CHROM. 6216

THIN-LAYER CHROMATOGRAPHY OF LYSERGIDE AND OTHER ERGOT ALKALOIDS

R. FOWLER, P. J. GOMM AND D. A. PATTERSON
Home Office Central Research Establishment, Aldermaston, Reading, Berkshire (Great Britain)

(Received April 26th, 1972; revised manuscript received June 19th, 1972)

SUMMARY

The chromatographic behaviour of lysergide and fourteen related ergot alkaloids has been investigated using eighteen thin-layer systems. Resolution and correlation between the systems is discussed in relation to the routine identification of lysergide.

INTRODUCTION

Those concerned with the routine identification of lysergide have come to rely principally upon the thin-layer chromatographic (TLC) technique, partly because of its sensitivity and convenience but also because of the limitations of techniques such as ultraviolet and fluorescence spectroscopy, gas chromatography and paper chromatography (PC). In these circumstances it is important that the chromatography system or combination of systems chosen, should adequately resolve lysergide from other chemically similar compounds, and further, since the procedure is to be used routinely, the system(s) should be simple to operate and should give R_F values which are as reproducible as possible.

Many TLC systems have been described which purport to allow identification of lysergide[1–8] but in some cases, compounds related to lysergide have not been run concurrently and the reliance which can be placed upon results obtained, using these systems, is open to some doubt.

In an attempt to assess the value of some TLC systems which are known to have been used for the identification of lysergide, we have run fifteen ergot alkaloids in each of eighteen recommended systems. Our collection of alkaloids, although not comprehensive, represents those which we have been able to obtain from the recognised sources of supply and the likelihood of encountering an illicit ergot preparation not included in our survey would hopefully be small.

EXPERIMENTAL AND RESULTS

For each experiment the reference substances (1–3 μg) were applied to a single

TABLE I

R_F VALUES × 100 FOR ERGOT ALKALOIDS

For description of systems used see text.

Compound		System																
No.	Name	A1	A1[a]	B1	B1[a]	C1	A2	A2[a]	C2	A3	A3[a]	A4	A4[a]	C4[a]	A5	A5[a]	C5	A6
1	D-Lysergic acid	00	00	00	02	00	00	00	00	03	00	00	00	00	00	00	00	02
2	Ergometrine maleate	10	10	26	30	18	07	14	14	44	42	00	00	01	00	00	02	36
3	*Iso*-LSD	11	18	66	55	29	08	07	18	30	26	00	00	03	00	02	08	32
4	Methylergometrine	13	14	31	32	25	09	16	18	51	48	00	00	01	00	01	02	42
5	Methysergide bimaleate	13	13	33	34	25	09	19	19	51	43	00	00	01	00	02	03	54
6	Dihydroergotamine	19	20	40	44	32	12	22	25	54	37	00	00	01	00	01	04	60
7	Lysergide	27	28	70	56	39	23	26	37	54	41	00	00	09	04	06	21	60
8	Ergotamine tartrate	32	31	48	44	39	23	29	33	63	45	00	00	02	02	02	08	62
9	Dihydroergocristine	35	37	64	54	46	27	30	39	64	48	00	00	03	02	02	11	65
10	Ergocristine	51	50	66	56	54	43	42	47	70	52	02	02	15	10	12	32	68
11	Ergocryptine	51	50	68	53	55	43	42	47	69	53	02	02	16	11	12	33	67
12	Ergotoxine ethanesulphonate	51	50	68	54	55	43	43	48	69	52	02	02	15	11	13	32	68
13	Ergosine	31	32	52	44	43	25	30	34	62	44	00	00	03	02	03	11	61
14	Ergocornine	50	50	67	54	53	42	42	45	68	52	03	02	12	11	12	32	67
15	Lysergamide	—	—	27	30	—	—	10	—	—	40	00	00	—	—	01	—	—

[a] Overspotted with NaOH

20 × 20 cm plate as a solution in methanol. The plates were developed in a 4-l, paper-lined tank containing approximately 100 ml of solvent and in each case the solvent was allowed to run 15 cm from the start line. Visualisation of the spots was by examination under 254 nm and 350 nm illumination followed by spraying with a solution of *p*-dimethylaminobenzaldehyde (1 g) in conc. HCl (10 ml) and ethanol (90 ml). Subsequent heating of the plates revealed blue spots.

Generally, commercially available coated glass plates (Merck), or polyester sheets (Eastman Kodak) were used because of the reproducibility which they offer but in a few cases hand-coated plates were prepared.

No special precautions were taken to ensure that the plates were activated before use.

