	99-90	99-90
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	R. Br	O. R
89-81 74-65	70-32 ⊕	70-32 (D)	94-84	93⊕	93⊕	97-90	96⊕ 33	96⊕ 33	97-90	97⊕ 33	97⊕ 33	97-91	98⊕	98⊕
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	R. Br	O. R
91-82 80-76	77-54	77-54 (D)	94-82	92⊕ 27	92⊕ 27	93-86	92-83 24 9 4	92-83 24 9 4	93-86	92-83 24 9 4	92-83 24 9 4	97-39	94-88 4	94-88 4
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	R. Br	O. R
96-89	96-89	96-89 (D)	91-88	96-87 33-26 18-11	96-87 33-26 18-11	31-26	31-26 10-4	31-26 10-4	89-81	38-93 26-22 12-7	38-93 26-22 12-7	12-7	12-7	12-7
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	R. Br	O. R
88-85 50-76	89-71	89-71 (D)	96⊕	89-79	89-79	98-87	96-87 72 44-39 14-0	96-87 72 44-39 14-0	93-88	96-87 72 59 19	96-87 72 59 19	93-88	96-87 59 14-4	96-87 59 14-4
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	R. Br	O. R
98-35	94-75	94-75 (D)	96⊕	92⊕ 70-62 57-53 37-30	92⊕ 70-62 57-53 37-30	97-79	92-82 71-66 58-53 34-25	92-82 71-66 58-53 34-25	96-82	92-85	92-85	97-92	93-85	93-85
B. F	DpB	O. R	Bk	Br	O. R	Bk	L. Br	O. R	Bk	L. B	O. R	Bk	L. B	O. R
94-84 82-75	89-70	89-70 (D)	81⊕	94⊕	94⊕	95-86	95-89 40-31 11-5	95-89 40-31 11-5	91-88	95-89 11-5	95-89 11-5	94-87	93-89 11-5	93-89 11-5
B. F	DpB	O. R	Bk	R. Br	O. R	Bk	L. Br	O. R	Bk	R. Br	O. R	Bk	R. Br	O. R

Fig. 1. Thin layer chromatogram of carbamate insecticides on silica gel G-60 (250-micron)



solvent cyclohexane : acetone
developed; (70 : 30)

cyclohexane : acetone
(70 : 30)

ether : hexane
(80 : 20)

spray reagent: ultraviolet light
temperature; 27°C(Approx.)

2,6-dibromoquinone-4-chlorimide
27°C(Approx.)

Dragendorff's reagent
27°C(Approx.)

* compounds are: 1) Farbam; 2) Mipcin; 3) Bassa; 4) Sevin; 5) Bux 2; 6) Dithane stenless; 7) Dithane M-15;
8) Dithane M-22; 9) Zineb Z-78

** dotted circle means disappeared

Table 2. Rf values and colorations of carbamate insecticides. (silica gel F-254, plastic roll)

