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STUDIES ON QUANTITATIVE *IN SITU* FLUOROMETRY OF LYSERGIC ACID DIETHYLAMIDE (LSD) ON THIN-LAYER CHROMATOGRAMS

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SUMMARY

The quantitative *in situ* fluorometry of LSD on thin-layer chromatograms was investigated. Reproducibilities for the range 0.1–2.0 μg of LSD on the same and different chromatograms were examined in both direct and internal standard methods. The linear relationship between fluorescence emission intensity and amount of LSD was found in both methods.

This technique has been applied to the determination of unchanged LSD in the photodecomposition process. It was noted that LSD was easily decomposed by ultraviolet irradiation.

INTRODUCTION

Lysergic acid amide, isolysergic acid amide and clavine alkaloids in morning glory seeds have been determined by densitometry after treatment with Van Urk reagent on thin-layer chromatograms[1]. It is known, however, that lysergic acid diethylamide (LSD) on a chromatogram is detected by fluorescence with much better sensitivity than by coloration with the reagent[2]. Recently, various instruments and methods for *in situ* fluorometric scanning of thin-layer chromatograms have been developed[3–8].

This paper describes the quantitative *in situ* fluorometry of LSD on thin-layer chromatograms, using an automatic scanner with a digital recorder, and the reproducibilities of both direct and internal standard methods are discussed. In addition, this technique has been applied to determination of unchanged LSD in the photodecomposition process.

EXPERIMENTAL

Materials

LSD was synthesized from *d*-lysergic acid (Sigma) by GARBRECHT's method[9]. Quinine was recrystallized from benzene.

Apparatus

A Hitachi MPF-2 type Autorecording Spectrophotofluorometer equipped with a scanning attachment for thin-layer chromatograms, a J201 integrator and a J301 digital recorder (Hitachi Ltd.) was used with the following settings: sensitivity, 6; slit width for excitation and emission, 10 and 10 mμ, respectively; slit for thin-layer chromatograms, 6 × 2 mm. The fluorescence emission profiles were obtained on a recorder, and at the same time the fluorescence emission intensities were printed out as numerals on a digital recorder. The scanning direction of the chromatograms was either parallel or perpendicular to the chromatographic flow.

Thin-layer chromatography

Thin-layer chromatography (TLC) was carried out on 250-μ layers of Silica Gel G. Solvent systems used for development were: (A) methanol–chloroform–*n*-hexane (1:4:2), (B) methanol–chloroform (1:4), and (C) acetone–chloroform (4:1). After development, the plates were dried for 5 min in a stream of warm air.

Direct method

5 μl of chloroform solution containing 2.0 μg of LSD were spotted at a starting point on a chromatographic plate and four more spots were prepared on the same plate, followed by development with three solvent systems. The fluorescence emission intensity of LSD on the chromatogram was measured and the relative standard deviation on the same chromatogram was calculated. The chromatography was repeated five times on different plates and the relative standard deviations on different chromatograms were calculated. In the same way, the relative standard deviations on both the same and different plates were obtained from solutions containing 1.5, 1.0, 0.5, 0.2 and 0.1 μg of LSD, respectively, and the relationship between emission intensity and the amount of LSD was examined.

Internal standard method

Quinine was used as an internal standard. 5 μl of ethanol solution containing 2.0 μg of LSD and 0.5 μg of quinine were spotted at a starting point on a chromatographic plate, four more spots were prepared on the same chromatographic plate, and the development was carried out in the solvent systems (A) and (B). The ratio of the fluorescence emission intensity of LSD to that of quinine was obtained. The relative standard deviation of this ratio on the same plate was calculated. This chromatography was repeated five times on different plates and relative standard deviations on different chromatograms were calculated. The same procedure was repeated on solutions containing 1.5, 1.0, 0.5, 0.2 and 0.1 μg of LSD, and 0.5 μg of quinine, respectively. The relationship between the above ratio and the amount of LSD was examined.

