acid. Dilute solutions of the dye in 2*N* hydrochloric acid show absorption peaks at 740 mμ and 688 mμ; in water the absorption peak of dilute solutions is at 660 mμ.

Method for Highly Saline Solutions. 1. For each determination prepare an adsorption column of 1 gram of analytical grade calcium carbonate supported on a grade 4 glass sinter in a 12-mm. bore tube. 2. Draw 5 ml. of sample through the column under light suction. The sample should contain less than 30 μg. of poly(acrylic acid). 3. Wash with 3 ml. of distilled water and discard the washings. 4. Draw 5 ml. of 32 p.p.m. methylene blue solution through the adsorbent. Dye-polyanion complexing occurs at the surface of the $CaCO_3$ during this step. 5. Elute the dye present in the complex with 10 ml. of distilled water. 6. Estimate the polyanion concentration by measuring the absorbance of the eluted dye against that of a blank at 660 mμ and compare with results obtained from standards.

If the sample contains more than 30 μg. of poly(acrylic acid), the amount of dye solution specified in step 4 is inadequate for full complexing. The success of the method depends upon the use of suction conditions at step 4 which leave only a small and reproducible residue of dye solution in the column. Apparently, in contrast to its behavior on borosilicate glass, the polyanion is so strongly adsorbed on calcium carbonate that it remains in the column during the elution of the dye from the complex.

The method is suitable for estimating the polyanions described above, at concentrations in sea water from 1 to 6 p.p.m., with a mean error of 0.1 p.p.m. The sensitivity could probably be increased by using a larger sample.

The absorbance *vs.* concentration plots were linear; the absorptivity of the eluted dye solutions was 2300, calculated as above, from the corresponding concentration of polyanion which in this method was half of the polyanion concentration in the standard sample.

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LSD 1470/DHE

Identification and Estimation of Lysergic Acid Diethylamide by Thin Layer Chromatography and Fluorometry

SIR: Recent police seizures of various materials suspected to contain lysergic acid diethylamide (LSD) prompted an investigation of some of the characteristics of the drug to devise rapid methods for its detection and estimation.

Genest and Farmilo (*4*) suggest that identification of LSD is unequivocal only after a study is made of the degradation products obtained by subjecting the parent compound to hydrolytic cleavage and prolonged ultraviolet irradiation. Our experiments were directed toward simplification of the identification process by eliminating these two steps.

EXPERIMENTAL

Thin Layer Chromatography (TLC). Migrational characteristics of LSD and structurally related bases were examined in a variety of solvents on commercially prepared silica gel plates of 100-micron thickness (Eastman Chromagram, Types K-301R with and K-301R2 without fluorescent indicator), and alumina G plates, 250 microns thick. The latter were prepared by mixing 35 grams of aluminum oxide G (according to Stahl) with 40 ml. of water and coating 20-cm.[2] glass plates with the slurry using the DeSaga adjustable spreader.

The most satisfactory solvent for the silica gel films was 1,1,1-trichloroethane: methanol (90:10) and 1,1,1-trichloroethane:methanol (98:2) was the most suitable for the alumina G plates. All chromatograms were developed in glass tanks.

Spots were detected by examination under ultraviolet light. Also, a modified van Urk reagent (*3*), prepared by dissolving 0.8 gram of *p*-dimethylaminobenzaldehyde in 100 ml. of ethanol containing 10% v./v. concentrated sulfuric acid was used as a spray reagent.

Fluorometry. LSD fluoresces strongly when irradiated by ultraviolet light. The excitation maximum is at 325 mμ. The fluorescence maximum reported by Udenfriend *et al.* (*5*) was 465 mμ and by Axelrod *et al.* (*2*), 445 mμ. Aghajanian and Bing (*1*) recommended 325 mμ and 445 mμ as excitation and emission wavelengths for measuring the rate of disappearance of LSD from human plasma while Genest and Farmilo (*4*) employed 335 mμ and 435 mμ for estimation of the compound in simulated narcotic seizures. These investigators used the Aminco-Bowman spectrophotofluorometer.

In the present study, quantitative data were secured with a Turner Model 110 filter fluorometer.

After TLC using the thin film silica gel layers or the glass-supported alumina G layers, LSD spots were either cut out or scraped off, then eluted with 1 ml. of methanol. The eluates were diluted with 0.001*M* HCl to provide concentrations in the range 0 (blank) to 0.1 μg. of LSD per ml. The blanks were obtained by an identical technique using areas of the chromatograms which had been irrigated by the solvent but which showed no ultraviolet fluorescence.

The diluted solutions were measured fluorometrically employing the following instrumental conditions: excitation source, GE 4W Blacklight; excitation slit, 1 × Position; sensitivity attenuation, Position 2; primary filter, 360 mμ, CS 7-60; and secondary filter, 436 mμ, CS 47-B.

Fluorometric measurements were also made directly on the flexible silica gel chromatograms with the Turner chromatogram scanning door, Model 110-861. The conditions were the same as those previously described except that the excitation slit was in the 3× position. The aperture of the mask on the door was 11 mm. in diameter. Background was attenuated with a neutral density filter, CS 2ND, 1%T (Kodak Wratten No. 96). Again, nonfluorescing areas of the chromatograms were employed as blanks.