Solv	CI SR Item	Farbam			Mipcicn			Bassa			Sevin		
		UV	1	2	UV	1	2	UV	1	2	UV	1	2
1	Rf	96-88	96-88	96-88	92-86	92-86	92-86	91	91	—	94-77	94-77	94-77
	CL	D. G	Br	D. R. Br	G. B	L. B	D. O. Y	L. G	B	—	Dp. Bv	BpBv	D. Bv
2	Rf	83-65	83-65	83-65	90-85 81	90-85 81	90-85 81	90	90	—	82-50	82-50	82-50
	CL	D. G	L. Y. Br	D. R. Br	G. B	L. B	O. R	L. G	B	—	Dp. Bv	DpBv	D. Bv
3	Rf	8-0	8-0	8-0	15-1	15-0	15-0	4-0	4-0	—	10-0	10-0	10-0
	CL	D. G	Br	G. B	G. B	B	O. R	L. G	B	—	Dp. Bv	Dp. Bv	D. Bv
4	Rf	71-59 48-37 35-27	71-59 48-37 35-27	71-59 48-37 35-27	48-18	48-18	48-18	27	27	27	42-15	42-15	42-15
	CL	D. G	L. Br	D. Y	G. B	L. B	O. R	L. G	B	O. R	Dp. Bv	Dp. Bv	Pu
5	Rf	93-91 26-9	93-91 26-9	93-91 26-9	93-92 41-26	93-92 41-26	93-92 41-26	36-32	36-32	—	32-28 24-0	32-28 24-0	32-28 24-0
	CL	D. G	Y. Br	L. Br	G. B	L. B	O. R	L. G	B	—	Dp. Bv	Dp. Bv	D. O. Y
6	Rf	36-11	36-11	36-11	50-31 28-2	50-31 28-2	50-31 28-2	40	40	—	23 23-0	33	33
	CL	D. G	L. Br	G. B	G. B	L. B	O. R	L. G	B	—	Dp. Bv	Dp. Bv	G. Y
7	Rf	64-41	64-41	64-41	89 79-68 23	89 78-68 23	89 79-68 23	77	77	—	82 68	82	82
	CL	D. G	Y. Br	D. O. Y	G. B	L. B	O. R	L. G	B	—	Dp. Bv	Dp. Bv	D. O. Y
8	Rf	83-77 69	83-77 69	83-77 69	77-68	77-68	77-68	79	79	—	83-79 69-38	83-79	83-79
	CL	D. G	Y. Br	D. Br	G. G	L. B	O. R	L. G	B	—	Dp. Bv	Dp. Bv	O. R
9	Rf	93-81	93-81	93-81	81-76	81-76	81-76	82-79	82-79	—	93-90 88-72	93-90	93-90
	CL	D. G	Y. Br	D. Br	G. B	L. B	O. R	L. G	B	—	Dp. Bv	Dp. Bv	D. O. Y
10	Rf	92-88 83-8	92-88 83-8	92-88 83-8	83-75	83-75	83-75	84-81	84-81	—	84-80 76-65	84-80	84-80
	CL	D. G	Y. Br	D. Br	G. B	B	O. R	L. G	B	—	Dp. Bv	Dp. Bv	O. R