UV irradiation for photodecomposition of LSD

2 μg of LSD spotted on a thin layer were irradiated with UV light from a National GL-10 lamp (2537 Å, distance 10 cm) for 1, 5, 15, 30 and 60 min. After irradiation, three or four standards containing 0.2–2.0 μg of LSD were spotted on the same plate and TLC was carried out using the solvent systems (A), (B) and (C). Amounts of unchanged LSD on the chromatograms were determined by the direct

method. As a control experiment, 2 μg of LSD spotted on thin layers were left to stand in darkness for 60 min or 17 h, followed by chromatography and determination of unchanged LSD as above.

RESULTS AND DISCUSSION

Direct method

The excitation and emission wavelength for the direct method were selected to give maximum emission intensity of the spot of LSD on the chromatogram. They were 330 and 410 mμ, respectively, as shown in Fig. 1. The reproducibilities for the range 0.1–2.0 μg of LSD in the direct method are shown in Table I.

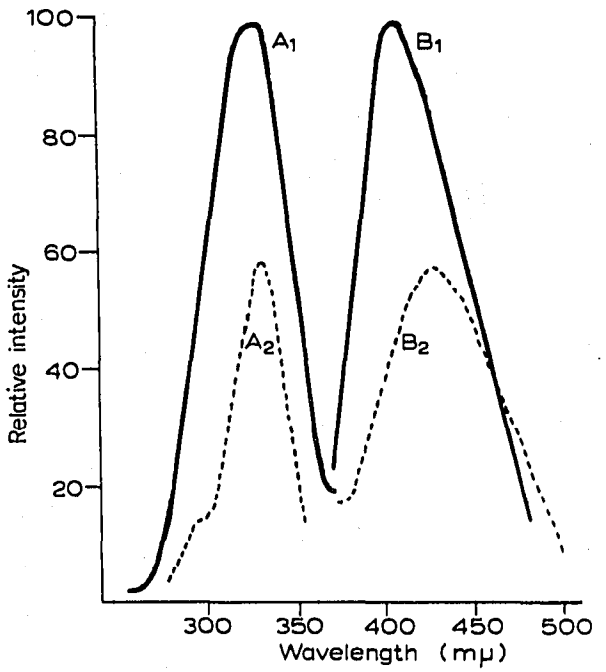

Fig. 1. Fluorescence excitation and emission spectra for LSD and quinine on a thin-layer chromatogram. Mixture containing 2.0 μg of LSD and 0.5 μg of quinine were spotted on the thin-layer plate, followed by development in the solvent system (A), MeOH–$CHCl_3$–*n*-hexane (1:4:2). (A_1) Fluorescence excitation spectrum for LSD by setting emission at 410 mμ; (B_1) fluorescence emission spectrum for LSD by setting excitation at 330 mμ; (A_2) fluorescence excitation spectrum for quinine by setting emission at 436 mμ; (B_2) fluorescence emission spectrum for quinine by setting excitation at 336 mμ.

Relative standard deviations obtained by scanning in the direction parallel to the chromatographic flow were 0.7–6.3% on the same chromatogram and 3.3–10.8% on different chromatograms. For the case of scanning in the direction perpendicular to the chromatographic flow, higher deviations were observed than those shown in Table I. Accordingly, in this experiment, scanning of the chromatograms was performed in the direction parallel to the chromatographic flow. The relationship between emission intensity and the amount of LSD was linear over the range 0.2–2.0 μg in each solvent system (Fig. 2).

