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CHROMATOGRAPHIC IDENTIFICATION OF PESTICIDES

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SUMMARY

The chromatographic properties of 51 common pesticides have been measured using seven different chromatographic systems involving gas–liquid chromatography (GLC), high-performance liquid chromatography (HPLC) with diode-array spectrophotometric detection and thin-layer chromatography (TLC) with different spray reagents. Correlation coefficients were calculated for combinations of all systems. The best combination of the chromatographic systems examined for the identification of an unknown compound is GLC on OV-17, HPLC on ODS-Hypersil with acetonitrile–water as eluent and TLC using an isooctane–ethyl acetate solvent system.

INTRODUCTION

An unavoidable consequence of the widespread use and availability of pesticides is the problem of accidental or intentional poisoning. While the annual number of deaths from pesticide poisoning is relatively small[1], accidental poisoning by pesticides or herbicides is a seasonal hazard faced by many agricultural workers. In addition, the availability of many proprietary garden chemicals provides a ready source of material for the would be criminal poisoner to adulterate food. Although few incidents of serious poisoning occur as a result of intentional food adulteration, mainly due to the unpleasant odours or presence of added colourants, a regular task of the forensic toxicologist involves the examination of foods, gastric contents and body tissues for the detection and identification of pesticides.

Because of the immense variety of chemical compounds possessing pesticidal properties, the identification of an "unknown" is complicated and although many analysis procedures have been published, the majority are directed at the identification of specific compounds or groups of compounds. Lawrence and Turton[2] reviewed the high-performance liquid chromatography (HPLC) literature for 166 pesticides and demonstrated the use of a wide range of column packing materials and mobile phases for the analysis of these compounds. Experience has shown that most HPLC

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separations may be achieved using either silica or octadecyl-silica, so the U.K. Forensic Science Laboratories have recently standardised on the use of two packing materials, Spherisorb S5W and ODS-Hypersil. Eluents have subsequently been developed for the separation of the major classes of pharmaceuticals encountered in forensic casework[3–7] using these standard phases.

In this paper we describe methods for the separation of 51 common pesticides using HPLC with Spherisorb S5W and ODS-Hypersil in combination with a rapid-scanning photodiode-array spectrophotometer. In order to assist with the confirmation of identify of pesticides, the retention indices of the 51 compounds have been measured on three gas–liquid chromatographic (GLC) stationary phases and $R_F \times 100$ values have been measured on two thin-layer chromatographic (TLC) systems.

EXPERIMENTAL

High-performance liquid chromatography

HPLC was performed with a Beckman 110A pump, a Rheodyne 7125 injection valve (fitted with a 20-μl loop) and a Hewlett-Packard HP1040A diode-array spectrophotometer. Reversed-phase HPLC of pesticides was performed using a 160 mm × 5 mm stainless-steel column (Shandon Southern Products, Runcorn, U.K.) packed with 5-μm ODS-Hypersil (Shandon Southern Products) by a slurry procedure. The packing material was dispersed in a small volume of isopropanol and hexane was used as the pressurising solvent. Optimum chromatographic separation was achieved with an acetonitrile–water mixture (60:40, v/v) at a flow-rate of 2 ml/min.

Separation of pesticides on Spherisorb S5W silica (Phase Separations, Queensferry, U.K.) was achieved using 250 mm × 5 mm columns (Shandon Southern Products) and an eluent comprising dichloromethane–isooctane (60:40, v/v) at a flow-rate of 2 ml/min. All HPLC eluents were prepared from HPLC-grade reagents and water and were degassed by ultrasonication prior to use. The diode-array spectrophotometer continuously collected spectra over the range 200–450 nm during each chromatographic separation.

Retention data are expressed as capacity factors, k', which are defined by $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the substance under investigation and a non-retained compound respectively.

Gas–liquid chromatography

GLC retention indices were measured using three low-polarity stationary phases: SE-30, OV-7 and OV-17. In each instance the stationary phase loading was 3% (w/w) on 80–100 mesh Chromosorb W HP (Perkin-Elmer, Beaconsfield, U.K.). Silanised glass columns (2 m × 4 mm) were used in each case. Oxygen-free nitrogen carrier gas was maintained at a constant flow-rate of 50 ml/min. The SE-30 column was housed in a Perkin-Elmer Sigma 3 gas chromatograph fitted with a flame ionisation detector. Chromatography on OV-7 and OV-17 was performed using Sigma 3B gas chromatographs and column effluent was split 50:50 to flame ionisation and nitrogen–phosphorus detectors. Straight-chain hydrocarbons were used for the calculation of retention indices[8].