CI; carbamate insecticides, CL; coloration

1) 2,6-dibromoquinone-4-chlorimide

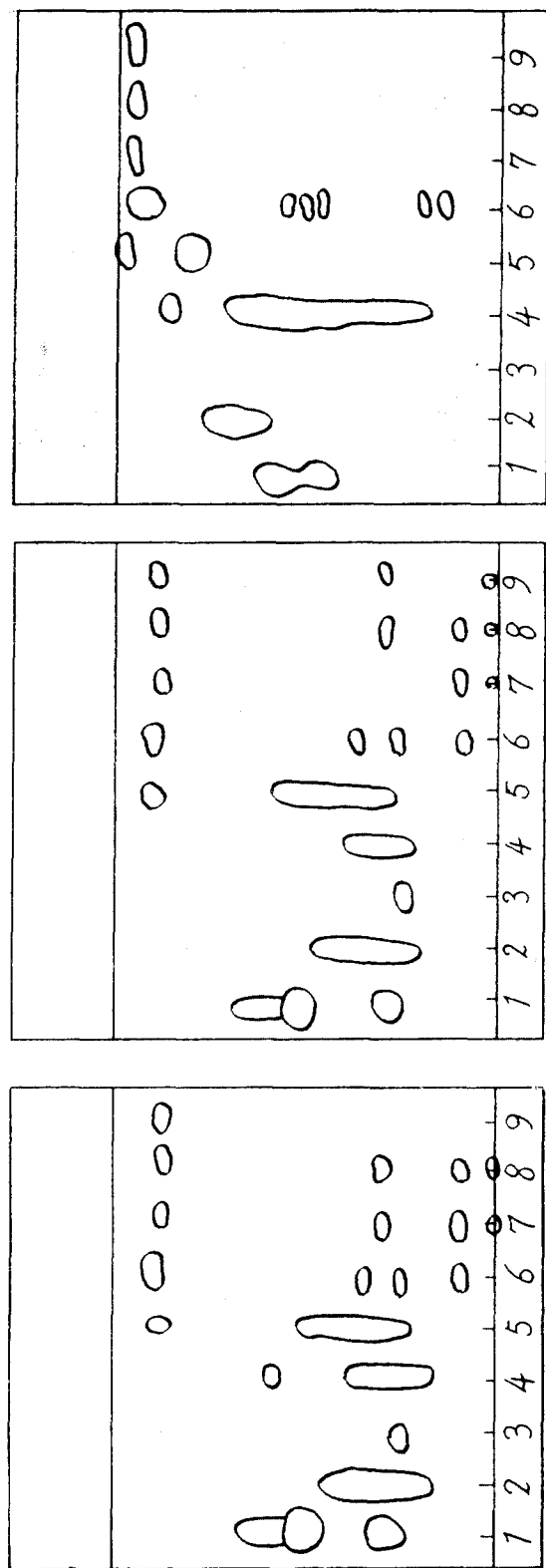
SR; spray reagent 2) Rhodamin B

3) Rhodamin B+ 0.5N-NaOH (solvent 1, 2, 3)

SOLV; solvent developed, (-); colorless, ⊕; tailing

Bux-2			Dithane stainless			Dithane M-45			Dithane M-22			Zineb Z-78		
UV	1	2	UV	1	2	UV	1	2	UV	1	2	UV	1	2
94-86	94-86	94-86	90 72-66 62-57	90 72-66 62-57	90 72-66 62-57	86 77 48 38	86 77 48 38	86	86 77 48 38	86 77 48 38	86	86 77 54 23	86 77 54 23	86
DpBv	DpB	D. R. Pu	D. G	L. R. Br	D. G	D. G	L. R. Br	D. G	D. G	LRBr	D. G	D. G	L. R. Br	D. G
92 80-12	92 89-12	92 89-12	95-81 69 63 36	95-81 69 63 36	95-81 69 63 36	96 94 82 68	96 94 82 68	96	96 94 89 82 68	96	96	94 17 12 8	89	94 89
Dp. Bv	DpB	D. G	Dp. Bv	L. R. Br	D. G	D. G	LRBr	D. G	D. G	LRBr	D. G	D. G	LRBr	D. G
17-6	17-6	17-6	96-92 12-0 ⊕	96-92 12-0	96-92 12-0	10	10	96	10	6	6	10	6	94
Dp. Bv	DpB	D. V	D. G	LRBr	D. G	D. G	LRBr	D. G	D. G	LRBr	D. G	D. G	LRBr	D. G
50-19	50-19	50-19	92-87 35-26 23-17 15-8⊕	92-87 35-26 23-17 15-8	92-87 35-26 23-17 15-8	92 31 12	92 31 12	92	92 31 12	31	92	92	31	92
Dp. Br	DpB	O. R	D. G	LRBr	D. Pu	D. G	LRBr	D. Pu	D. G	LRBr	D. Pu	D. G	LRBr	D. Pu
92-87 51-32	92-87 51-32	91-87 51-32	96-74 39-37 31-28 9-0	96-74 39-37 31-28 9-0	96-74 39-37 31-28 9-0	99-93 5-3	99-93 5-3	99	99-93 96-93 96-93 96-93	96-93 96-93 96-93 96-93	96-93 96-93 96-93 96-93	40	96-93	96-93