TABLE I

REPRODUCIBILITIES IN THE DIRECT METHOD

Solvent system	*LSD (μg)*	*Standard deviation (%)*			
		Parallel to chromatographic flow		*Perpendicular to chromatographic flow*	
		Same chromatogram	*Different chromatograms*	*Same chromatogram*	*Different chromatogram*
(A)	2.0	0.7–3.4	5.6	0.3– 3.8	5.6
	1.5	2.9–5.4	10.1	2.8– 5.6	10.8
	1.0	2.3–2.7	9.7	1.8–11.3	14.5
	0.5	3.2–4.5	6.6	2.5–12.1	4.7
	0.2	1.2–3.3	6.4	4.6– 8.7	9.9
	0.1	3.7–6.3	8.9	5.3– 8.0	3.8
(B)	2.0	1.6–4.0	5.2	0.8–11.2	5.9
	1.5	3.6–4.5	9.3	2.8– 4.1	14.6
	1.0	2.1–4.7	10.8	5.5– 8.6	16.2
	0.5	1.0–4.0	8.0	1.0–11.2	15.8
	0.2	3.3–5.9	4.3	6.1– 8.2	21.3
	0.1	2.8–5.1	5.5	2.1– 7.4	11.5
(C)	2.0	0.9–3.2	3.3	0.3–10.7	12.2
	1.5	3.4–4.4	9.4	3.4– 9.7	11.0
	1.0	1.6–4.9	6.1	3.1– 7.0	8.1
	0.5	3.1–4.5	9.0	6.9–13.7	5.5
	0.2	2.0–4.4	6.5	0.9–15.0	10.0
	0.1	3.9–5.6	5.9	5.1–14.9	8.5

Internal standard method

The excitation and emission wavelength described for the direct method were used in the internal standard method, although the maximum emission intensity of quinine which was used as an internal standard compound was obtained at 436 mμ by excitation at 336 mμ, as shown in Fig. 1. The intensity of quinine decreased to about 75% of the maximum intensity by excitation at 330 mμ and emission at 410

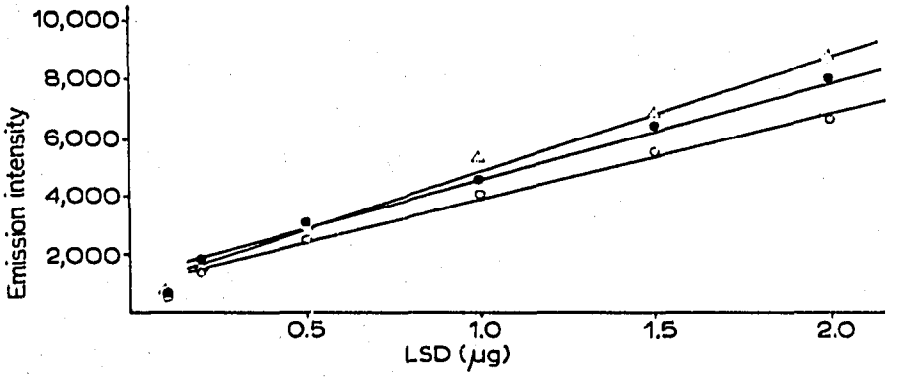

Fig. 2. Relationship between fluorescence emission intensity and amount of LSD. ●—●, solvent system (A), MeOH–$CHCl_3$–*n*-hexane (1:4:2); ○—○, solvent system (B), MeOH–$CHCl_3$ (1:4); △—△, solvent system (C), acetone–$CHCl_3$ (4:1).

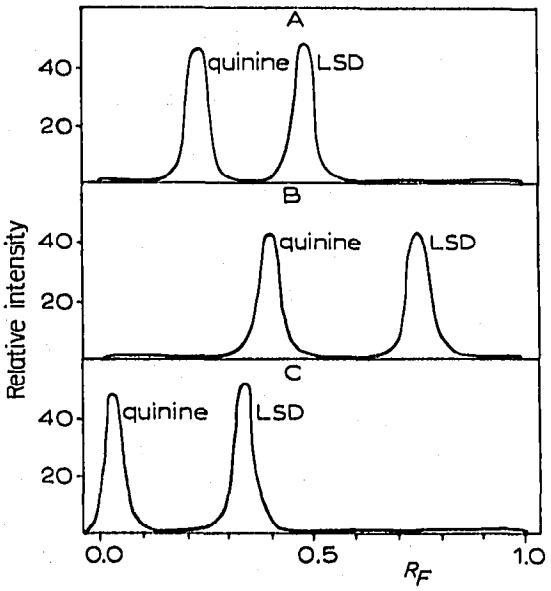

Fig. 3. Fluorescence emission profiles of a mixture containing 1.0 μg of LSD and 0.5 μg of quinine. (A), Solvent system (A); (B), solvent system (B); (C), solvent system (C).