Thin-layer chromatography

TLC was performed on glass plates precoated with silica gel 60 F254 (E. Merck; Art. No. 5714). Compounds were chromatographed using two independent solvent systems, *viz.* (a) dichloromethane and (b) ethylacetate–isooctane (15:85, v/v), respectively. Compounds were located by examination of the plates under ultraviolet radiation followed by further treatment with one of the two following spray systems[9]. Spray system 1 was a solution containing 0.7 g diphenylamine and 0.7 g zinc chloride in 100 ml of acetone. After spraying, plates were heated at 200°C until no further colour change was observed. A positive reaction was indicated by a coloured spot on a white background.

Visualisation system 2 required the chromatographically developed plates to be placed in a chamber containing bromine vapour for 1 min prior to sequential spraying with two solutions (A and B). Spray A comprised 1 ml of a 0.25% (w/v) solution of fluorescein in dimethylformamide diluted to 50 ml with ethanol. Spray B contained 1.7 g silver nitrate in 5 ml of water, 10 ml of phenyl cellosolve (2-phenoxyethanol) plus 185 ml of acetone. Yellow spots on a pink background were obtained which, after exposure to ultraviolet light for 10 min, produced yellow spots on a black background. $R_F \times 100$ values were recorded.

RESULTS AND DISCUSSION

The compounds examined during this work were classified into six groups according to their uses and chemical properties, *viz.* organophosphate insecticides, organochlorinated insecticides, carbamate insecticides, botanical insecticides, herbicides and fungicides. A list of capacity factors measured on both HPLC systems is presented in Table I together with the ultraviolet absorption maxima recorded by the diode-array spectrophotometer in each of the different mobile phases. Capacity factors listed as 99.99 have been used to indicate compounds which were retained or were insoluble in the mobile phase. The transparency of the acetonitrile–water mobile phase as low as 200 nm enabled full ultraviolet spectra to be measured. Because of the high cut-off of the isooctane–dichloromethane mobile phase (230 nm) compounds which absorbed at wavelengths below 230 nm were not detectable. The rapid-scanning photodiode-array spectrophotometer offers a wide range of options to aid in peak identification without the requirement of interrupting solvent flow and encountering all of the frustrations inherent to stopped flow procedures.

Simultaneous multiwavelength scanning of HPLC eluents reduces the chance of missing eluting compounds whilst the facility to scan rapidly and to superimpose spectra taken on the up-slope, apex and down-slope of an eluting peak provides an indication of peak purity.

A list of GLC retention indices on SE-30, OV-7 and OV-17 is presented as Table II. Paraquat, diquat and glyphosate were not examined by GLC owing to their water-solubility. Of the remaining 48 compounds asulam, chlordane, dalapon, dicamba and trichlorphon did not elute from the columns under the conditions used.

A list of $R_F \times 100$ values for the 51 compounds in two TLC systems is presented on Table III together with the colours obtained after visualisation with the two spray reagent systems. The spray reagents exhibited a degree of selectivity. The diphenylamine spray (spray 1) reacted with organochlorine insecticides and was nega-

TABLE I

HPLC CAPACITY FACTORS AND ULTRAVIOLET ABSORPTION DATA FOR PESTICIDES IN ALPHABETICAL ORDER

$HPLC_1$ = Spherisorb S5W with dichloromethane–isooctane (60:40, v/v); $HPLC_2$ = ODS-Hypersil with acetonitrile–water (60:40, v/v). UV_1 and UV_2 = ultraviolet spectrum maxima in HPLC systems 1 and 2, respectively. Classes: 1 = organophosphorus insecticide; 2 = organochlorine insecticide; 3 = carbamate insecticide; 4 = botanical insecticide; 5 = herbicide; 6 = fungicide. M = Maximum absorbance; MP = multiple peaks. 2,4-D = (2,4-Dichlorophenoxy)acetic acid; 2,4,5-T = (2,4,5-Trichlorophenoxy)-acetic acid.