Dp. Bv	DpB	O. R	D. G	LRBr	DRBr	D. G	LRBr	D. Br	D. G	LRBr	D. Br	D. G	LRBr	D. Br
88 59-35	88 59-35	88 59-35	92-87 42-15	92-87	92-87	92 37 25 5	92 37 25 5	92	92 37 25 18	38	92	92	38	92
Dp. Bv	DpB	O. R	D. G	LRBr	DRBr	D. G	LRBr	D. Br	D. G	LRBr	D. Br	D. G	LRBr	D. Br
98 82-73	98	98	94-81 52 45 42	94-81 52 45 42	94-81 52 45 42	94 42 19	94 42 19	94	94 42 19	94 75	94	94	75	94
Dp. Bv	DpB	O. R	D. G	L. R. Br	D. R. Br	D. G	LRBr	D. Br	D. G	LRBr	D. Br	D. G	LRBr	D. Br
86 83-75	86	86	94-90 77 69-46 35	94-90 77 69-46 35	94-90 77 69-46 35	94 75 67 55	94 75 67 55	94	94 75 67 55	89	94	94	89	94
Dp. Bv	DpB	O. R	D. G	R. Br	DRBr	D. G	R. Br	D. Br	D. G	LRBr	D. Br	D. G	LRBr	D. Br
96-94 83-78	96-94	96-94	96-94 88-85 78-73 63-58	96-94 88-85 78-73 63-58	96-94 88-85 78-73 63-58	96-94 78-73 63-58 52-49	96-94 78-73 63-58 52-49	96-94	96-94 78-73 63-58 52-49	96-94 78-73 63-58 52-49	96-94 78-73 63-58 52-49	96-94 96-94 96-94 96-94	96-94 96-94 96-94 96-94	96-94 96-94 96-94 96-94
Dp. Bv	DpB	O. R	D. G	R. Br	D. R. Br	D. G	R. Br	D. Br	D. G	LRBr	D. Br	D. G	LRBr	D. Br
95-93 86-78	95-93	95-93	97-92 80-75 70-65 55-50	97-92 80-75 70-65 55-50	97-92 80-75 70-65 55-50	97-92 80-75 70-65 55-50	97-92 80-75 70-65 55-50	97-92	97-92 80-75 70-65 55-50	82	97-92	97-92 82 70-65 55-50	97-92 82 70-65 55-50	97-92
Dp. Bv	DpB	O. R	D. G	D. R. Br	D. R. Br	D. G	L. R. Br	L. Br	D. G	L. R. Br	D. Br	D. G	LRBr	D. Br