Supports

A Merck Silica Gel F_{254} (0.25 mm pre-coated)
B Merck Aluminium Oxide F_{254} (type E, 0.25 mm pre-coated)
C Eastman Chromagram Sheets 6060 (silica gel with fluorescent indicator)
D Eastman Chromagram Sheets 6063 (alumina with fluorescent indicator)
E Merck Silica Gel F_{254} (0.25 mm hand-coated)
F Merck Cellulose sprayed with 5% sodium dihydrogen citrate and dried

Solvents

1 Acetone
2 Acetone–chloroform (4:1)
3 Acetone–methanol (4:1)
4 Chloroform
5 Chloroform–acetone (6:1)

	System																			
No.	A6[a]	A7	A7[a]	A8	A9	A10	A11	A12	A12[a]	B12	B12[a]	B13	B13[a]	D13	B14	B14[a]	D14	B15	B15[a]	C16	F17
1	00	00	00	77	01	00	00	01	02	01	00	00	00	00	00	00	00	00	00	00	17
2	39	10	15	66	52	07	01	00	11	21	14	04	06	03	00	04	00	00	00	18	31
3	33	08	10	66	84	34	21	10	12	50	53	30	30	40	14	22	35	05	08	50	66
4	45	13	20	69	68	00	02	12	14	24	14	05	10	05	00	06	00	00	02	22	42
5	54	26	31	68	57	17	05	21	27	45	34	17	21	23	02	10	13	00	03	40	47
6	50	30	36	68	73	00	02	20	26	54	40	20	26	24	03	10	10	00	04	19	79
7	61	36	42	68	83	36	25	21	27	61	54	36	47	41	19	28	37	12	14	54	59
8	62	35	40	69	73	11	02	20	32	52	40	20	28	23	04	10	10	00	04	30	77
9	66	38	44	72	82	10	05	24	35	63	52	31	40	33	07	16	24	01	05	34	83
0	60	50	55	72	84	20	14	26	36	67	56	30	49	38	13	22	31	04	08	55	83
1	68	40	54	72	85	20	16	27	31	65	55	34	48	37	12	22	31	04	08	58	83
2	68	40	54	72	84	30	17	28	30	65	56	34	48	35	12	22	30	04	08	52	82
3	60	36	45	68	75	12	04	23	24	49	38	20	30	20	03	14	08	00	04	37	75
4	68	40	50	70	83	20	17	28	32	04	54	33	46	27	11	20	20	04	06	54	81
5	38	10	—	—	—	—	—	—	10	—	17	—	08	—	—	04	—	—	00	—	25

6 Chloroform–methanol (4:1)
7 Chloroform–methanol (9:1)
8 Methanol–ammonia (0.88) (100:1.5)
9 Methanol–acetate buffer (pH 4.5) (9:1)
10 Chloroform–cyclohexane–isopropylamine (5:5:1)
11 Chloroform–cyclohexane–diethylamine (5:5:1)
12 1,1,1-Trichloroethane–methanol (9:1)
13 1,1,1-Trichloroethane–methanol (96:4)
14 1,1,1-Trichloroethane–methanol (98:2)
15 1,1,1-Trichloroethane–methanol (99:1)
16 Toluene–morpholine (9:1)
17 *n*-Butanol–citric acid–water (870 ml:4.8 g:130 ml)

The ergot alkaloids used, together with the code numbers given to them for use on the plates, are listed in Table I.

The R_F values obtained for each system (mean of three runs) are also recorded in Table I. For each of the systems being assessed, Merck plates were used and where specifically recommended, Chromagram sheets were run in addition.

In a preliminary series of experiments to determine the effect on the resulting chromatogram of using different procedures for obtaining alkaline conditions on the plate, and indeed, whether alkaline conditions were necessary at all, a series of silica gel plates (0.25 mm) were prepared. Each was then developed in chloroform–methanol (9:1) and the spots were located as described previously. Correlation coefficients between systems were calculated by the method described by SMALLDON[12]. The plates were: (a) hand-coated, using distilled water in the mixing stage, dried (105°), and the reference substances then applied (system E7(a)); (b) hand-coated, using 0.1 *N* NaOH in the mixing stage, dried and the reference substances applied (system E7(b));

(c) hand-coated, using distilled water, dried, and the reference substances subsequently over-spotted with 0.1 *N* NaOH (system E7(c)); (d) pre-coated Merck F_{254}, sprayed with 0.1 *N* NaOH, dried and the reference substances applied (system A7(d)); (e) pre-coated Merck F_{254}, and the reference substances subsequently over-spotted with 0.1 *N* NaOH (system A7*); (f) pre-coated Merck F_{254}, and the reference substances then applied (system A7).

