CHROM. 4218

GAS–LIQUID AND THIN-LAYER CHROMATOGRAPHY OF PHORATE, DISULFOTON AND FIVE OF THEIR OXIDATION PRODUCTS

D. L. GRANT, C. R. SHERWOOD AND K. A. McCULLY
Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa 3 (Canada)

(Received June 9th, 1969)

SUMMARY

Phorate, phorate sulfoxide, phorate sulfone, phoratoxon, phoratoxon sulfoxide, phoratoxon sulfone, disulfoton, disulfoton sulfoxide, disulfoton sulfone, disulfoton oxygen analog, disulfoton oxygen analog sulfoxide and disulfoton oxygen analog sulfone were resolved by gas–liquid chromatography with temperature programming on a column containing Chromosorb W AW-DMCS coated with 5% stabilized DEGS. The limit of detection of the organophosphorus compounds after thin-layer chromatography was determined for eleven detection methods. An enzyme inhibition method with 5-bromoindoxyl acetate as substrate was found to be the most sensitive method. Bromocresol Green–$AgNO_3$ reagent detected all the compounds at the sub-microgram level. Sixteen mobile phases and six sorbents were compared for the TLC resolution of the organophosphorus compounds. The mobile phase methanol–benzene (10:90) with the sorbent MN-Kieselgel G-HR gave the best resolution of phorate, disulfoton and their oxidation products.

INTRODUCTION

A large number of column supports and stationary phases have been used for the gas–liquid chromatographic (GLC) analyses of organophosphorus pesticides and their metabolites[1–11]. McLEOD *et al.*[11] separated phorate and five of its metabolites with a column of 5% DEGS on 80–100 mesh HMDS-treated Chromosorb W. An oven temperature of 150° was used for the phorate and phoratoxon separation and 195° for the phorate sulfoxide, phorate sulfone, phoratoxon sulfoxide and phoratoxon sulfone separation.

Many mobile phases and sorbents have been evaluated for the thin-layer chromatographic (TLC) resolution of organophosphorus pesticides[12–20]. BLINN[17] resolved phorate and five of its oxidation products on thin-layer plates with a 1.75% methanol in chloroform mobile phase. MENZER AND DITMAN[19] employed a 2.5% methanol in chloroform mobile phase with Silica Gel G for the resolution of disulfoton, phorate and five of their oxidation products.

Many reagents have been used for the detection of organophosphorus compounds on paper and thin-layer chromatograms. WATTS[21] recently published an extensive review on the chromogenic spray reagents for organophosphorus pesticides.

A comprehensive study on the GLC and TLC of phorate, phoratoxon, phorate sulfoxide, phorate sulfone, phoratoxon sulfoxide, phoratoxon sulfone, disulfoton, disulfoton oxygen analog, disulfoton sulfoxide, disulfoton sulfone, disulfoton oxygen analog sulfoxide and disulfoton oxygen analog sulfone is reported here.

EXPERIMENTAL

Organophosphorus compounds

Phorate, phoratoxon, phorate sulfoxide, phorate sulfone, phoratoxon sulfoxide and phoratoxon sulfone were obtained from American Cyanamid Company. Disulfoton, disulfoton oxygen analog, disulfoton sulfoxide, disulfoton sulfone, disulfoton oxygen analog sulfoxide and disulfoton oxygen analog sulfone were obtained from Chemagro Corporation. The phorate and disulfoton were analytical grade, 97.8 and 96.8%, respectively, while their oxidation products were technical grade. Phorate and disulfoton were dissolved in hexane while the oxidation products were dissolved in acetone. All standards were diluted with hexane to give appropriate concentrations for GLC and TLC analyses.