Fig. 2. Thin layer chromatogram of carbamate insecticides on silica gel F-254, plastic roll (200-micron)



solvent cyclohexane : acetone
developed; (80 : 20)

cyclohexane : acetone
(80 : 20)

ether : hexane
(80 : 20)

spray reagent; ultraviolet light
temperature; 27°C (Approx.)

2, 6-dibromoquinone-4-chlorimide
27°C (Approx.)

Rhodamin B (ultraviolet)
27°C (Approx.)

* compounds are: 1) Farbam; 2) Mipcin; 3) Bassa; 4) Sevin; 5) Bux 2; 6) Dithane stentless;
7) Dithane M-45; 8) Dithane M-23; 9) Zineb Z-78

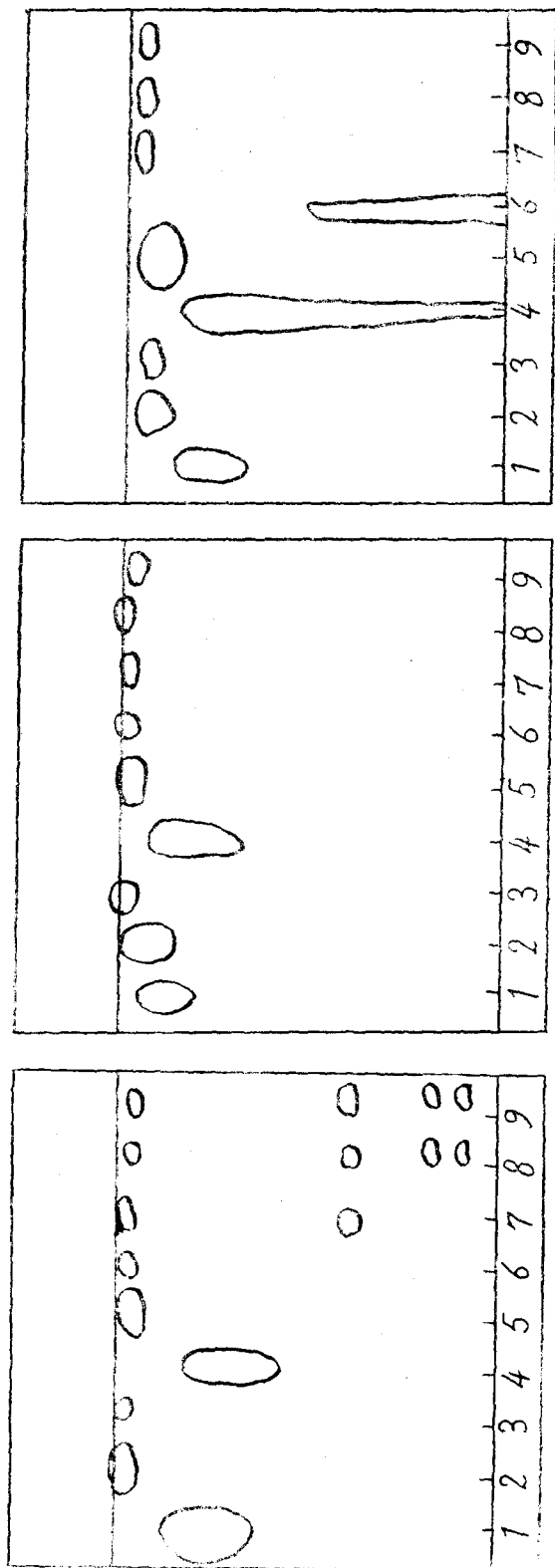
Table-3. Rf values and colorations of carbamate insecticides. (aluminium oxide 150 F-254, glass plate)

Solv	Item	CI	Farbam			Mipcin			Bassc			Sevin		
		SR	UV	1	2	UV	1	2	UV	1	2	UV	1	2
1	Rf		100-96	100-96	97-93	100-93	100-93	96-81	95	95	98-91	99-91	99-91	97-64
	CL		D. G	O. R	D. Br	G	L. B	G. Y	D. G	D. G	Bk	V	B. V	B
2	Rf		98-95 12-0	98-95	96 15Ⓢ	98-95	98-95	98-88	—	—	97-93	99-93	99-93	97-42
	CL		D. G	O. R	D. Br	G	L. B	G. Y	—	—	Bk	B. V	B. V	B
3	Rf		91-73 58-0	91-73 58-0	86-69	100-91	100-91	97-85	—	—	93	83-37	83-27	80Ⓢ
	CL		G	P	D. Br	K.G.B	B	G. Y	—	—	Bk	B. V	B. V	B
4	Rf		100-99 58-0	100-95 58-0	95	97-88	97-88	95-85	—	—	89	96-77	96-77	92-69
	CL		D. G	D. R	D. Br	G	LB	G. Y	—	—	Bk	DGB	B. V	B
5	Rf		89-62 50-0	89-62 50-0	81-59 0.21Ⓢ	100-93	100-93	95-80	—	—	95	75-54	75-54	74-03
	CL		G	O. R	D. Br	G	LB	G. Y	—	—	Bk	B. V	B. V	B
6	Rf		94-82 20-0	94-82 20-0	94-82 52Ⓢ	99-85	99-85	99-89	100-95	—	96	92-71	92-71	87-69
	CL		G	O. R	D. Br	G	LB	G. Y	DG	—	Bk	B. V	B. V	B
7	Rf		97-92 10-0	97-92	93-66 9-Ⓢ	99-93	99-93	99-83	—	—	96	99-90	99-90	93-37
	CL		DG	O. R	D. Br	G	L. B	G. Y	—	—	Bk	B. V	B. V	B
8	Rf		99-95	99-95	96-91	98-88	98-88	91-80	97-96	—	94	97-83	97-83	92-69
	CL		DG	O. R	D. Br	G	L. B	G. Y	DG	—	Bk	B. V	B. V	B
9	Rf		99-95	99-95	98	99-93	99-93	97	98-96	—	86	99-91	99-91	97-87
	CL		DG	OR	D. Br	G	L. B	G. Y	DG	—	Bk	B. V	B. V	B
10	Rf		99-92	99-92	97-91	96-77	96-77	92-81	98-95	—	97-85	95-82	95-82	92-69
	CL		DG	O. R	D. Br	G	L. B	G. Y	DG	—	Bk	B. V	B. V	B