In the resulting chromatograms the "neutral" plates (E7(a) and A7) gave similar resolution to one another and to the basic plates (E7(b), E7(c), A7(d), A7*). Correlation coefficients within the series were also very high (Table II) and it appeared that for these compounds it was not essential to run basic plates although slight tailing of spots was evident under "neutral" conditions. In order to confirm this observation it was considered necessary in the subsequent assessment of the other systerms to run two series of plates in which the conditions were either basic or neutral.

TABLE II

CORRELATION COEFFICIENTS FOR THE SILICA GEL/CHLOROFORM–METHANOL (9:1) SYSTEM USING PLATES PREPARED IN DIFFERENT WAYS

For description of systems used see text.

	E7(a)	*E7(b)*	*E7(c)*	*A7(d)*	*A7**
E7(a)					
E7(b)	0.93				
E7(c)	0.98	0.88			
A7(d)	0.99	0.95	0.98		
*A7**	0.99	0.92	0.98	0.99	
A7	0.99	0.90	0.99	0.98	0.99

A further observation from this preliminary experiment was that the method by which basic conditions were obtained on the plate did not significantly affect the chromatogram, and it was therefore considered that, of the methods available for obtaining basic conditions, the use of the over-spotting technique (A7*), which adequately minimises tailing and which is simple and convenient to perform, was the method of choice. This technique has previously been recommended by PHILLIPS AND GARDINER[5].

Five of the TLC systems being assessed here (A8, A9, A10, A11, C16) required either basic or acidic solvents for development and in these cases the reference materials were not over-spotted with alkali. For the others, using neutral solvents, however, the two series of Merck plates were run in which the reference materials were, or were not over-spotted, and when the original method had specified the use of chromagram sheets, these were run in addition, but using only the conditions stated. Differences in R_F value were observed between the two series of Merck plates but the degree of resolution of lysergide from the other compounds on the plate remained almost unchanged and, again, the correlation coefficients for the two series (given in Table III) were high.

DISCUSSION

Perhaps the most significant observation emerging from our survey is that no

TABLE III

CORRELATION COEFFICIENTS BETWEEN "NEUTRAL" AND NaOH OVER-SPOTTED PLATES
For description of systems used see text.

System	*Correlation coefficient*
A1	0.99
B1	0.98
A2	0.97
A3	0.96
A4	1.00
A5	0.99
A6	0.99
A7	0.99
A12	0.94
B12	0.99
B13	0.99
B14	0.96
B15	0.92

single TLC system which we have tried unequivocally resolves lysergide from the other ergot alkaloids and it follows that a compromise must be found in which either a combination of systems is used or one system is used in combination with an alternative technique. Several pairs of systems tried here could be effective. For example, a number of systems based on a silica gel adsorbent with a single, or mixture of, neutral solvent(s) allow resolution of lysergide from all but ergotamine (8) and ergosine (13). The two dihydro alkaloids (6 and 9) included in our chromatograms run near to lysergide in these cases but these would not be confused with lysergide because they do not fluoresce under 350 nm light, absorb differently to lysergide in 254 nm light and occur as purple spots on spraying (*cf.* lysergide is blue). Either acetone alone or chloroform–methanol (9:1) would suffice (see systems A1, A7 and Figs. 1a and 1b).

The alternative system could be based on either a silica adsorbent together with a basic developing solvent mixture (see systems A10, A11, C16 and Fig. 1c) or on an alumina adsorbent using one of the 1,1,1-trichloroethane–methanol mixtures (see systems B12–B15 and Fig. 1d) or acetone (system B1, Fig. 1e). Each of these latter groups allows resolution of lysergide from all the alkaloids run except *iso*-LSD (3) and the ergotoxine group, ergocristine (10), ergocryptine (11) and ergocornine (14).

Of the several pairs of systems outlined as being suitable, the simplest and most convenient for routine use would in our opinion consist of silica–acetone (A1) and alumina–acetone (B1). This would be advantageous in that: (a) pre-coated silica and alumina plates could be used without activation; (b) a single tank containing acetone could be used for development of both plates; and (c) a single-component developing solvent obviates the danger of incorrect mixing and of changing composition of the developing solvent in the tank due to different vapour pressures of the components.

The correlation coefficient for this combination of systems is fairly high, however, (0.78), and it would consequently be unsuitable for identification of a number of the individual ergot alkaloids, although for lysergide, we feel that it does allow unequivocal identification.