Gas–liquid chromatography

A Varian Aerograph 2100 gas chromatograph fitted with a phosphorus detector was operated as follows: (1) 55 cm × 2 mm bore capillary U-shaped glass column containing Chromosorb W (80–100 mesh) coated with DC-200 and QF-1 (0.4 g of DC-200 and 0.6 g of QF-1 per 10 g of Chromosorb W); the nitrogen, hydrogen and compressed air flow rates were 20, 14 and 170 ml per min, respectively; the injector, column and detector temperatures were 200, 190 and 210°, respectively. (2) 110 cm × 1 mm bore capillary U-shaped glass column containing Chromosorb W AW-DMCS (high performance, 80–100 mesh) coated with 5% (w/w) stabilized DEGS; the nitrogen, hydrogen and compressed air flow rates were 20, 14 and 170 ml per min, respectively; for phorate and its oxidation products, the injector and detector temperatures were 190° and the column temperature was programmed at 2° per min from a starting temperature of 162° to 182° and then isothermal at 182°, for disulfoton and its metabolites the injector and detector temperatures were 195° and the column temperature was programmed at 1° per min from a starting temperature of 173° to 193° and then isothermal at 193°. The column supports were coated according to the method of MENDOZA *et al.*[22].

Thin-layer chromatography

The following sorbents and water were used to prepare the thin-layer plates: MN-Kieselgel G-HR (1:2, w/v), Silica Gel G (1:2, w/v), SilicAR TLC-7 (1:2, w/v), Aluminum Oxide G (1:2, w/v), Adsorbosil-M-2 (1:2.25, w/v), and Silica Gel H (1:2.42, w/v). The sorbents were shaken with the required amount of water and spread, 400 μ or 250 μ thick, with a Desaga applicator on acetone-rinsed glass plates (20.5 cm × 20.5 cm). Silica Gel G thin-layer plates were also prepared with pH 6 buffer[17]. The freshly coated plates were allowed to stand at room temperature for the following

TABLE I

TLC MOBILE PHASES

No.	*Composition*	*No.*	*Composition*
1	Chloroform	9	Methanol–benzene (5.0:95.0)
2	Methanol–chloroform (1.0:99.0)	10	Methanol–benzene (7.5:92.5)
3	Methanol–chloroform (1.5:98.5)	11	Methanol–benzene (10.0:90.0)
4	Methanol–chloroform (2.0:98.0)	12	Methanol–benzene (15.0:85.0)
5	Methanol–chloroform (2.5:97.5)	13	Acetone–hexane (20.0:80.0)
6	Methanol–chloroform (3.0:97.0)	14	Acetone–hexane (25.0:75.0)
7	Methanol–chloroform (4.0:96.0)	15	Cyclohexane–acetone–chloroform (70.0:25.0:5.0)
8	Methanol–chloroform (5.0:95.0)	16	Acetone–benzene (15.0:85.0)

periods: MN-Kieselgel G-HR, 10 min; Silica Gel G, 45 min; SilicAR TLC-7, 10 min; Aluminum Oxide G, 10 min; Adsorbosil-M-2, 30 min, and Silica Gel H, 45 min. The plates were then placed in a vertical position in an oven at 110° for 1 h. The mobile phases (Table I), prepared from glass distilled solvents, were placed in the glass chambers (Arthur H. Thomas Co.) 10 min or 1 h, in the case of mobile phases containing methanol, before developing the plates. Filter paper liners were placed in the chambers, when methanol was a constituent of the mobile phase. The plates were developed at room temperature. The organophosphorus compounds were applied 1.5 cm from the bottom of the thin-layer plate and developed until the mobile phase had reached a line drawn at a predetermined distance, usually 15 cm, from the starting point. The organophosphorus compounds were detected on the thin-layer plates by one of the methods shown in Table II. The reproducibility of the hR_F values[23], effect of thickness of sorbent and length of solvent travel on resolution of the organophosphorus compounds were studied.

RESULTS AND DISCUSSION

GLC tracings obtained with the organophosphorus compounds are shown in Figs. 1 and 2. Phorate, disulfoton and their five oxidation products were resolved

