

Effective Application of the Relative Retention Time Diagram for Gas Chromatographic Analysis of Pesticides

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To graphically grasp the behavior of pesticides among the liquid phases and to establish efficient operating conditions for gas chromatographic (GC) analysis of pesticides, the relative retention times (RRT) of 58 pesticides vs chlorpyrifos were determined on 8 liquid phases. An RRT diagram was prepared by plotting the RRT of each pesticide on the horizontal axis and the McReynolds constant on the vertical axis. By using chlorpyrifos as a reference compound, which has wide sensitivity for several GC detectors, the RRT diagram method was found to be a useful compass for GC analysis. These results demonstrated that the RRT diagram could be used to determine the optimal operating conditions for qualitative GC analysis of residual pesticides and to know their properties in liquid phase. Thus, the RRT diagram was found to be very effective tool for confirmation of each pesticide in GC analysis.

INTRODUCTION

In previous papers, we proposed an application of two relative retention time (RRT) diagrams for GC analysis of pesticides (Omura et al., 1988, 1990). One is the RRT diagram of organophosphorus pesticides with parathion as a reference compound for FPD-GC (Omura et al., 1988). The other is the RRT diagram of organochloric pesticides with aldrin as a reference compound for ECD-GC (Omura et al., 1990). Both are simple and efficient tools for qualitative analysis of well-known pesticides in Japan.

But these diagrams are not fully convenient for comparison of the data because of the different reference compounds used. In the analysis of the pesticides containing prothiophos, which is sensitive for ECD and FPD, an analyst needs to convert the RRT diagram data for ECD into those for FPD.

In this paper, we propose a new RRT diagram using a chlorpyrifos as a reference compound. As chlorpyrifos consists of Cl, P, S, N, C, and H atoms, it has wide sensitivity to several kinds of detectors such as flame ionization detector (FID), flame photometric detector (FPD), flame thermionic detector (FTD), and electron capture detector (ECD) of GC. This chemical is also used as a reference compound in other papers (Fehring and Walters, 1984; Saxton, 1987; *Pesticide Analytical Manual*, 1987).

In this experiment, we tried to prepare the RRT diagram for 58 pesticides by using 8 kinds of liquid phases and to generalize the diagram theory as a compass for a correlation between the RRTs of pesticides and the polarity of liquid phases. We also examined the application of the diagram for establishment of operating conditions in GC analysis.

MATERIALS AND METHODS

Apparatus and Reagents. (a) *Gas Chromatography.* Two gas chromatographic apparatus were used: a GC-263-70 (Hitachi, Tokyo) with FPD and a GC-14A (Shimadzu, Kyoto) with a

⁶³Ni ECD. Retention times and peak heights were calculated with a data processor (Chromatopac CR-4A, Shimadzu).

(b) *Pesticides.* Fifty-eight pesticides used are alphabetically listed in Table I according to their International Standards Organization (ISO) name (Tomizawa et al., 1989). Among them, 22 pesticides were measured by GC-FPD and 36 by GC-ECD. Twenty-one pesticides are regulated in usage by Japanese food sanitation laws.

All pesticide standards and solvents used were of analytical grade (purchased from Wako Pure Chemical Co., Osaka, and GL Sciences Co., Tokyo). A standard solution of each pesticide was prepared by dissolving it in acetone and/or hexane at 1000 µg/mL and then diluting to a predetermined working level (generally 25-75% full scale deflection).

Chlorpyrifos was used as a reference compound for comparison with the data in other papers (Fehring and Walters, 1984; Saxton, 1987; *Pesticide Analytical Manual*, 1987).

(c) *Chromatographic Columns.* The length and inside diameter of silanized columns were standardized to 2 m and 3 mm, respectively. Gas Chrom Q (80-100 mesh, GL Sciences Co.) coated with 2% liquid phase was used for the column material. The flow rate of nitrogen carrier gas was ca. 40-50 mL/min. The detector temperature was regulated at 250 °C for FPD and 300 °C for ECD. The column oven temperature was controlled at 198-237 °C (Table II).

(d) *Liquid Phases.* On the basis of the McReynolds constant (MC, $0 \leq MC < \sim 4500$) (McReynolds, 1970), eight liquid phases were classified into three groups with the part of 1000 MC units for the RRT diagram. OV-1, DC-550, and OV-17 were selected as nonpolar or low-polar liquid phases with $MC \leq 1000$. QF-1 and XE-60 were selected as medium-polar liquid phases ($1000 < MC \leq 2000$). PEG-20M and DEGS were utilized as high-polar liquid phases ($2000 < MC < 4500$). All liquid phases used are shown in Table II.

