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A novel thin-layer chromatography system for lysergide (LSD)

A continually increasing demand for the rapid identification of substances suspected of containing lysergide (LSD) prompted an investigation into the possibilities of faster and more reliable forensic methods. Screening tests have already been used which eliminate many "innocent" samples from further investigation. The use of ultraviolet induced fluorescence (Government Chemist, 1969) together with a modified field test (Alliston, Bartlett & others, 1971) can restrict the problem to certain erganes and tryptamines within a few minutes.

Thin-layer chromatography has been widely employed for the final stage of the identification of lysergide. Phillips & Gardiner (1969) investigated a number of systems and recommended that of Genest & Farmilo (1964), which employs development with chloroform–methanol (9:1) on NaOH treated silica gel plates, for the separation of a number of natural and synthetic erganes. This is not convenient for commercial silica gel coated polyester sheets such as Eastman 'Chromagram' on which it is necessary to spot 2 μl 0·1N NaOH at the origin. Moreover, with certain common (and licit) preparations of the natural ergot base ergotamine, coextraction of the other ingredients in compound preparations may cause mobility to be modified to such an extent that, in some cases, ergotamine is confusible with lysergide. A favoured alternative—development with chloroform–methanol (1:4) on silica plates (Martin & Alexander, 1967)—is not an improvement in practice; low mobility and heavy streaking are observed. Separation can be achieved with 1,1,1-trichloroethane–methanol (96:4) on alumina plates (dal Cortivo, Broich & others, 1966) but mobilities are low and "eyebrow" shaped spots (indicating movement on a secondary solvent front) are observed. This reduction in the proportion of the plate available for separation occurs with many other systems, especially where more than two solvents are employed.

In seeking an entirely new system for use with "Chromagram" sheets, incorporation of an organic base into the mobile phase seemed desirable. Development with systems containing varying proportions of diethylamine, aniline, quinoline, morpholine, picoline and ethanolamine in a series of solvents was investigated. In most cases the substances examined did not move in the system or were carried with the solvent front. Where separation did occur, the ergotamine spot had a large tail (even after an equilibrium between the epimers had been established) or, in the case of 5% diethylamine in chloroform, 8β-ergotamine and 8α-lysergide were resolved but 8α-ergotamine and 8β-lysergide moved with the solvent front. Morpholine appears to be the best base and 1:9 the optimum admixture with toluene. Morpholine–xylene (1:4) or morpholine–benzene (1:9) are also suitable developing solvents but the extra drying time required for xylene and the health hazard of benzene would preclude them from routine use.

The following procedure is recommended. Place 15–20 mg of the crushed sample in an ignition tube and add 1–2 drops of reagent grade methanol. Stir with a pointed glass rod, stopper and allow to stand for 5 min. Spot 0·5 μl of the supernatant liquor onto a 8 cm $\times$ 4 cm "Chromagram 6060" sheet (silica gel with fluorescent indicator).

Table 1. *R_F values ($\times 100$) for erganes and some tryptamine derivatives.*

	††	Solvent I	Solvent II	Visualization (*a*) 360 nm	Visualization (*a*) 254 nm	Visualization (*b*) colour
Psilocybin	+	00	01	—	A	blue-grey
Lysergic acid		00	02	F	F	purple
8β-Ergometrine		11	25	F	F	purple
8β-Lysergamide	+	12	24	F	F	purple
Methylergometrine		14	31	F	F	purple
Dihydroergotamine		15	50	—	A	violet
8α-Ergometrine		22	39	F	F	purple
8β-Ergotamine		22	58	F	F	purple
Bufotenine	+	22	07	—	A	purple
Methysergide		33	51	F	F	violet
8α-Ergotamine		36	76	F	F	purple
8α-Lysergide	+	42	34	F	F	purple
8β-Ergocristine		44	70	F	F	purple
Psilocin	+	46	—	—	A	blue-grey
8β-Lysergide	+	51	60	F	F	purple
NN-Dimethyltryptamine	+	54	16	—	A	violet
NN-Diethyltryptamine	+	61*	16	—	A	violet
1-Acetyl-lysergide		61*	74	—	A	faint purple
Ibogaine		71**	—	—	A	none

T.l.c. systems: Eastman "Chromagram 6060" sheets —0·1 mm silica gel layer incorporating a fluorescent indicator.
Solvents: I = morpholine–toluene (1 : 9); II = methanol–chloroform (1 : 9).
Visualization: *a*: ultraviolet induced fluorescence (F) or absorbance (A) at 254 and 360 nm. *b*: spraying with 5% w/v 4-dimethylaminobenzaldehyde in methanol–HCl (1 : 1).