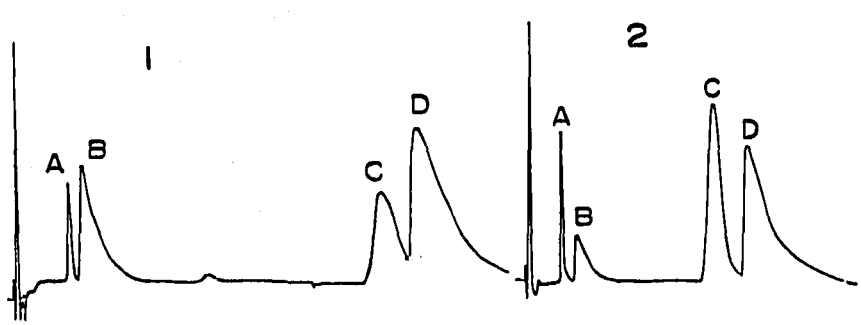

Fig. 1. GLC tracings obtained from the chromatography of disulfoton, phorate and some of their oxidation products on Chromosorb W coated with DC-200 and QF-1. Tracing 1: (A) 2×10^{-3} μg disulfoton, (B) 4×10^{-3} μg disulfoton oxygen analog, (C) 1.8×10^{-2} μg disulfoton sulfone, (D) 1×10^{-1} μg disulfoton oxygen analog sulfone. Tracing 2: (A) 2×10^{-3} μg phorate, (B) 1×10^{-2} μg phoratoxon, (C) 1×10^{-2} μg phorate sulfone, (D) 1×10^{-1} μg phoratoxon sulfone. Detection with Varian phosphorus detector.

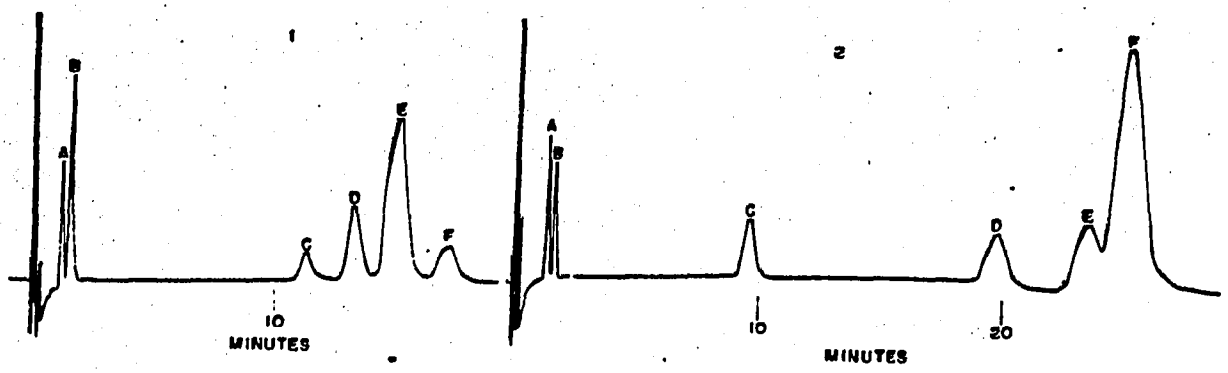

Fig. 2. GLC tracings obtained from the chromatography of disulfoton, phorate and some of their oxidation products on Chromosorb W AW-DMCS high performance coated with 5% DEGS. Tracing 1: (A) 1×10^{-3} μg phorate, (B) 2.5×10^{-3} μg phoratoxon, (C) 5×10^{-4} μg phorate sulfoxide, (D) 1×10^{-2} μg phoratoxon sulfoxide, (E) 5×10^{-3} μg phorate sulfone, (F) 2×10^{-3} μg phoratoxon sulfone. Tracing 2: (A) 1.5×10^{-3} μg disulfoton, (B) 1.2×10^{-3} μg disulfoton oxygen analog, (C) 3×10^{-3} μg disulfoton sulfoxide, (D) 6×10^{-2} μg disulfoton oxygen analog sulfoxide, (E) 1×10^{-3} μg disulfoton sulfone, (F) 4×10^{-3} μg disulfoton oxygen analog sulfone. Detection with Varian phosphorus detector.

on the 5% DEGS column. Phorate sulfoxide, phoratoxon sulfoxide, disulfoton sulfoxide and disulfoton oxygen analog sulfoxide were not detected when chromatographed on the DC-200 and QF-1 column. The oxygen analogs did not tail on the DEGS column. Temperature programming of the column had the advantage that the parent pesticide and its five oxidation products were resolved in a single run whereas McLEOD *et al.*[11] had to use two runs to resolve the six compounds. The 5%