The retention time of chlorpyrifos as a standard substance in each liquid phase was adjusted to exactly 5.0 min. A gas chromatograph was operated by means of isothermal analysis to maintain the thermal stability of ECD and FPD and to determine the accuracy of retention times during operation. Other GC conditions were the same as those shown in previous papers (Omura et al., 1988, 1990).

Table I. Pesticides Used in the Experiment

no. ^a	compound ^{b,c}	no. of				MW	other names
		Cl	P	S	N		
1	aldrin	6				365	
2	α -BHC	6				291	α -HCH
3	β -BHC	6				291	β -HCH
4	γ -BHC	6				291	γ -HCH
5	δ -BHC	6				291	δ -HCH
6	captafol	4		1	1	349	Difolatan
7	captan	3		1	1	301	Orthocide
8	α -chlorfenvinphos	3	1			360	α -CVP, Vinylphate
9	β -chlorfenvinphos	3	1			360	β -CVP, Vinylphate
10	chlormitrofen	3			1	319	CNP, MO
11	chlorobenzilate	2				325	Akar
12	chlorothalonil	4			2	266	TPN, Daconil
13	chlorpyrifos	3	1	1	1	351	Dursban
14	chlorpyrifos-methyl	3	1	1	1	323	Reldan, Dowreldan
15	cyanofenphos*		1	1	1	303	CYP, Surecide
16	<i>o,p'</i> -DDD	4				320	
17	<i>p,p'</i> -DDD	4				320	
18	<i>o,p'</i> -DDE	4				318	
19	<i>p,p'</i> -DDE	4				318	
20	<i>o,p'</i> -DDT	5				354	
21	<i>p,p'</i> -DDT	5				354	
22	demeton-methyl*		1	2		230	Metasystox
23	diazinon*		1	1	2	304	
24	dichlobenil	2			1	172	DBN, Casoron
25	dichlorvos	2	1			221	DDVP, Vapona
26	dicofol	5				370	Kelthane
27	dieldrin	6				381	
28	dimethylvinphos	3	1			332	Rangard
29	dimethoate*		1	2	1	229	
30	disulfoton*		1	3		274	Disyston, Ethylthiometon
31	edifenphos*		1	2		310	EDDP, Hinosan
32	α -endosulfan	6		1		407	Malix, α -Benzoepin
33	β -endosulfan	6		1		407	Malix, β -Benzoepin
34	endrin	6				381	
35	EPN*		1	1	1	323	
36	ethion*		2	4		384	
37	fenitrothion*		1	1	1	277	MEP, Sumithion
38	fenthion*		1	2		279	MPP, Baycid
39	formothion*		1	2	1	257	Anthio
40	heptachlor	7				373	
41	iprofenfos*		1	1		288	IBP, Kitazin P
42	isoxathion*		1	1	1	313	Karphos
43	malathion*		1	2		330	Marathon
44	methidathion*		1	3	2	302	DMTP, Supracide
45	parathion*		1	1	1	291	
46	parathion-methyl*		1	1	1	263	
47	phenthoate*		1	2		320	PAP, Paphthion, Elsan
48	phosalone	1	1	2	1	368	Rubitox
49	phosmet*		1	2	1	317	PMP, Imidan
50	propaphos*		1	1		304	Kayaphos
51	prothiophos	2	1	2		345	Tokuthion
52	pyridaphenthion*		1	1	2	340	Ofunack
53	quintozene	5			1	295	PCNB
54	salithion*		1	1		216	Dioxabenzofos
55	tetrachlorophthalide	4				272	Fthalide, Rabcide
56	tetrachlorvinphos	4	1			366	CVMP, Gardicide
57	tetradifon	4		1		356	Tedion
58	thiobencarb	1		1		258	Benthiocarb, Saturn

^a Numbers used for identification in tables and figures. ^b An asterisk indicates pesticides measured by GC-FPD (others were by GC-ECD). ^c The name of the international standards organization.