CI; carbamate insecticides, CL; coloration
 SR; spray reagent 1) 2,6-Dibromoquinone-4-chlorimide
 2) Rhodamin B
 SOLV; solvent developed, (-); colorless, ⊕; tailing

Bux-2			Dithane stainless			Dithane M-45			Dithane M-22			Zineb Z-78		
UV	1	2	UV	1	2	UV	1	2	UV	1	2	UV	1	2
99-94	99-94	97-81	100-86 38 8	100-86 38 8	96-92 37⊕	99 95 37	99 95 37	96-92 92-88	98 95 31	98 95 31	96-92 92-88	99 96 37	99 96 37	96-93
G	B	Bk	DG	DRPu	G. Y	D. G	DPu	Bk	D. G	DPu	Bk	DG	DPu	Bk
99-95	99-95	91-79	99-95 11-0	99-95 11-0	97 7	95 74	95 74	98-95 66	99 94 92	99 94 92	98-95 66	98 45 45 10	98 45 45 10	97
G	B	Bk	DG	DRPu	G. Y	D. G	D. Pu	Bk	DG	DPu	Bk	DG	DPu	Bk
100-92	100-92	97-80	100-96 11-0	100-96 11-0	56⊕	100-95 20 3	100-95 20 3	93 17	100-99 21 2	100-99 21 2	93 17	98 21 8	98 21 8	93
LGB	B	Bk	DG	DRPu	G. Y	DG	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk
97-90	97-90	95-80	100-99 98-96	100-99 98-96	97 93 11	100-99 97 79 38	100-99 97 79 38	97 93	100-99 98 83 38	100-99 98 83 38	97 93	100-99 16 10	100-99 16 10	96
LGB	B	Bk	DG	DRPu	G. Y	DG	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk
97-91	97-91	98-80	100-99 43-16 16-11	100-99 43-16 16-11	51⊕	100-99 18 14	100-99 18 14	97 15	100-99 46 17 12	100-99 46 17 12	97 15	100-99 17 12	100-99 17 12	96
G	B	Bk	DG	DRPu	G. Y	D. G	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk
99-95	99-95	98-87	99-98	99-98	97 58⊕	99-98 42-32	99-98 42-32	96	99-98 40-35 22-20	99-98 40-35 22-20	96	99-98 42-37 24-21	99-98 42-37 24-21	96
LGB	B	Bk	DG	DRPu	G. Y	DG	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk
99-95	99-95	98-89	97-94 82	97-94 82	99-97 7⊕	99-98 83-79 44-42	99-98 83-79 44-42	99-97 94 33	99-98 83-80 47-42	99-98 83-80 47-42	97 94 33	99-96 49-45	99-96 49-45	96
LGB	B	Bk	DG	DRPu	G. Y	DG	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk
99-88	99-88	92-81	99-85 20-14	99-85 20-14	97-89 21	99-95	99-95	97	99-96 49-46	99-96 49-46	97 94	97-95 90-86 21-17	97-95 90-86 21-17	96
LGB	B	Bk	DG	DRPu	G. Y	DG	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk
99-92	99-92	97-87	99-92	99-92	97 53	99-96 94-92	99-96 94-92	97 89	99-96 95-92 94-81	99-96 95-92 94-81	97 89	99-98 96-92 55-50	99-98 96-92 55-50	97
LGB	B	Bk	DG	DRPu	G. Y	DG	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk
97-87	97-87	93-82	99-82	99-82	97 93 15⊕	99-97	99-97	97	99-97 86-82	99-97 86-82	97	99-98 87-82 20-14	99-98 87-82 20-14	97
LGB	B	Bk	DG	DRPu	G. Y	D. G	DPu	Bk	DG	DPu	Bk	DG	DPu	Bk