ABLE II

MIT OF DETECTION (μg) OF THE ORGANOPHOSPHORUS COMPOUNDS ON MN-KIESELGEL G-HR THIN-LAYER ATES, 450 μ THICK

ey: (A) Enzyme inhibition[24]; (B) palladium chloride[17]; (C) Bromocresol Green–$AgNO_3$[25]; (D) iodoplatinate[26];) Brilliant Green–bromine[14]; (F) bromine–ferric chloride–2-(*o*-hydroxyphenyl)benzoxazole-UV[27]; (G) bro- ne–ferric chloride–2-(*o*-hydroxyphenyl)benzoxazole–Congo Red[27]; (H) 2,6-dibromobenzoquinone-4-chloro- ide[29]; (J) iodine[30]; (K) 4-(*p*-nitrobenzyl)pyridine[31].

mpound	*Detection method*										
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*
iorate	0.075	0.5	0.2	0.1	0.5	1	1	1	0.5	2	>20[b]
iorate sulfoxide	0.075	0.5	0.2	2	0.5	1	1	1	0.5	>20	>20
iorate sulfone	0.005	0.5	0.5	5	0.5	1	1	1	0.5	>20	>20
ioratoxon	0.025	2	0.2	0.5	5	5	5	5	10	2[a]	>20
ioratoxon sulfoxide	0.010	5	0.5	5	>20	>20	>20	>20	>20	>20	>20
ioratoxon sulfone	0.005	5	0.5	5	>20	>20	>20	>20	>20	>20	>20
sulfoton	0.075	2	0.5	0.2	0.5	1	1	1	0.5	2	>20
sulfoton sulfoxide	0.050	0.5	0.5	1	0.5	1	1	1	0.5	>20	>20
sulfoton sulfone	0.400	2	0.5	2	1	1	1	1	0.5	>20	>20
sulfoton oxygen analog	0.050	2	0.5	0.5	2	5	5	5	20	2[a]	>20
sulfoton oxygen analog sulfoxide	0.025	5	0.5	5	>20	>20	>20	>20	>20	>20	>20
sulfoton oxygen analog sulfone	0.400	5	0.5	>20	>20	>20	>20	>20	>20	>20	>20

[a] Spot only visible for about 10 min.
[b] Did not test quantities above 20 μg.

TABLE III

hR_F[a] VALUES OBTAINED WHEN PHORATE AND ITS OXIDATION PRODUCTS WERE CHROMATOGRAPHED ON M KIESELGEL G-HR, 400 μ THICK

Mobile phase: No. 11 (*cf.* Table I).

Compound	*Plate No. 1*		*Plate No. 2*		*Plate No. 3*		*Plate No. 4*		*Plate No. 5*		*Av.*
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	
Phorate	82	83	85	85	84	84	79	79	83	82	82.7 ± 2.0
Phorate sulfone	71	71	73	73	71	71	69	69	71	71	71.0 ± 1.7
Phoratoxon	62	62	66	65	60	61	61	61	61	62	62.1 ± 1.9
Phorate sulfoxide	46	46	51	51	42	43	49	49	45	45	46.7 ± 2.9
Phoratoxon sulfone	39	39	44	43	35	35	43	43	37	38	39.6 ± 3.4
Phoratoxon sulfoxide	22	21	26	26	20	20	23	23	21	21	22.3 ± 2.2

[a] Ratio of distance travelled by the substance and the mobile phase front × 100.

DEGS column has been found to be satisfactory for a number of other oxygen analogs of organophosphorus pesticides (unpublished results).