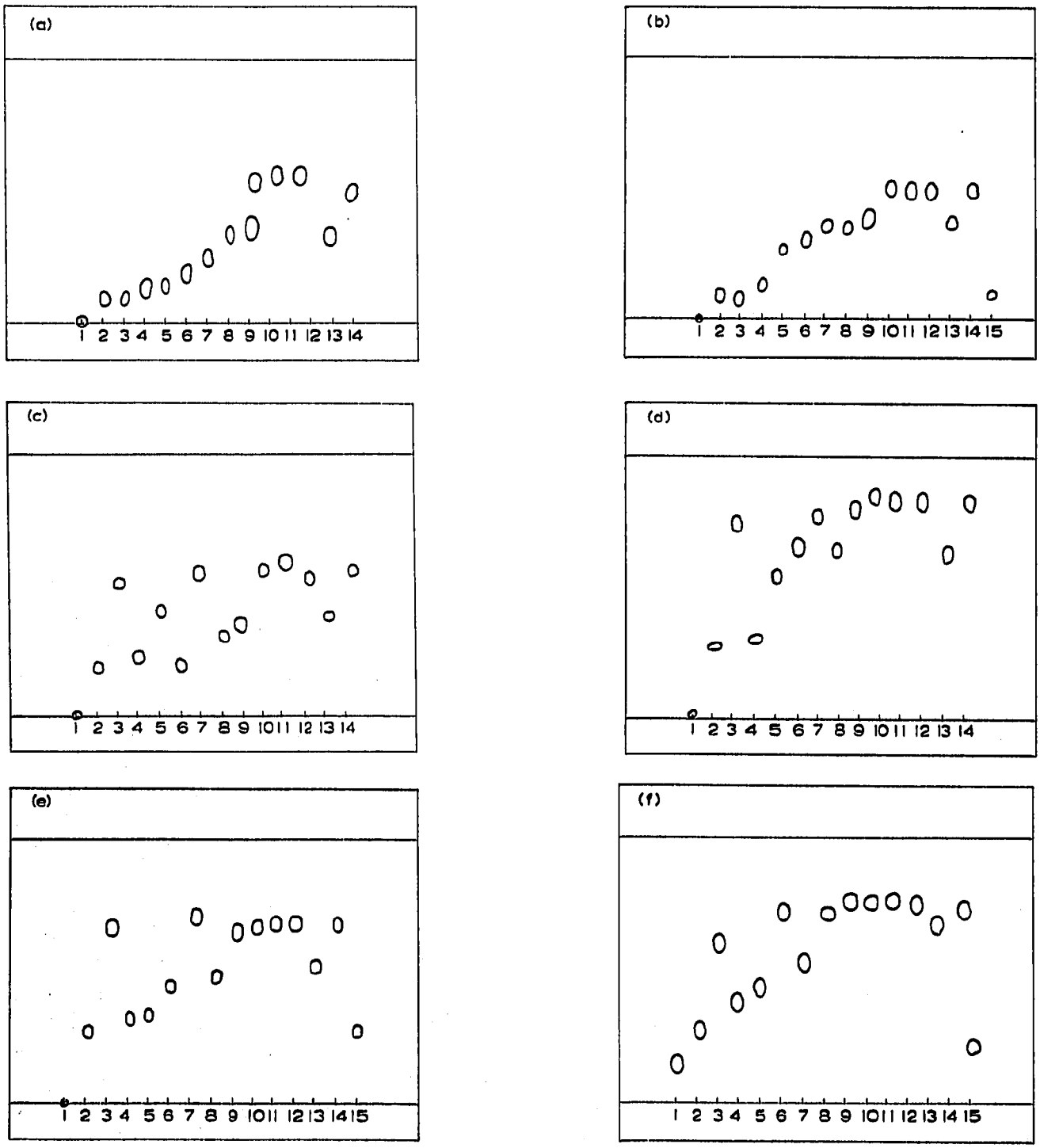

Fig. 1. Chromatograms of ergot alkaloids. (a) System A1; (b) system A7; (c) system C16; (d) system B12; (e) system B1; (f) system F17. See Table I for compound identification.

Where prior indication has not been given that the preparation being analysed may contain lysergide, it is likely that the preliminary stages in the identification will have included PC using the CURRY AND POWELL system[9] or its thin-layer modification[10,11]. This is commonly used as a screening procedure for bases, and R_F values

for over seven hundred basic compounds are recorded. For the ergot alkaloids, using the thin-layer modification, a good spread of R_F values is obtained (system F17, Fig. 1f) and it is interesting to note that lysergide is resolved from the other ergot alkaloids to at least the same extent as the best of the other systems which have been commended previously, although for routine identification of lysergide, the time taken for development (approx. 3 h to run 10 cm) could be considered to be disadvantageous.

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