Preparation of RRT Diagram. The RRTs of 58 pesticides vs chlorpyrifos were calculated. As shown in Table III, the RRTs of the 8 liquid phases were related in sequence on the basis of the RRTs on an OV-1 column. From these results, an RRT diagram was prepared by plotting RRTs on the horizontal axis and MC values on the vertical axis to examine the behavior of 58 pesticides.

RESULTS AND DISCUSSION

Determination of RRT. On the basis of use frequency and polarities, we selected eight typical liquid phases for the effective and practical confirmation of pesticides as

shown in Table II (Omura et al., 1979, 1988, 1990; Koda, 1984). In this experiment, the retention time of chlorpyrifos was adjusted to exactly 5.0 min in all liquid phases. The RRTs of other pesticides in eight liquid phases were then obtained with respect to chlorpyrifos (1 RRT). The separating efficiency was excellent for all pesticides in OV-1 liquid phase. So, the RRTs shown in Table III were sorted in the column of OV-1. In the columns filled with nonpolar or low-polar liquid phase, RRT values were analogous to each other. As the polarity of liquid phases increases, the RRTs of pesticides also increased and their peak tailing phenomena became more pronounced. Sev-

Table II. McReynolds Constant and Column Temperature in Liquid Phases Used

no.	liquid phase	McReynolds constant	column temp, °C
1	OV-1	222	205
2	DC-550	620	237
3	OV-17	884	226
4	QF-1	1500	201
5	XE-60	1785	198
6	PEG-20M	2308	228
7	DEGA	2764	201
8	DEGS	3543	210

eral pesticides have relatively large molecular weight, and the compounds with polar radicals in the molecules had large RRTs in high-polar liquid phases. These results seem to reflect a possible correlation between the polarity of liquid phase and the physical property of the pesticide.

The general sensitivity and separation degrees have been reported in our previous papers (Omura et al., 1988, 1990). The range of minimum detection limits was also from 0.01 to 0.1 ng in this experiment. High sensitivity and favorable separation were observed in the liquid phases of OV-1, DC-550, OV-17, QF-1, and XE-60 ($0 < MC \leq 2000$). The RRT distribution of most pesticides fell within 10-fold units compared to that of chlorpyrifos.

On the other hand, the high-polar liquid phases such as PEG-20M, DEGA, and DEGS ($2000 < MC$) often failed to separate the pesticides. In general, an increase of the polarity of the liquid phase resulted in a decrease of the detection limits. These pesticides are marked by (-) or (+) in Table III: (-) is assigned for the pesticides showing no noticeable peak, and (+) is assigned for the pesticides showing a decomposition peak. This phenomenon may be caused by thermal decomposition of the pesticides in the column and/or in the injection port, and by their sorption with the liquid phase. In addition, the pesticides having relatively large molecular weight or high polarity could not be analyzed in the columns packed with high-polar liquid phase because of the limited working temperature.

Comparison of RRT Data. The Food and Drug Administration (FDA) in the United States compiled the relative retention time data in its *Pesticide Analytical Manual* (1987) for the tentative identification of pesticides and industrial chemical residues. These data were determined isothermally at 200 °C on 6-f packed columns.

A study was undertaken to determine that the RRT diagram could be used for identification of chemical residue analysis. The comparison experiment was conducted between the FDA's RRT data and our RRT data. The FDA's data were described in four liquid phases such as OV-101, OV-17, OV-225, and DEGS. The MC value (229) and chemical structure of OV-101 are very similar to those of OV-1 ($MC = 222$). Among them, the data from three liquid phases were much the same as ours. The relationships between the RRT data of the FDA (Y) and our RRT data (X) in OV-1, OV-17, and DEGS were investigated and are shown in Table IV. The results adequately approximated each other. In the OV-1 column, the number of data points was 33, the correlation constant was 0.996, and the equation of the regression line was $Y = 1.05X - 0.07$. Both laboratory data had extremely high relationship in all three liquid phases.

In the literature, a linear relationship was observed between the column temperature and the relative retention time for aldrin for GC-ECD or parathion for GC-FPD (Thompson et al., 1975). Furthermore, the RRTs have been found to decrease with an increase of column temperature

in all compounds eluting later than the reference compound. Variations of RRTs were relatively small.

On the other hand, the majority of the FDA's retention time ratios obtained in packed columns can be used for the tentative identification of pesticides by using capillary columns at isothermal conditions for the four coatings tested in their papers (Fehring and Walters, 1984).

