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Note

Direct quantitative analysis of lysergic acid diethylamide (LSD) and 2,5-dimethoxy-4-methylamphetamine (STP) on thin-layer chromatograms

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Lysergic acid diethylamide (LSD) sold on the illicit drug market usually contains impurities and in some instances another hallucinogen. For quantification of these drugs, it is necessary to separate the components quantitatively. Recently, a densitometer for the direct measurement of the UV absorption of spots on thin-layer chromatograms has been developed. The base-line noise caused by local irregularities of thickness of the thin layer is eliminated and errors due to the unevenness of size and shape of the spot are minimized[1], as the instrument is equipped with accessories for the dual-wavelength and zig-zag scanning methods.

This paper describes the direct quantitative densitometry of LSD and 2,5-dimethoxy-4-methylamphetamine (STP) on thin-layer chromatograms.

EXPERIMENTAL

Apparatus

A Shimadzu CS-900 dual-wavelength scanner was used with the following settings: slit for thin-layer chromatograms, 1.25 × 1.25 mm; scanning speed, 5 mm/min with zig-zag scanning; reflection mode. The developed thin-layer plate was scanned in the direction parallel to the chromatographic flow. UV absorption profiles of the thin-layer chromatograms and the integration curves of the peaks in the profiles were obtained simultaneously on a recorder.

Materials

LSD was synthesized from *d*-lysergic acid (Sigma, St. Louis, Mo., U.S.A.) by the method of Nakahara and Niwaguchi[2], and STP was kindly supplied by the Bureau of Narcotics and Dangerous Drugs (Washington, D.C., U.S.A.). All other chemicals used were special-grade materials.

Thin-layer chromatography

Thin-layer chromatography (TLC) was carried out on 250-μm layers of silica gel G (E. Merck, Darmstadt, G.F.R.). The solvent systems used for development were: (A) chloroform–ethanol–methanol (9:2:1); (B) acetone–chloroform–methanol (3:1:1). After development, the plates were dried for 5 min in a stream of warm air.

Quantitative analysis of LSD

A 5-μl volume of chloroform containing 2.0 μg of LSD was spotted at the starting point on a chromatographic plate and development was carried out in solvent systems A and B. The absorption intensity of LSD on the chromatograms was measured. The chromatography was repeated five times on different plates and the relative standard deviation was calculated. In the same way, the relative standard deviations were obtained from solutions containing 1.5, 1.0, 0.5, 0.2 and 0.1 μg of LSD and the relationship between absorption intensity and the amount of LSD was examined.

Quantitative analysis of LSD and STP

The relationship between the absorption intensity of the spot on the thin-layer chromatograms and the amount of STP was investigated in the same manner as for LSD.

Mixtures of 10–30 μg of LSD, 400 μg of STP and 10 mg of starch were prepared as samples. LSD and STP in the samples were determined as follows: 10 ml of 1% tartaric acid solution were added to a sample and the solution was made alkaline with sodium hydrogen carbonate, followed by extraction three times with chloroform. The chloroform layers were collected, dried over anhydrous sodium sulphate and filtered, and the chloroform was evaporated *in vacuo*. The residue obtained was dissolved in 500 μl of chloroform, and of 5 μl of the solution were spotted on a thin-layer plate. After development, LSD and STP on the chromatograms were simultaneously determined.

RESULTS AND DISCUSSION

Quantitative analysis of LSD

For the measurement of the UV absorption intensity of the LSD spot on the chromatogram, sample and reference beams (λ_s and λ_r) were set at 310 and 400 nm, respectively, as the LSD spot showed maximum absorption at 310 nm and minimum at 400 nm (Fig. 1).

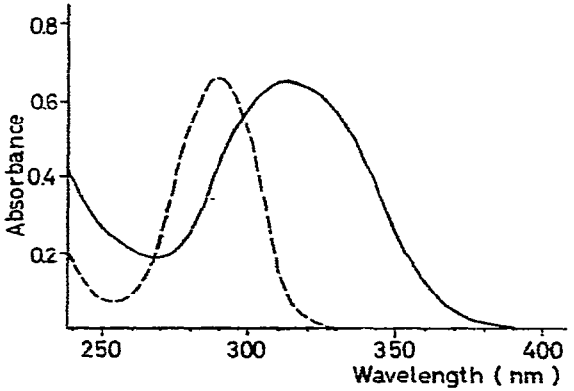

Fig. 1. UV absorption spectra on a thin-layer chromatogram of LSD and STP. Mixtures containing 1.5 μg of LSD and 4.0 μg of STP were applied using the solvent system A. ———, LSD; — — —, STP.