Pesticide	*k'*		*Absorption maxima (nm)*		
	$HPLC_1$	*$HPLC_2$*	*UV_1*	*UV_2*	*Class*
Aldicarb	7.63	0.69	250	249	3
Aldrin	0.00	33.86	No UV	212	2
Asulam	99.99	0.00	Insoluble	246 + 268	5
Atrazine	10.75	1.24	263	222 + 265	5
Barban	0.42	2.56	275 + 285	239M + 277 + 284	5
Bromoxynil octanoate	0.19	31.65	282 + 292	211M + 290	5
Captan	0.54	99.99	249	Insoluble	6
Carbaryl	2.39	0.97	270 + 280 + 290	221M + 270 + 279	3
Carbofuran	5.93	0.87	279	225 + 279	3
Carbophenothion	0.00	15.26	262	226 + 265	1
Chlorbromuron	1.35	2.05	247 + 285 + 295	212 + 248 + 289	5
Chlordane	0.00	99.99	No UV	MP in $HPLC_2$	2
Chlorpropham	0.41	2.68	278 + 285	237M + 278 + 286	5
Chlorpyrifos methyl	0.00	6.18	289	230 + 289	1
2,4-D	99.99	0.00	Insoluble	230 + 283 + 290	5
Dalapon	99.99	0.00	Insoluble	No UV	5
Dazomet	2.19	0.47	285M + 245	211 + 245 + 283M	6
DDT (O–P)	0.00	31.72	279	235 + 275	2
DDT (P–P)	0.00	29.20	279	235 + 275	2
Diazinon	2.13	4.92	249	248 + 288	1
Dicamba	99.99	0.00	Retained	205 + 230 + 278	5
Dichloroprop	5.84	0.00	280	231 + 285 + 292	5
Dichlorovos	5.22	0.86	No UV	No UV	1
Dieldrin	0.11	12.04	No UV	217	2
Dimethoate	0.81	0.46	No UV	No UV	1
Dinoseb	0.17	0.79	270 + 350	263 + 371 + 416	5
Diquat dibromide	99.99	0.00	Insoluble	257	5
Disulfoton	0.10	6.48	No UV	No UV	1
Endrin	0.20	11.24	No UV	224	2
Fenitrothion	0.22	2.86	268	268	1
Flampropisopropyl	4.29	5.71	274	245 + 275	5
Gamma-BHC	0.00	0.00	NoUV	No UV	2
Glyphosate acid	99.99	0.00	Insoluble	257	5
Heptachlor	0.00	20.83	No UV	No UV	2
Iodofenphos	0.00	11.45	283 + 292	213M + 238 + 285	1
Malathion	0.00	0.00	–	–	1
MCPA	0.78	0.00	280	230M + 278 + 285	5
MCPB	0.47	0.00	280	230M + 280 + 288	5
Mecoprop	7.67	0.00	280 + 285	230M + 280 + 288	5
Methidathion	0.81	1.84	No UV	216	1
Methiocarb	1.93	1.63	265	225 + 265	3
Methomyl	12.64	0.34	No UV	232	3
Mevinphos	1.37	0.45	No UV	218	1
Paraquat dichloride	99.99	0.00	Insoluble	257	5
Permethrin	0.00	51.96	272	275	4
Phosalone	0.35	5.94	235 + 283	235M + 283	1
Phosphamidon	0.97	0.44	No UV	216	1
Pirimicarb	99.99	1.13	Retained	246 + 311	3
2,4,5-T	99.99	0.00	Insoluble	232 + 288 + 295	5
Terbutryne	12.09	1.68	265	227M + 267	5
Trichlorphon	99.99	99.99	Retained	Retained	1

TABLE II

ALPHABETICAL LIST OF PESTICIDE RETENTION INDICES ON SE-30, OV-7 AND OV-17

Classes: 1 = organophosphorus insecticide; 2 = organochlorine insecticide; 3 = carbamate insecticide; 4 = botanical insecticide; 5 = herbicide; 6 = fungicide. 9999 represents compounds which did not elute.