In general, the quality of the chromatogram and the retention time greatly depend on the chromatographic parameters such as isothermal or temperature-programming techniques and capillary or packed column usage. Fluctuations of RRT data were very few when a reference compound was used. From these findings, if the same liquid phase was used, the RRTs of pesticides would be very similar to each other. Thus, the RRT values in GC analysis might be reliable for constant values.

Preparation and Application of RRT Diagram. In GC analysis, it is very useful to know a relationship between the qualities of compounds and liquid phases. The RRT data from our experiment could be graphically represented and will show a possible correlation among pesticides, RRTs, and liquid phases.

The preparation method for the RRT diagram of 58 pesticides based on the RRTs is shown in Figure 1 for GC-FPD analysis with 31 pesticides and in Figure 2 for GC-ECD analysis with 36 pesticides. The set MC (McReynolds, 1970) indicates the polarity of liquid phase on the vertical axis. The next set of RRTs is plotted on the horizontal axis. Then, eight horizontal liquid-phase lines are drawn from nonpolar or low-polar OV-1 to high-polar DEGS. The RRTs of 58 pesticides are plotted on the liquid-phase lines, and the RRT points of each pesticide are connected on the liquid-phase lines to draw a zigzag curve. One curve is peculiar to one pesticide in this diagram. Therefore, the peculiarity is very useful to determine the pesticide.

By using this diagram, it is possible to demonstrate the identical behavior patterns among the liquid phases for given pesticides. These patterns can be broadly classified into three groups: In group A, with low polarity, containing aldrin and heptachlor, the RRTs tend to become small, deviating from that of chlorpyrifos due to an increase of the polarity of the liquid phase. In group B containing thiobencarb and prothiophos, the pesticides have polarity, chemical composition, and molecular weight similar to those of chlorpyrifos, and therefore the RRT behavior curve is almost parallel to that of chlorpyrifos. In group C containing chlorobenzilate and EPN, the RRTs tend to become extremely large in polar liquid phases. These pesticides have high polarity and relatively large molecular weight.

In general, it is necessary to use more than two liquid phases for identification of chemical compounds in GC analysis. Furthermore, usage of a combination of liquid phases whose elution sequence is reversed will give more reliable results. In our study of RRT diagrams, the identification efficiency is improved on liquid phases with 500 MC units apart or greater.

From the point of view of practical chromatography, it is believable that such liquid-phase combination would be a very useful means for identification and separation of residual pesticides. If the selection of two liquid phases from eight materials occurred, the RRT line could be contributed to identify the compound.

Except these eight liquid phases, if a new liquid phase for which the MC value is known is used, one can set the MC value into this diagram. Then, an approximate RRT of the new liquid phase will be obtained from the

Table III. Relative Retention Times of Pesticides to Chlorpyrifos in Eight Liquid Phases