Fig. 3. Thin layer chromatogram of carbamate insecticides on aluminium oxide 150 F-25A (250-micron)



solvent hexane : acetone
developed; (70 : 30)

cyclohexane : acetone
(70 : 30)

chloroform : benzene:
carbon tetrachloride
(60 : 30 : 25)

spray reagent : ultraviolet light
temperature : 27°C (Approx.)

2,6-dibromoquinone-4-chlorimide
27°C (Approx.)

Rhodamin B (ultraviolet light)
27°C (Approx.)

* compounds are : 1) Farbam ; 2) Mipcin ; 3) Bassa ; 4) Sevin ; 5) Bux 2 ; 6) Dithane stenless ;
7) Dithane M-45 ; 8) Dithane M-22 ; 9) Zineb Z-78

blue-violet; BV	dark reddish-purple; DRPu
brown; Br	dark violet; DV
colorless; C	dark yellow; DY
dark black; DBK	deep blue; DpB
dark blue-violet; DBV	deep blue-violet; DpBV
dark brown; DBr	grey; G
dark grey; DG	greyish-blue; GB
dark greyish-blue; DGB	
greyish-brown; GBr	light yellow-brown, LYBr
greyish-yellow; GY	orange-red; OR
light blue; LB	pink; P
light brown; LBr	purple; Pu
light grey; LG	reddish-brown; RBr
light greyish-blue; LGB	sky blue; SB
light orange-yellow; LOY	violet; V
light reddish-brown; LRBr	yellow-brown; YBr

Results and Discussion

A) Observation on silica gel G-60, glass plate

Generally, 10-solvent systems were developed satisfactory on this plate, as shown in Table 1 and Fig. 1. The result observed on this plate are summarized as follows.

- 1) Sevin and Bux-2 showed blue-violet and blue fluorescence under ultraviolet light.
- 2) After spraying of 2,6-Dibromoquinone-4-chlorimide, light blue on upper part and light brown colorations on lower part were observed on Bassa and Dithane stenless.
- 3) Spot was not detected by examination under ultraviolet light on Bassa.
- 4) Colorations were observed for Mipcin, Bassa, Sevin and Bux-2 after spraying of Dragendorff's reagent, but these were disappeared immediately.
- 5) The satisfactory solvents for the silica gel G-60 plate were cyclohexane: acetone (70 : 30) and ether: hexane(80 : 20) and 2,6-Dibromoquinone-4-chlorimide was the most suitable spray reagent on this plate.

B) Observation on silica gel F-254, plastic roll

The results were achieved satisfactory light silica gel G-60, glass plate, as shown in Table 2 and Fig. 2. And summarized as follows.

- 1) Dithane M-22 and Zineb Z-78 showed blue coloration in the middle of chromatogram after spraying of 2,6-Dibromoquinone-4-chlorimide.
- 2) Spots were detected by examination under ultraviolet light for Dithane M-45, Dithane M-22 and Zineb Z-78. When the plate was sprayed by Rhodamin B observed the coloration only for upper spot.
- 3) Bassa was not colored by Rhodamin B.
- 4) Each insecticides showed different coloration between Rhodamin B and 0.5N-sodium hydroxide in methanol+Rhodamin under ultraviolet light on the solvent systems, ether: hexane (90 : 10), ethyl acetate: chloroform (60 : 40) and chloroform: benzene: carbon tetrachloride (60 : 30 : 25).
- 5) The satisfactory solvent systems on this plate were cyclohexane: acetone (80 : 20) and ether: hexane (80 : 20) also, 2,6-Dibromoquinone-4-chlorimide comparatively suitable reagent for silica gel F-254 plate.