mμ, and 0.5 μg of quinine on a chromatogram gave an intensity about half that of 2.0 μg of LSD.

LSD and quinine were separated on the chromatograms in the solvent systems (A), (B) and (C) as shown in Fig. 3. However, systems (A) and (B) were used since quinine stayed at the starting point of the chromatogram in system (C).

Relative standard deviations for the range 0.1–2.0 μg of LSD were 0.2–6.2% on the same chromatogram and 1.6–9.3% on different chromatograms, as shown in Table II.

TABLE II

REPRODUCIBILITIES IN THE INTERNAL STANDARD METHOD

Solvent system	*LSD (μg)*	*Standard deviation (%)*	
		Same chromatogram	*Different chromatograms*
(A)	2.0	0.2–5.8	7.9
	1.5	1.9–4.8	1.7
	1.0	3.4–5.2	4.2
	0.5	0.8–5.6	9.3
	0.2	2.7–6.2	6.1
	0.1	3.6–4.2	5.5
(B)	2.0	1.7–4.6	2.0
	1.5	3.4–5.6	1.6
	1.0	2.7–3.1	5.9
	0.5	4.3–4.5	5.2
	0.2	1.7–5.2	6.6
	0.1	2.5–5.2	3.6

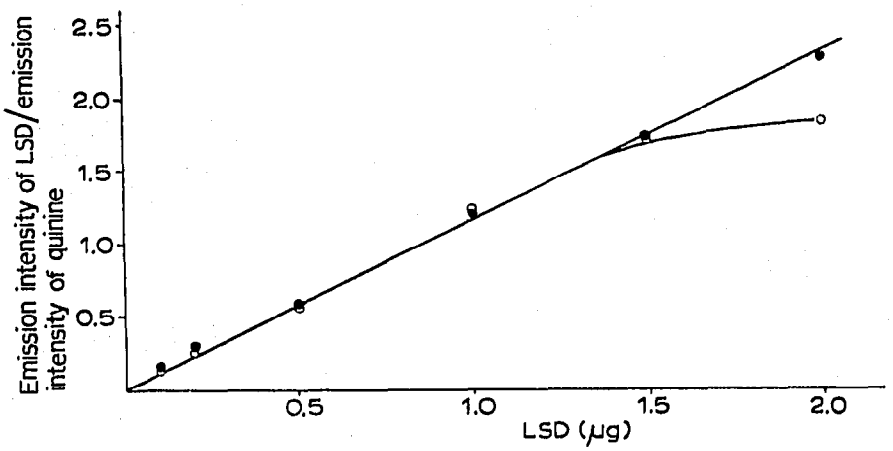

Fig. 4. Relationship between the ratio of the fluorescence emission intensity of LSD to that of quinine and the amount of LSD. ●—●, solvent system (A); ○—○, solvent system (B).

The relationship between the ratio of the emission intensity of LSD to that of quinine and the amount of LSD was linear over the range 0 – 1.5 μg (Fig. 4).

The reproducibilities of scanning on the same plate were found to be less than 6% in both the direct and internal standard methods, whilst those on different plates were less than 9% in the internal standard methods and less than 11% in the direct method.