no. ^a	compound	OV-1 ^b	DC-550	OV-17	QF-1	XE-60	PEG-20M	DEGA	DEGS
25	dichlorvos	0.21	0.22	0.15	0.25	0.20	0.19	0.20	0.22
24	dichlobenil	0.25	0.27	0.21	0.32	0.28	0.29	0.29	0.32
22	demeton-methyl	0.39	0.43	0.38	0.60	0.59	0.57	0.61	0.73
54	salithion	0.44	0.51	0.50	0.62	0.75	0.97	0.95	1.22
2	α -BHC	0.48	0.52	0.45	0.49	0.59	0.41	0.67	0.74
3	β -BHC	0.52	0.64	0.63	0.65	1.96	1.40	2.82	2.87
4	γ -BHC	0.55	0.60	0.57	0.58	0.80	+	0.98	1.09
5	δ -BHC	0.58	0.75	0.74	0.71	2.05	+	2.73	+
23	diazinon	0.58	0.56	0.50	0.52	0.47	0.45	0.41	0.41
53	quintozene	0.59	0.61	0.56	0.61	0.54	0.49	0.56	0.56
30	disulfoton	0.60	0.63	0.58	0.64	0.66	0.59	0.57	0.60
29	dimethoate	0.62	0.61	0.65	1.09	1.80	2.00	— ^d	3.17
12	chlorothalonil	0.66	+	0.79	1.35	1.65	+	+	+
41	iprofenfos	0.68	0.68	0.64	0.87	0.77	0.67	0.71	0.73
39	formothion	0.72	0.80	0.89	1.55	2.53	—	—	4.37
14	chlorpyrifos-methyl	0.77	0.81	0.81	0.82	0.89	1.02	1.03	1.12
46	parathion-methyl	0.79	0.85	0.88	1.55	1.81	—	2.20	2.78
40	heptachlor	0.83	0.78	0.69	0.66	0.61	0.65	0.59	0.62
37	fenitrothion	0.92	0.96	1.02	1.69	1.95	+	2.29	2.72
43	malathion	0.92	0.97	1.01	1.48	1.55	1.43	1.53	1.78
38	fenthion	0.97	1.06	1.17	1.11	1.39	1.74	1.82	1.99
58	thiobencarb	0.98	0.98	0.99	0.93	1.02	1.12	1.18	1.18
13	chlorpyrifos	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	aldrin	1.03	0.94	0.85	0.77	0.66	0.71	0.61	0.63
45	parathion	1.03	1.04	1.06	1.97	2.04	1.87	2.08	2.35
28	dimethylvinphos	1.09	+	1.17	1.58	+	1.97	+	+
7	captan	1.22	1.50	1.75	2.13	3.27	—	—	—
26	dicofol	1.25	1.07	1.10	1.22	1.33	1.65	1.82	1.84
47	phenthoate	1.26	1.41	1.59	1.57	1.94	2.14	+	2.49
55	tetrachlorophthalide	1.27	1.35	1.53	2.03	2.69	2.54	4.12	3.96
8	α -chlorfenvinphos	1.28	1.18	1.22	1.74	1.67	1.55	1.67	1.75
50	propaphos	1.36	1.46	1.62	2.39	2.45	2.08	2.34	2.49
44	methidathion	1.44	1.65	1.97	2.26	3.12	—	+	5.25
9	β -chlorfenvinphos	1.44	1.31	1.40	1.91	1.95	1.82	2.11	2.14
18	<i>o,p'</i> -DDE	1.47	1.42	1.45	1.09	1.26	1.58	1.48	1.46
32	α -endosulfan	1.52	1.48	1.48	1.53	1.46	1.52	1.66	1.78
56	tetrachlorvinphos	1.61	+	+	2.34	+	+	+	+
51	prothiophos	1.72	1.63	1.69	1.56	1.69	1.65	1.59	1.49
27	dieldrin	1.76	1.73	1.79	1.81	1.94	2.02	2.07	2.23
19	<i>p,p'</i> -DDE	1.79	1.66	1.72	1.37	1.61	1.97	1.94	1.85
42	isoxathion	1.93	2.13	2.48	3.14	4.09	+	+	+
34	endrin	1.96	2.03	2.18	2.10	2.18	2.20	2.38	2.52
33	β -endosulfan	2.01	2.28	2.55	2.53	3.96	3.93	5.11	5.38
11	chlorobenzilate	2.23	2.08	2.24	2.28	3.26	4.56	5.26	5.31
36	ethion	2.28	2.46	2.83	2.74	3.53	3.12	3.19	3.11
17	<i>p,p'</i> -DDD	2.28	2.23	2.50	2.15	3.84	4.19	1.99	5.17
16	<i>o,p'</i> -DDD	2.29	1.83	2.00	1.57	2.41	2.72	3.20	3.09
20	<i>o,p'</i> -DDT	2.31	2.19	2.42	1.72	2.11	2.60	3.10	3.00
10	chlornitrofen	2.83	2.68	3.07	3.83	4.45	5.23	6.72	6.70
21	<i>p,p'</i> -DDT	2.88	2.71	3.03	2.40	3.53	+	5.33	5.02
15	cyanofenphos	2.89	3.08	3.82	5.20	7.40	8.12	9.84	10.49
31	edifenphos	2.94	3.17	4.09	3.71	5.08	—	—	—
6	captafol	3.06	—	+	4.99	—	—	—	—
52	pyridaphenthion	3.96	4.56	6.11	8.46	12.29	15.26	23.44	18.12
49	phosmet	4.16	+	6.78	7.49	+	+	+	+
35	EPN	4.20	4.36	5.50	7.11	10.20	11.06	+	14.88
57	tetradifon	5.19	5.10	6.41	8.25	11.31	11.45	16.71	16.50
48	phosalone	5.46	5.69	7.06	9.42	15.72	—	—	20.02

^a Identified in Table I. ^b Listed in order of relative retention time on the OV-1. Absolute retention time for chlorpyrifos was adjusted to 5.0 min. ^c +, showed multiple peaks. ^d —, not determined.