C) Observation on aluminium oxide 150 F-254, glass plate

The result were not achieved satisfactory for this plate, as shown in Table 3 and Fig. 3.

- 1) Generally, Rhodamin B was suitable spray reagent under the ultraviolet light.
 - 2) Farbam and Sevin were easily detected when sprayed 2,6-Dibromoquinone-4-chlorimide on this plate.
 - 3) Spots were detected satisfactorily by examination under ultraviolet light for Farbam, Bassa, Dithane M-45, Dithane M-22 and Zineb Z-78.
 - 4) Chloroform: benzene: carbon tetrachloride (60 : 30 : 25) and cyclohexane: acetone (70 : 30) were comparatively satisfactory solvent and Rhodamin B was suitable spray reagent for this plate.
- D) Compare with foreign literature^{2,3,8,12)}

Some of the foreign literatures have suggested a Thin-layer chromatographic method of carbamate insecticides using 0.05% p-Nitrophenyldiazonium fluoborate in methanol as a spray reagent. Solvent systems used for development were; hexane: acetic acid(90 : 10), Rf-0.28 and hexane: acetic acid: chloroform(85 : 15:5), Rf-0.66. And also described, as little as 100mg of Sevin⁹⁾ is directly directly detectable, on Thin-layer chromatographic plate when sprayed with p-Nitrophenyldiazonium fluoborate.

However, the method suffers from the disadvantages of poor selectivity. Practically, this spray reagent can be measured only for the Sevin and the other carbamate insecticides were not visualized.

It is considered that the 2,6-Dibromoquinone-4-chlorimide is more suitable spray reagent for carbamates than thep-Nitrophenyl-diazonium fluoborate.

Result

Finally, observations on experiment are summarized as following:

- 1) The silica gel G-60, glass plate and silica gel F-254 were excellent for this experiment.
- 2) The satisfactory solvent systems for the above plates were chloroform: benzene: carbon tetrachloride (60 : 30 : 25), cyclohexane: acetone (80 : 20), cyclohexane: acetone (70 : 30) and ether: hexane (80 : 20).
- 3) The 2,6-Dibromoquinone-4-chlorimide and ultraviolet light were suitable as a spray reagent for detecting the carbamate insecticides.
- 4) Blue-violet and blue fluorescence were detected by examination under ultraviolet light for Sevin and Bux-2 on the silica gel G-60 plate.
- 5) It is considered that carbamate insecticides in samples containing various or in some biological materials can be determined without purification with the above method and applied to the forensic chemistry.

References

- 1) Soc. Anal. Chem., Anal. Public Nuisance. 1973
- 2) J. Fac. Pham. Ankata, 6, 102. 1976
- 3) J. Chromatographic Science. Vol. 13, 223. 1975
- 4) Agaure, R.J. Shimanukt, H and Al. Varoz C.C. : J. Agr. Food Chem. 18, 668. 1970
- 5) Argauer, R.J. and Webb, R.E. : J. Agr. Food Chem. 20, 732. 1972
- 6) Ishii. Y. : Bull., Agr. Chem. Insp. sta. Jap 10,51. 1970
- 7) Ott, D.E. Ittig, M. and Friestad, H. :J. Assoc. office Anal. Chem. 54, 160. 1971
- 8) Ishii and Yamashita : Bull. Agr. Chem. Inspect stn. No. 12, 63-70 1972
- 9) H. Ansan. Moyo : J. Agric Food Chem. Vol. 23 No. 3. 1975
- 10) Chang, P.S. : New Pestology, 274 1974
- 11) Ministry of Agr. and Fishery : Manual of Pesticides
- 12) National Research Institute of Police Science : Anal. Chem. Subs. 1975