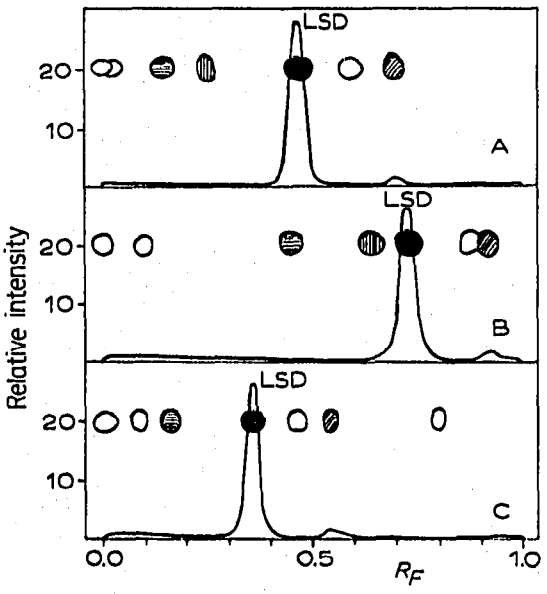

Fig. 5. Fluorescence emission profiles of UV-irradiated LSD. (A) Solvent system (A); (B) solvent system (B); (C) solvent system (C). 2 μg of LSD spotted on a thin-layer plate were irradiated with UV light (2537 Å) for 15 min. After development, spots were detected by observation under UV light at 3650 Å and then by coloration with *p*-dimethylaminobenzaldehyde reagent. ●, Detected by blue fluorescence under UV light and blue color with the reagent; ○, detected by blue fluorescence under UV light and orange color with the reagent; ◍, detected by yellow fluorescence under UV light and blue color with the reagent; ◍, detected by UV light but not by the reagent; ◍, detected by the reagent but not by UV light.

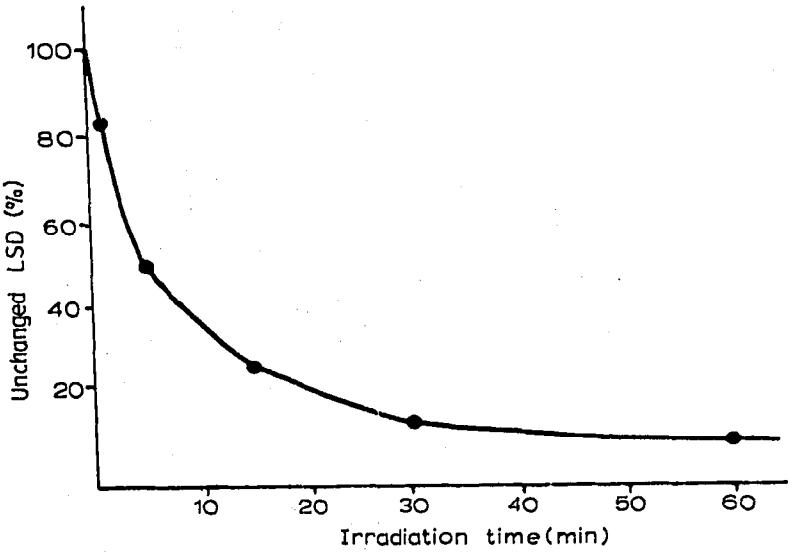

Fig. 6. Photodecomposition curve of LSD.

Photodecomposition of LSD on thin layers

The fluorescence emission profiles of chromatograms of LSD irradiated for 15 min are shown in Fig. 5. Five additional spots were detected by observation under UV light. The amounts of unchanged LSD at each irradiation time were determined by the direct method, and the photodecomposition curve is shown in Fig. 6.

The amount of 2 μg of LSD initially spotted on a thin layer was decreased to 0.2 μg (10%) by irradiation for 60 min, whilst in the control experiment, LSD in darkness was unchanged after standing for 60 min but decreased to 1.8 μg (90%) after 17 h. Structures of the products of the photodecomposition of LSD are now under investigation.

It is considered that LSD in samples containing some impurities or in biological materials can be determined practically without purification by either the direct or the internal standard method described in the present work.

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