Extraction of Drug from Seizure Materials. Sugar cubes, material from capsules, and a crude solution contain-

Table I. R_f Values of LSD and Related Substances

Silica gel (100 microns); 1,1,1-trichloroethane:methanol (90:10)

Compound	R_f
Quinine	0.35
Quinidine	0.30
Tryptamine	0.06
Indole	1.00
Etryptamine	0.11
LSD	0.55
Ergotamine	0.47
Ergonovine	0.21
Dihydroergotamine	0.45
Methylergonovine	0.25
Methyl-methylergonovine	0.42

ing LSD were dissolved in or mixed with about 20 ml. of water. The solutions were adjusted to pH 8.5–9.0 with alkali, saturated with sodium chloride, and extracted 3 times with 15-ml. portions of spectral quality chloroform. Each extraction was permitted to proceed for 10 minutes on a mechanical shaker. The combined chloroform layers were back-extracted with 5 ml. of 0.01*M* HCl for 5 minutes. The HCl solutions were made alkaline and extracted with 10 to 15 ml. of chloroform. After careful evaporation of the solvent, the residues were dissolved in 100 μl. of methanol and portions containing about 0.05 to 0.1 μg. of drug taken for TLC. In instances where direct fluorometric measurements of TLC films were made, the aliquots taken for chromatography contained about 0.1 to 1 μg. of LSD.

RESULTS AND DISCUSSION

On silica gel films, 1,1,1-trichloroethane:methanol (90:10) produced compact spots and minimal tailing of the various bases examined; an exception was indole which migrated with the solvent and tended to spread laterally along the front. Time necessary for development of a 10-cm. chromatogram was about 30 minutes. R_f values observed are presented in Table I.

Figure 1 illustrates the excellent separation of LSD from related substances achieved in 30 minutes on alumina G with 1,1,1-trichloroethane:methanol (98:2). LSD, which appears in position 6, is readily identified in crude mixtures using this system.

Reproducibility of R_f's from day to day is excellent for both silica gel and alumina systems.

As little as 0.05 μg. of LSD is distinctly observed on nonfluorescent thin layer plates when sprayed with van Urk's reagent or examined under ultraviolet illumination. If plates with fluorescent indicator are employed, sensitivity of detection by the ultraviolet method can be increased; however, quantitation by fluorometry is not possible because of the extremely high background fluorescence.

The elution-fluorometric technique furnished a linear relationship between concentration and fluorescence for the range 0 to 0.1 μg. of LSD per ml. with a sensitivity of 0.01 μg. per ml. of final solution. Greater sensitivity can be achieved by decreasing photomultiplier attenuation and/or increasing excitation energy. Neither of these steps was considered necessary in our experiments.

The relationship between emission and concentration is linear over the range 0 to 2 μg. when measurements are made directly on the silica gel TLC films. Sensitivity is in the neighborhood of 0.1 μg. of LSD. It is difficult to increase sensitivity in this kind of measurement because of the relatively high background contributed by the silica gel.

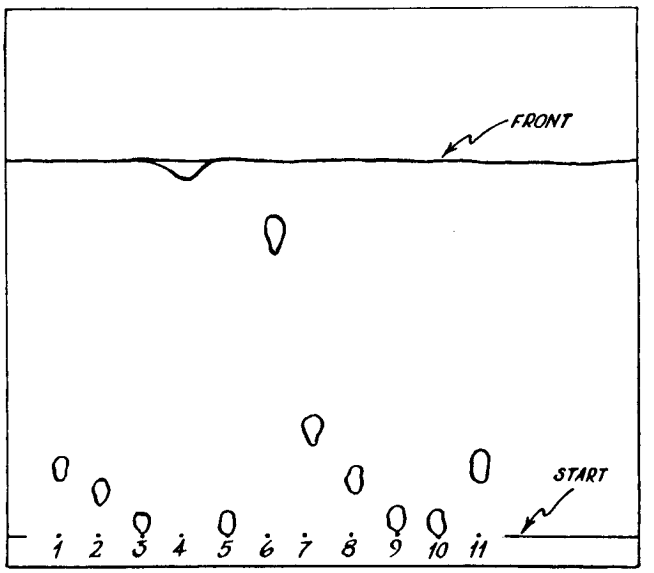

Figure 1. Thin layer chromatogram of LSD and related substances on alumina G (250 microns) with TCE:methanol (98:2) as developing solvent

Compounds are: 1. quinine; 2. quinidine; 3. tryptamine; 4. indole; 5. etryptamine; 6. LSD; 7. ergotamine; 8. methyl-methylergonovine; 9. ergonovine; 10. methylergonovine; 11. dihydroergotamine

Recoveries of 20 μg. of LSD added to water were consistently in the vicinity of 95% when either the direct fluorometric or the elution-fluorometry procedure was used.

More than 100 sugar cubes were confiscated, of which 12 were assayed for LSD content. The lowest concentration found was 52 μg., the largest, 95 μg.; the average quantity was about 70 μg. Several capsules were found to contain from 40 to 50 μg. of LSD. Tests made on these seized materials proved negative for the presence of other, commonly encountered drugs. A confiscated solution has a reddish color and contains 1.5 mg. of LSD per ml. TLC gives evidence for the presence of at least 4 other compounds, none of which has been identified at this time.

Fluorescence intensity of LSD is pH dependent. We found maximum emission intensity to occur at hydrogen ion concentrations between $10^{-3}M$ and $10^{-5}M$. An acid concentration of $10^{-3}M$ was considered the lowest practicable for dilution of TLC eluates. HCl of 0.01 molarity was selected for back-extraction of LSD from chloroform to ensure complete removal of the drug.

LSD can be measured photometrically at 312 mμ; however, the method suffers from the disadvantages of low sensitivity (at least 10 μg. of LSD per ml. is required) and poor selectivity. This observation confirms that of Genest and Farmilo (*4*).

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