Results of TLC detection methods are shown in Table II. The enzyme inhibition method of MENDOZA *et al.*[24] (A) was the most sensitive. The palladium chloride method[17] (B) detected all the organophosphorus compounds tested. The compounds were detected immediately and the sensitivity was increased if the plates were subsequently sprayed with 5 *N* NaOH. The Bromocresol Green–$AgNO_3$ spray reagent[25] (C) detected small quantities but at times it was difficult to clear the background with the acetate buffer spray. To decrease the background, one-half the recommended amount of Bromocresol Green was used in the spray. The iodoplatinate reagent[26] (D) detected all the compounds tested except disulfoton oxygen analog sulfone and is particularly useful because the reagent is stable indefinitely. Methods (E)[14], (F)[27], (G)[27], (H)[28] and (I)[29] are useful for the detection of phorate, disulfoton and their sulfoxide and sulfone but did not detect the oxygen analog sulfoxide and the oxygen analog sulfone at the 20 μg level. The iodine spray[30] (J) detected only phorate, phoratoxon, disulfoton and disulfoton oxygen analog and the oxygen analogs

TABLE IV

EFFECT OF MOBILE PHASE, THICKNESS OF SORBENT (μ) AND LENGTH OF RUN (cm) ON THE hR_F VALUES OF PHORATE AND ITS OXIDATION PRODUCTS

Compound	*12*[a]				*11*			
	400 μ		*250 μ*		*400 μ*		*250 μ*	
	15 cm	*10 cm*	*15 cm*	*10 cm*	*15 cm*	*10 cm*	*15 cm*	*10 cm*
Phorate	89	90	85	86	85	90	88	89
Phorate sulfone	76	79	78	77	75	80	78	79
Phoratoxon	65	70	75	71	67	72	71	71
Phorate sulfoxide	45	52	70	60	53	56	61	58
Phoratoxon sulfone	41	45	63	51	44	45	52	48
Phoratoxon sulfoxide	35	35	47	37	27	26	31	23

[a] For mobile phases, see Table I.

TABLE V

hR_F VALUES OBTAINED WHEN PHORATE AND ITS OXIDATION PRODUCTS WERE CHROMATOGRAPHED ON 400 μ

Compound	Silica Gel G (buffer)[a]							Silica Gel G (water)[a]					Silica Gel H[a]							
	1[c]	2	3	4	5	13	15	4	9	11	12	13	3	4	5	6	9	10	11	14
Phorate	67	72	73	73	71	19	53	81	73	77	80	83	72	75	80	77	70	74	74	74
Phorate sulfone	31	53	61	63	65	15	33	63	55	66	69	40	47	58	72	66	55	60	60	32
Phoratoxon	15	37	51	53	57	18	35	43	37	55	60	47	23	37	63	57	41	50	50	33
Phorate sulfoxide	11	21	35	37	47	6	16	23	19	37	47	30	7	17	45	35	23	35	37	14
Phoratoxon sulfone	4	13	27	29	42	5	13	16	15	31	41	19	3	11	39	29	19	30	33	14
Phoratoxon sulfoxide	0	5	10	11	21	1	4	5	3	17	31	3	1	3	16	10	5	11	18	3

[a] Length of mobile phase travel was 15 cm.
[b] Length of mobile phase travel was 12 cm.
[c] For mobile phases, see Table I.

were only visible for about 10 min. At the 20 μg level, 4-(*p*-nitrobenzyl)pyridine[31] (K) did not detect any of the organophosphorus compounds tested on the MN-Kieselgel G-HR or Silica Gel G (water) plates. GETZ AND WHEELER[20] noted that the sensitivity may be reduced by a factor of 5 if the 4-(*p*-nitrobenzyl)pyridine or tetraethylenepentamine are off color. This may account for the failure to detect any of the organophosphorus compounds tested, although the tetraethylenepentamine was filtered through charcoal as recommended[20]. GUTH[16] did not detect 10 μg of phorate but did detect 5 μg of disulfoton on Kieselgel GF_{254} with the 4-(*p*-nitrobenzyl)-pyridine reagent. The TCNE reagent (2% tetracyanoethylene in benzene), DDG reagent (2% 2,3 dichloro-5,6-dicyano-1,4-benzoquinone in benzene) and chloranil (1% tetrachloro-*p*-benzoquinone in benzene) have been used to detect sulfoxides, sulfones and sulfides[32] but failed to detect, at the 20 μg level, any of the organophosphorus compounds used in this study.