Table IV. Correlation of Relative Retention Times between Our Data and the FDA's Data in Three Liquid Phases

liquid phase	coefficient correlation, ^b $Y = AX + B$			
	N^a	r	A	B
OV-1	33	0.996	1.05	-0.07
OV-17	32	0.999	1.30	-0.34
DEGS	13	0.997	1.36	-0.61

^a Number of pesticides used. ^b $Y = AX + B$, where Y is the FDA's data and X is our data.

intersections with the predetermined RRT curve of the two liquid phases. From this intersection, it is also possible to estimate the GC conditions of the objective compound.

Moreover, when other compounds except the 58 pesticides mentioned above are used, a plot of the obtained RRTs on the diagram produces a zigzag curve of the compound. Compare this with the RRT behavior of the other pesticides. By doing the comparison, it is possible to estimate the approximate properties of this compound.

The RRT diagram provides a convenient visual impression of the relationship among the separation efficiency, RRTs, and liquid phases. Gas chromatographic separation of adjacent peaks can be easily accomplished on the diagram. Also, the diagram not only is useful for screening the compound in GC analysis but also may be used to establish the operating conditions for GC-MS analysis that are essential to identify the compound.

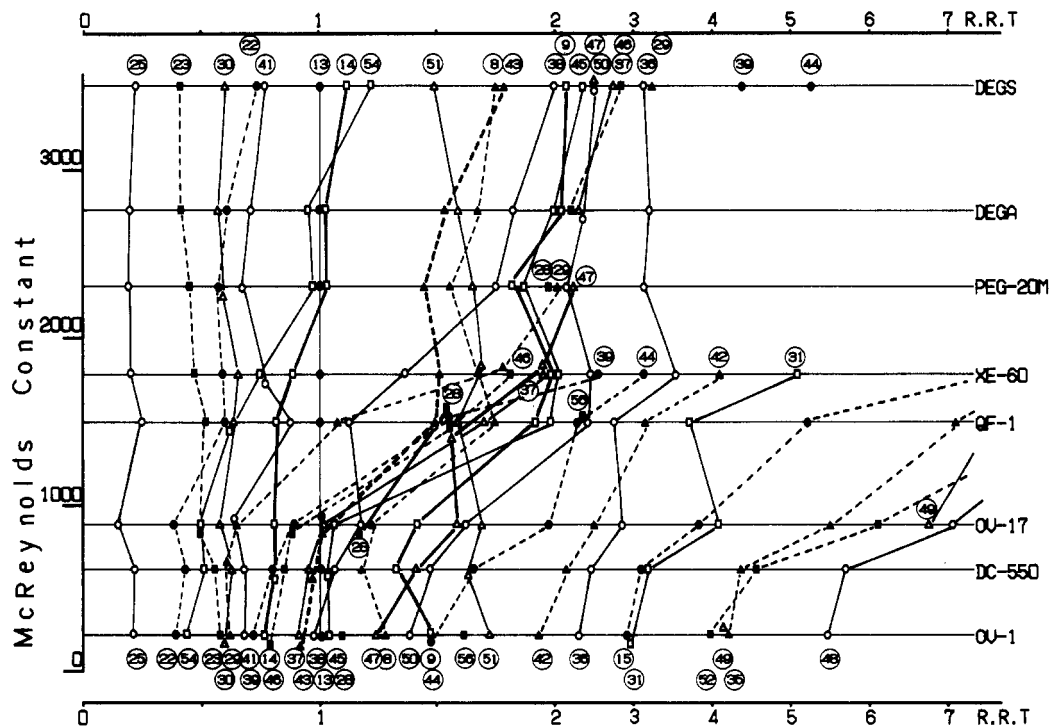


Figure 1. Relative retention time diagram 1 of FPD-sensitive pesticides. 8, α -chlorfenvinphos; 9, β -chlorfenvinphos; 13, chlorpyrifos; 14, chlorpyrifos-methyl; 15, cyanofenphos; 22, demeton-methyl; 23, diazinon; 25, dichlorvos; 28, dimethylvinphos; 29, dimethoate; 30, disulfoton; 31, edifenphos; 35, EPN; 36, ethion; 37, fenitrothion; 38, fenthion; 39, formothion; 41, iprofenfos; 42, isoxathion; 43, malathion; 44, methidathion; 45, parathion; 46, parathion-methyl; 47, phenthoate; 48, phosalone; 49, phosmet; 50, propaphos; 51, prothiophos; 52, pyridaphenthion; 54, salithion; 56, tetrachlorvinphos.