Notes: †† indicates control under the Drugs (Prevention of Misuse) Act, 1964—Modification Order 1970.

* substance travels on secondary solvent front.

** substance travels in phase ahead of secondary solvent front.

The spot area should not exceed 1 mm in diameter. Develop the sheet with morpholine–toluene (1 : 9) using an unequilibrated tank (a tall beaker is suitable). When the solvent front reaches the top of the sheet, remove the sheet and, before it is completely dry (2–3 min), observe under 360 and 254 nm ultraviolet radiation. Allow the plate to dry (about 5 min) and then observe the sheet under 254 nm ultraviolet radiation again. Spray the sheet with a solution of 5% w/v 4-dimethylaminobenzaldehyde in methanol–hydrochloric acid (1 : 1). To preserve the sheet, place it inside a re-sealable polythene bag to prevent yellowing due to oxidation.

Table 1 shows the data obtained from a range of erganes and certain tryptamine derivatives after development with morpholine–toluene (1 : 9) on "Chromagram 6060" sheets. Previous results (Phillips & Gardiner, 1969) with methanol–chloroform (1 : 9) are included for comparison.

The only compounds found to have mobilities comparable with the epimers of lysergide are 8β-ergocristine, *NN*-dimethyltryptamine (DMT) and psilocin. Ergocristine is so rarely encountered alone that the problem of resolving 8α-lysergide and 8β-ergocristine is not likely to occur. Moreover, we have never encountered 8α-lysergide without the 8β epimer (although 8β-lysergide often occurs by itself) so the absence of a spot due to 8β-lysergide would suggest the presence of 8β-ergocristine. If any doubt still remains, 8α-lysergide and 8β-ergocristine can be readily resolved using methanol–chloroform (1 : 9) (Phillips & Gardiner, 1969). Although 8β-lysergide,

DMT and psilocin have similar mobilities, the latter pair are easily identified by their lack of fluorescence at 360 nm whereas they absorb 254 nm radiation. Differences in colour response to the chromogenic agent are also observed. As with most other t.l.c. systems investigated, morpholine–toluene (1 : 9) exhibits a secondary solvent front. However, only 1-acetyl-lysergide and *NN*-diethyltryptamine travel on this front (R_F 0·61) and ibogaine, an hallucinogen with a slight history of abuse, runs in the solvent phase ahead of this front. The enhanced mobility of tryptamine derivatives in this new t.l.c. system may have some advantages over the four systems investigated by Phillips and Gardiner; this aspect is being pursued.

The system was tried with conventially prepared plates as well as other commercially available sheets. None of these gave acceptable results. The uniformity and fineness of texture of "Chromagram" sheets appears to be essential for the success of this system.

Using a covered tank prepared 24 h previously, the spread of mobilities for the series of compounds investigated became progressively smaller throughout the day. However, a tank newly prepared each day with these low volatility solvents i.e. essentially unequilibrated, gives results that are extremely reproducible. de Faubert Maunder (1969) and de Zeeuw (1970) have also reported good reproducibility in unsaturated t.l.c. chambers.

The risk of modification of the relative mobilities of the bases in compound preparations was investigated. Cafergot "Q"—a proprietary product containing 1 mg ergotamine tartrate B.P. and 100 mg caffeine B.P. within a chocolate flavoured shell and outer sugar coating—was completely ground (although in normal practice the inner, active tablet would have been separated physically before grinding), and a methanol extract was spotted on a "Chromagram 6060" sheet; a methanol extract of 8β-ergotamine tartrate was spotted on the same sheet. On development with morpholine–toluene (1 : 9) no differences in mobility were observed. The extracts were retained until some epimerization of the 8β-ergotamine had occurred and then rechromatographed: there were no changes in the mobility of 8α-ergotamine either.

The use of this new system facilitates rapid analysis of materials suspected of containing lysergide without problems of interference from compound preparations. Good separation can be obtained with an 8 cm development. Time and costly full scale t.l.c. apparatus are saved. The limit of detection for lysergide using this system was found to be 4 nanograms.

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April 26, 1971

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