The main difficulty in the resolution by TLC of phorate, disulfoton and five of their oxidation products is in separating the sulfone from the oxygen analog and the sulfoxide from the oxygen analog sulfone. Detection method B was used for all

TABLE VI

hR_F VALUES OBTAINED WHEN DISULFOTON AND ITS OXIDATION PRODUCTS WERE CHROMATOGRAPHED ON 400 μ THICK PLATES

Compound	Silica Gel G[a]		Silica Gel H[a]		MN-Kieselgel G-HR[a]			SilicAR TLC-7[a]	
	4[b]	12	9	10	9	11	13	10	11
Disulfoton	78	79	76	77	83	85	69	82	83
Disulfoton sulfone	60	65	55	59	55	71	29	63	70
Disulfoton oxygen analog	45	61	48	49	41	62	35	55	64
Disulfoton sulfoxide	21	44	23	25	15	35	15	28	41
Disulfoton oxygen analog sulfone	17	41	21	21	12	30	11	26	35
Disulfoton oxygen analog sulfoxide	5	31	4	8	3	19	2	13	24

[a] Length of mobile phase travel was 15 cm.
[b] For mobile phases, see Table I.

ICK PLATES

MN-Kieselgel G-HR[a]								*SilicAR TLC-7*[a]								*Aluminum Oxide G*[a]								*Adsorbosil-M-2*[b]			
4	*6*	*7*	*8*	*9*	*11*	*12*	*13*	*3*	*4*	*9*	*10*	*11*	*12*	*13*	*15*	*1*	*4*	*11*	*12*	*13*	*14*	*16*		*1*	*3*	*13*	*15*
89	91	91	91	83	84	89	73	79	75	77	76	79	73	58	65	78	84	82	84	89	68	80		85	85	88	73
72	83	84	87	56	73	76	30	67	65	63	62	68	69	34	34	71	84	82	84	37	40	69		55	73	55	65
57	74	79	83	20	63	65	35	51	55	45	50	59	65	37	37	70	84	82	84	49	46	63		43	76	64	66
35	56	66	75	8	46	45	21	32	37	31	35	43	53	17	17	56	84	82	84	24	21	39		23	57	38	50
27	48	59	70	6	39	41	12	27	31	24	27	37	47	14	14	—	84	82	84	2	20	36		8	48	21	38
9	20	32	45	2	23	35	4	7	10	7	12	23	35	0	3	—	81	75	84	1	1	0		3	17	6	12

studies on hR_F values of the organophosphorus compounds. Table III shows the reproducibility of hR_F values for phorate and the five oxidation products when chromatographed in duplicate, on five MN-Kieselgel G-HR plates. Although there was some variation in the hR_F values from plate to plate, all plates gave good resolution of the compounds.

The effects of sorbent thickness and length of run on the resolution of phorate and its oxidation products are shown in Table IV. Mobile phase No. 11 gave superior results to that obtained with No. 12. Phorate and its oxidation products were adequately resolved on both the 250 and 400 μ thick layers when chromatographed with mobile phase No. 11.

The hR_F values reported in Tables V and VI were obtained from single chromatographic separations and are a guide in the selection of conditions for the TLC of phorate, disulfoton, and their oxidation products. The hR_F values obtained for phoratoxon and disulfoton oxygen analog were greater than the hR_F values obtained for phorate sulfone and disulfoton sulfone with mobile phase No. 13. The hR_F values which were obtained for phorate and five of its oxidation products are shown in Table V. The following combinations of sorbent and mobile phase gave the best resolution of phorate, phorate sulfoxide, phorate sulfone, phoratoxon, phoratoxon sulfoxide and phoratoxon sulfone: Silica Gel G (buffer) with mobile phases No. 3 or No. 4; MN-Kieselgel G-HR with mobile phase No. 4, No. 6 or No. 11 and Silica Gel H with mobile phase No. 10. On SilicAR TLC-7 with mobile phases No. 9 or No. 10 phorate and its oxidation products were resolved but the spots tended to streak. Aluminum Oxide G did not give satisfactory resolution with any of the mobile phases. The mobile phases moved much slower in the sorbent Adsorbosil-M-2 than in the other sorbents and so the length of the run was terminated after 12 cm. Of the mobile phases tested No. 13 provided the best resolution with Adsorbosil-M-2. The hR_F values which were obtained with disulfoton and its oxidation products are shown in Table VI. The following combinations of sorbent and mobile phase gave the best resolution of disulfoton, disulfoton sulfone, disulfoton oxygen analog, disulfoton sulfoxide, disulfoton oxygen analog sulfone and disulfoton oxygen analog sulfoxide: Silica Gel H with mobile phase No. 10 and MN-Kieselgel G-HR with mobile phase No. 11.