Pesticide	*SE-30*	*OV-7*	*OV-17*	*Class*
Aldicarb	866	966	1088	3
Aldrin	2008	2096	2226	2
Asulam	9999	9999	9999	5
Atrazine	1710	1885	2048	5
Barban	1102	1174	1282	5
Bromoxynil octanoate	2316	2472	2620	5
Captan	2030	2281	2490	6
Carbaryl	1494	1706	1816	3
Carbofuran	1294	1390	1498	3
Carbophenothion	2310	2494	2708	1
Chlorbromuron	1408	1510	1610	5
Chlordane	9999	9999	9999	2
Chlorpropham	1626	1748	1888	5
Chlorpyrifos methyl	1866	2060	2234	1
2,4-D	1766	1954	2046	5
Dalapon	9999	9999	9999	5
Dazomet	1676	1902	2200	6
DDT (O–P)	2242	2428	2624	2
DDT (P–P)	2330	2502	2692	2
Diazinon	1769	1899	2032	1
Dicamba	9999	9999	9999	5
Dichloroprop	1756	1982	2055	5
Dichlorvos	1220	1334	1454	1
Dieldrin	2170	2304	2528	2
Dimethoate	1690	1888	2120	1
Dinoseb	1802	1926	2068	5
Diquat dibromide	–	–	–	5
Disulfoton	1776	1906	2080	1
Endrin	2236	2356	2600	2
Fenitrothion	1944	2112	2278	1
Flampropisopropyl	2228	2412	2588	5
Gamma-BHC	1728	1840	2088	2
Glyphosate acid	–	–	–	5
Heptachlor	1968	2068	2188	2
Iodofenphos	2150	2342	2564	1
Malathion	1917	2107	2287	1
MCPA	1672	1808	1962	5
MCPB	1820	1970	2262	5
Mecoprop	1740	1862	1962	5
Methidathion	2092	2268	2475	1
Methiocarb	1530	1660	1780	3
Methomyl	1059	1150	1266	3
Mevinphos	1394	1532	1680	1
Paraquat dichloride	–	–	–	5
Permethrin	2634	2836	3056	4
Phosalone	2488	2736	2960	1
Phosphamidon	1850	2016	2212	1
Pirimicarb	1816	1984	2164	3
2,4,5-T	1766	2110	2302	5
Terbutryne	1906	2064	2246	5
Trichlorphon	9999	9999	9999	1

TABLE III

TLC $R_F \times 100$ VALUES FOR COMMON PESTICIDES

TLC_1 = dichloromethane; TLC_2 = Isooctane–ethyl acetate (15:85, v/v). Compound classification: 1 = organophosphate insecticide; 2 = organochlorine insecticide; 3 = carbamate insecticide; 4 = botanical insecticide; 5 = herbicide; 6 = fungicide. Colours: LB = light blue; DB = dark brown; B = brown; Bl = black; Y = yellow; G = green; W = weak reaction to spray 2; +VE = positive; −VE = negative.