TABLE I

REPRODUCIBILITIES IN DIRECT UV SPECTROMETRY ON THIN-LAYER CHROMATOGRAMS

Solvent system	*LSD (μg)*	*Integrated value*	*Standard deviation (%)*
A	2.0	107.4	1.6
	1.5	85.2	4.7
	1.0	58.9	1.7
	0.5	32.5	1.1
	0.2	13.4	3.5
	0.1	5.3	2.8
B	2.0	106.5	1.8
	1.5	86.2	4.0
	1.0	59.2	2.5
	0.5	32.4	1.3
	0.2	13.2	2.1
	0.1	5.4	1.5

The relationship between the absorption intensity and the amount of LSD was linear over the range 0.1–2.0 μg in both developing solvents. Relative standard deviations obtained from the same amount of LSD on different chromatograms were 1.1–4.7% (Table I) and reproducible values were obtained.

Quantitative analysis of LSD and STP

Recently, mixtures of LSD and STP have appeared on the illicit drugs market. Sperling[3] has described a method for the determination of LSD and STP in mixtures, in which drugs were separated by column chromatography and then quantified by UV spectrophotometry. In the present investigation, direct UV spectrometry on thin-layer chromatograms was applied to the quantification of LSD and STP in mixtures.

As the maximum intensity of UV absorption of an STP spot on the chro-

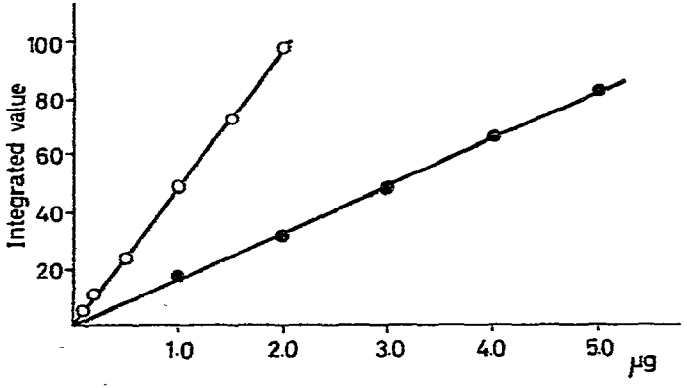

Fig. 2. Relationship between UV absorption intensity and amount of LSD or STP by setting λ_s at 300 nm and λ_r at 400 nm. Solvent system A or B was used. ○, LSD; ●, STP.

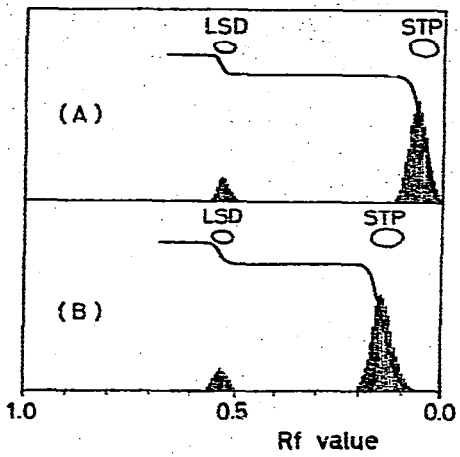

Fig. 3. UV absorption profiles and integration curves of a mixture containing 0.2 μg of LSD and 4.0 μg of STP by setting λ_s at 300 nm and λ_r at 400 nm. (A) Solvent system A; (B) solvent system B.

matogram was obtained 290 nm (Fig. 1), a spot containing less than 5 μg of STP was not detectable under the conditions for the quantification of LSD (λ_s 310 nm, λ_r 400 nm). On the other hand, when 290 nm was chosen as λ_s, the absorption intensity of the LSD spot decreased to about 70% of the maximum intensity obtained at 310 nm. Accordingly, 300 and 400 nm were used as λ_s and λ_r, respectively, for the simultaneous quantification of LSD and STP. Under these conditions, the intensity of LSD and STP decreased to 92% and 80%, respectively. The calibration curves for LSD and STP are shown in Fig. 2.

UV absorption profiles of the chloroform extract of the mixture are indicated in Fig. 3. Recoveries of LSD and STP in the mixtures were 93–95%, as shown in Table II.

TABLE II

RECOVERIES OF LSD AND STP IN MIXTURES

Added (μg)		*Determined (μg)*		*Recovery (%)*	
LSD	*STP*	*LSD*	*STP*	*LSD*	*STP*
10	400	9.4	380	94	95
20	400	18.9	373	95	93
30	400	28.4	371	95	93

By the method described here, LSD in mixtures or in samples containing some impurities can be determined quantitatively without purification. The method is simple, sensitive, reproducible and applicable to other drugs isolated from mixtures.

ACKNOWLEDGEMENT

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