REFERENCES

1 J. BURKE AND W. HOLSWADE, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 845.
2 J. KANAZAWA AND T. KAWAHARA, *J. Agr. Chem. Soc. Japan*, 40 (1966) 178.
3 C. A. BACHE AND D. J. LISK, *J. Assoc. Offic. Anal. Chemists*, 49 (1966) 647.
4 J. RUZICKA, J. THOMSON AND B. B. WHEALS, *J. Chromatog.*, 30 (1967) 92.
5 I. H. SUFFET, S. D. FAUST AND W. F. CAREY, *Environ. Sci. Technol.*, 1 (1967) 639.
6 M. C. BOWMAN AND M. BEROZA, *J. Assoc. Offic. Anal. Chemists*, 50 (1967) 1228.
7 C. A. BACHE AND D. J. LISK, *J. Assoc. Offic. Anal. Chemists*, 51 (1968) 1270.
8 R. R. WATTS AND R. W. STORHERR, *82nd Ann. Mtg. Assoc. Offic. Anal. Chemists, 1968.*
9 J. S. THORNTON AND C. A. ANDERSON, *J. Agr. Food Chem.*, 16 (1968) 895.
10 M. C. BOWMAN, M. BEROZA AND C. R. GENTRY, *J. Assoc. Offic. Anal. Chemists*, 52 (1969) 157.
11 H. A. MCLEOD, G. MULKINS AND S. L. N. RAO, *Bull. Environ. Contamin. Toxicol.*, 4 (1969) in press.
12 K. C. WALKER AND M. BEROZA, *J. Assoc. Offic. Agr. Chemists*, 46 (1963) 250.
13 C. W. STANLEY, *J. Chromatog.*, 16 (1964) 467.
14 D. C. ABBOTT, N. T. CROSBY AND J. THOMSON, *Proc. Soc. Anal. Chem. Conf., Nottingham*, (1965) 121.
15 M. F. KOVACS, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 1097.
16 J. A. GUTH, *Pflanzenschutz Ber.*, 35 (1967) 129.
17 R. C. BLINN, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 641.
18 R. C. BLINN, *J. Agr. Food Chem.*, 16 (1968) 441.
19 R. E. MENZER AND L. P. DITMAN, *J. Econ. Entomol.*, 61 (1968) 225.
20 M. E. GETZ AND H. G. WHEELER, *J. Assoc. Offic. Anal. Chemists*, 51 (1968) 1101.
21 R. R. WATTS, *Residue Rev.*, 18 (1967) 105.
22 C. E. MENDOZA, K. A. MCCULLY AND P. J. WALES, *Anal. Chem.*, 40 (1968) 2225.
23 E. STAHL, *J. Chromatog.*, 33 (1968) 273.
24 C. E. MENDOZA, P. J. WALES, H. A. MCLEOD AND W. P. MCKINLEY, *Analyst*, 93 (1968) 34.
25 M. E. GETZ, *J. Assoc. Offic. Agr. Chemists*, 45 (1962) 393.
26 H. F. MACRAE AND W. P. MCKINLEY, *J. Agr. Food Chem.*, 11 (1963) 174.
27 M. T. H. RAGAB, *J. Assoc. Offic. Anal. Chemists*, 50 (1968) 1088.
28 A. IRUDAYASAMY AND A. R. NOTARAJAN, *Analyst*, 90 (1965) 503.
29 J. STENERSEN, *J. Chromatog.*, 38 (1968) 538.
30 H. K. MANGOLD, *J. Am. Oil Chemists' Soc.*, 38 (1961) 708.
31 R. R. WATTS, *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 1161.
32 L. FISHBEIN AND J. FAWKES, *J. Chromatog.*, 22 (1966) 323.