Pesticide	*R_F = 100*		*Colour*		*Class*
	TLC_1	*TLC_2*	*Spray 1*	*Spray 2*	
Aldicarb	4	3	+VE LB	+VE	3
Aldrin	67	52	+VE LB	−VE	2
Asulam	1	0	+VE LB	+VE	5
Atrazine	4	8	−VE	W	5
Barban	39	16	+VE LB	W	5
Bromoxynil octanoate	47	39	−VE	−VE	5
Captan	25	5	+VE DB	−VE	6
Carbaryl	12	4	+VE B	+VE	3
Carbofuran	5	4	−VE	+VE	3
Carbophenothion	58	37	+VE LB	+VE	1
Chlorbromuron	20	8	+VE G	W	5
Chlordane	64	44	+VE Bl	−VE	2
Chlorpropham	42	29	−VE	+VE	5
Chlorpyrifos methyl	60	35	−VE	+VE	1
2,4-D	2	3	−VE	−VE	5
Dalapon	0	0	+VE LB	−VE	5
Dazomet	13	0	+VE Y	+VE	6
DDT (O–P)	65	42	+VE DB	−VE	2
DDT (P–P)	67	45	+VE DB	−VE	2
Diazinon	30	20	−VE	+VE	1
Dicamba	4	5	−VE	−VE	5
Dichloroprop	3	4	−VE	−VE	5
Dichlorvos	0	0	+VE DB	−VE	1
Dieldrin	51	27	+VE DB	−VE	2
Dimethoate	3	1	−VE	+VE	1
Dinoseb	51	18	+VE Y	+VE	5
Diquat dibromide	0	0	+VE DB	+VE	5
Disulfoton	52	35	−VE	+VE	1
Endrin	55	35	+VE DB	−VE	2
Fenitrothion	50	17	−VE	+VE	1
Flampropisopropyl	9	10	−VE	−VE	5
Gamma-BHC	0	0			2
Glyphosate acid	0	0	+VE DB	+VE	5
Heptachlor	69	51	+VE DB	−VE	2
Iodofenphos	62	31	−VE	+VE	1
Malathion	24	14	−VE	+VE	1
MCPA	4	2	−VE	−VE	5
MCPB	6	2	−VE	−VE	5
Mecoprop	3	4	−VE	W	5
Methidathion	25	13	+VE LB	+VE	1
Methiocarb	14	5	−VE	+VE	3
Methomyl	4	1	−VE	W	3
Mevinphos	3	2	−VE	W	1
Paraquat dichloride	0	0	+VE LB	+VE	5
Permethrin	63	40	+VE G	W	4
Permethrin	58	37	+VE G	W	4
Phosalone	39	17	+VE DB	+VE	1
Phosphamidon	4	2	−VE	W	1
Pirimicarb	6	5	−VE	+VE	3
2,4,5-T	4	2	−VE	−VE	5
Terbutryne	7	12	−VE	−VE	5
Trichlorphon	2	1	−VE	−VE	1

TABLE IV

SPEARMAN'S RANK CORRELATION COEFFICIENTS

	SE-30	*OV-7*	*OV-17*	*$HPLC_1$*	*$HPLC_2$*	*TLC_1*	*TLC_2*
SE-30	1.00	0.9798	0.9602	−0.6389	0.6734	0.6732	0.7058
OV-7		1.000	0.9827	−0.5780	0.6144	0.6125	0.6324
OV-17			1.000	−0.5829	0.5800	0.5864	0.5711
$HPLC_1$				1.000	−0.6664	−0.8584	−0.7759
$HPLC_2$					1.000	0.8404	0.8341
TLC_1						1.000	0.9179
TLC_2							1.000

tive with respect to most carbamate and organophosphate compounds. Spray 2 was negative for all of the organochlorine compounds and positive for most organophosphate and carbamate insecticides.

Spearman's correlation coefficients were calculated for combinations of the different systems (GLC, HPLC and TLC) and are presented as Table IV. The three non-polar GLC stationary phases were, as anticipated, closely correlated and would be unsuitable to use in combination for the unequivocable identification of unknown compounds. The two TLC systems were also closely correlated with one another although the additional use of group-specific spot-locating reagents would provide an added useful criterion for aiding identification of an unknown compound. Analysts attempting the identification of an unknown pesticide by chromatographic methods should therefore ideally use a combination of methods based on different physical and/or chemical parameters and which should not be closely correlated. Of the systems examined, a combination of GLC using OV-17, HPLC on ODS-Hypersil with acetonitrile–water mobile phase and TLC using an isooctane–ethyl acetate solvent system provides the greatest degree of discrimination.

REFERENCES

1 M. D. Osselton, R. C. Blackmore, L. A. King and A. C. Moffat, *Hum. Toxicol.*, 3 (1984) 201.
2 J. F. Lawrence and D. Turton, *J. Chromatogr.*, 159 (1978) 207.
3 R. Gill, A. H. Stead and A. C. Moffat, *J. Chromatogr.*, 204 (1981) 275.
4 R. Gill, S. P. Alexander and A. C. Moffat, *J. Chromatogr.*, 218 (1981) 639.
5 R. Gill, R. W. Abbott and A. C. Moffat, *J. Chromatogr.*, 301 (1984) 155.
6 B. Law, R. Gill and A. C. Moffat, *J. Chromatogr.*, 301 (1984) 165.
7 R. Gill and J. A. Key, *J. Chromatogr.*, 346 (1985) 423.
8 E. Kovats, *Helv. Chim. Acta*, 41 (1958) 195.
9 E. Stahl (Editor), *Thin Layer Chromatography*, Springer, Berlin, Heidelberg, New York, 2nd ed., 1969, p. 642.