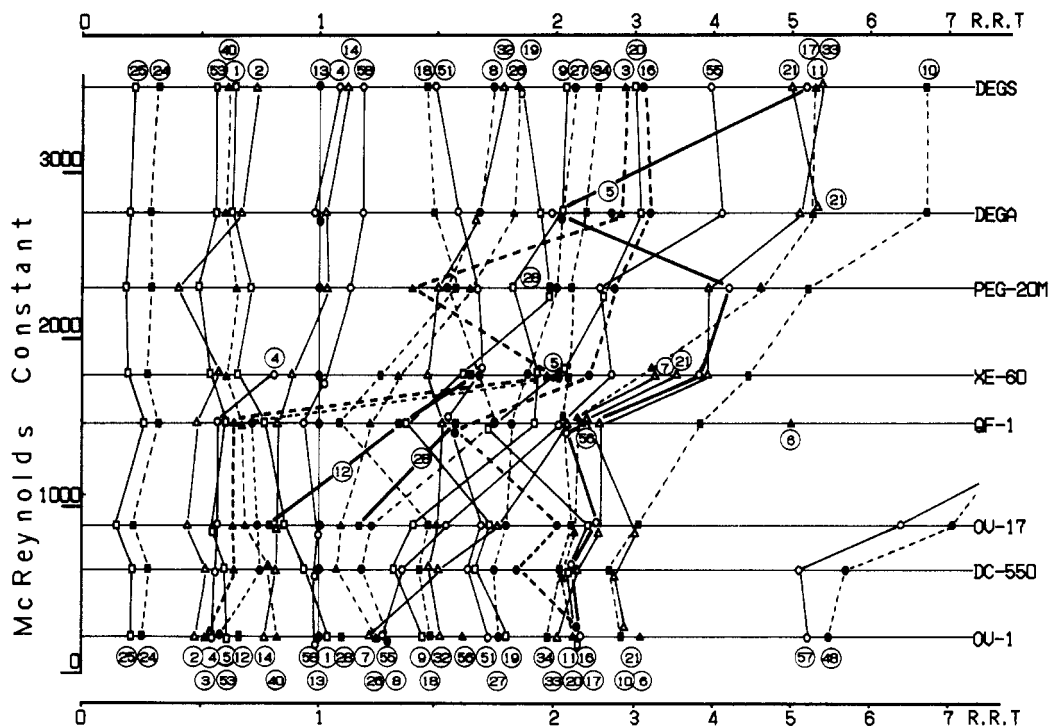


Figure 2. Relative retention time diagram 2 of ECD-sensitive pesticides. 1, aldrin; 2, α -BHC; 3, β -BHC; 4, γ -BHC; 5, δ -BHC; 6, captafol; 7, captan; 8, α -chlorfenvinphos; 9, β -chlorfenvinphos; 10, chlornitrofen; 11, chlorobenzilate; 12, chlorothalonil; 13, chlorpyrifos; 14, chlorpyrifos-methyl; 16, *o,p'*-DDD; 17, *p,p'*-DDD; 18, *o,p'*-DDE; 19, *p,p'*-DDE; 20, *o,p'*-DDT; 21, *p,p'*-DDT; 24, dichlobenil; 25, dichlorvos; 26, dicofol; 27, dieldrin; 28, dimethylvinphos; 32, α -endosulfan; 33, β -endosulfan; 34, endrin; 40, heptachlor; 48, phosalone; 51, prothiophos; 53, quintozene; 55, tetrachlorophthalide; 56, tetrachlorvinphos; 57, tetradifon; 58, tiobencarb.

Recently, a new RRT method was developed by Paoli et al. (1991). By using the system, 15 chlorinated pesticides have been identified on fused-silica capillary columns (Paoli et al., 1991). Simultaneous determination of organophosphorus pesticides was reported by FPD-GC with capillary columns (Tonogai et al., 1990). Also, most of the

RRT data for pesticides compiled using packed columns matched Fehring's data using capillary columns (Fehring and Walters, 1984), so our RRT data would be used with capillary gas chromatography. Therefore, the RRT diagram could be evaluated as an effective tool for GC analysis.

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