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Quantitative Determination by Circular Dichroism of Lysergic Acid Diethylamide in Confiscated Material

REFERENCE: Bowen, J. M., McMorrow, H. A., and Purdie, N., **"Quantitative Determination by Circular Dichroism of Lysergic Acid Diethylamide in Confiscated Material,"** *Journal of Forensic Sciences*, JFSCA, Vol. 27, No. 4, Oct. 1982, pp. 822-826.

ABSTRACT: D-Lysergic acid diethylamide has been quantitated in a variety of confiscated specimens by circular dichroism spectropolarimetry. Separation of the drug from the specimens was unnecessary. The minimum quantity detected was approximately 2 μg.

KEYWORDS: toxicology, chemical analysis, dichroism

A series of recent articles [*1-8*] has described and critically discussed the application of circular dichroism (CD) spectropolarimetry in the ultraviolet (UV) spectral range to the qualitative analysis and quantitative determination of dangerous drugs. Greater discrimination is obtained among members of the same drug families when the results from CD are compared with those from conventional UV spectrophotometric methods [*3*]. Modification of the solvent system further broadens the discriminatory capabilities [*1,2*]. The method has all of the attributes of spectrophotometry in terms of speed and technical simplicity.

A library of CD spectra is being assembled for opiates, amphetamines, tryptamines, ergotamines, and numerous miscellaneous compounds. Some of the compounds that have been successfully quantitated include morphine [*3*], heroin [*7*], opium, and cocaine [*5,6*] in anonymous confiscated materials, codeine in analgesic prescriptions and cough syrups, and tetracycline in human urine [*8*]. In all of these determinations, prior separation of the drugs from the sample or specimen was unnecessary.

In this work we describe our results for D-lysergic acid diethylamide (D-LSD). This is a particularly difficult drug to quantitate by any judicially acceptable analytical method, such as gas chromatography or mass spectrometry, because of its rapid degradation at either elevated temperatures or under high vacuum. Since legal conviction requires only qualitative identification, that is the usual extent of the analysis performed by federal and state criminalistic laboratories. With CD spectropolarimetry the quantitative analysis can be completed with ease and in a shorter time than is currently required for qualitative identification.

Received for publication 22 Dec. 1981; revised manuscript received 1 Feb. 1982; accepted for publication 4 Feb. 1982.

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Materials and Methods

A standard sample of D-LSD was obtained as the hydrochloride salt from the National Institute for Drug Abuse via the laboratories of the Research Triangle Institute (Batch 2167-36-4B). Stock solutions were prepared in distilled water and in 1*M* hydrochloric acid.

Confiscated specimens were provided by the criminalistic laboratories of the Oklahoma State Bureau of Investigation and the Oklahoma City Police Department. The specimens were of two basic types. Some were either pressed tablets, pale or intensely colored microdots; others were absorbent paper impregnated with the drug. Individual specimen weights varied from 2 to 150 mg. Solutions were prepared by vigorously shaking the entire specimen in a 10-mL aliquot of 1*M* hydrochloric acid. Undissolved material was removed by centrifugation. The CD spectra were measured on a recording spectropolarimeter (Cary 61). Solutions were placed in 1-cm quartz cells, and measurements were made against a solvent blank.

To be CD active, a substance must satisfy two requirements: (1) the molecule of the compound must be optically active, but the substance cannot be a racemic mixture, and (2) the molecule must contain an absorbing chromophore. Bond unsaturation leads to absorption in the visible to UV spectral range. The principal chromophore for drug molecules is the aromatic ring, extended in the case of D-LSD to include the pyrrole moiety. Unlike optical rotation, which is observed at all wavelengths, CD activity is observed only over the range of the absorption bands. Outside these ranges the CD signal is coincident with the instrument's baseline.

The incident light consists of two in-phase, circularly polarized components that rotate in the opposite sense from each other. Both are absorbed by the chromophore of the optically active compound but to different extents. The spectropolarimeter is designed to measure the difference in absorption as a function of wavelength to produce a CD spectrum. The quantity is customarily displayed on the ordinate as an ellipticity, which is quantitatively related to the absorption difference. The term *ellipticity* is descriptive of the cross-sectional plane of the transmitted light, which after unequal absorptions is no longer circularly polarized. Simply put, CD is a modification of absorption spectrophotometry and is as technically simple to perform.

Results and Discussion

The CD spectrum of D-LSD in 1*M* hydrochloric acid is shown in Fig. 1. Principal features are a positive Cotton band with a maximum around 317 nm and a negative Cotton band with a maximum at 236 nm. The 317-nm band corresponds to a weak shoulder in the UV absorption spectrum (Fig. 2a). At wavelengths less than 300 nm, where absorption is strong, there is little to no gain in the intensity of the Cotton bands. Analytically, the weak absorption maximum at 310 nm could be used to quantitate the drug from measured absorptions but only in the absence of interfering compounds. The 317-nm band is a better choice for quantitating the drug by CD.

The lack of correlation between intensities of absorption bands and Cotton bands has long been appreciated and is not difficult to understand, since the experimental ellipticity ψ_{exp} is directly proportional to the difference between the molar absorption coefficients of the left ϵ_L and right ϵ_R circularly polarized components according to

$$[\theta] = \frac{\psi_{exp}}{C} = 3300\,(\epsilon_L - \epsilon_R)$$

where $[\theta]$ is the molar ellipticity coefficient and C is the molar concentration of drug expressed in moles per litre. Values of $[\theta]$ calculated from linear correlations of ψ_{exp} versus C are $+165$ at 317 nm (Fig. 3) and -181 at 236 nm.

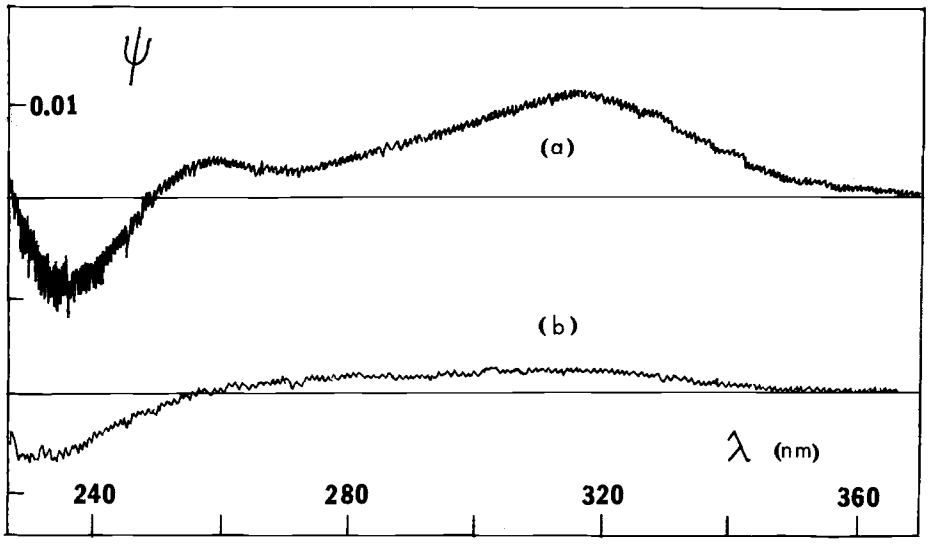

FIG. 1—*The CD spectrum in 1*M *hydrochloric acid of* (a) *6.4* × 10^{-5}M *standard* D-*LSD and* (b) *pink microdot Specimen 4. Calculated concentration of* D-*LSD is 1.10* × 10^{-5}M.

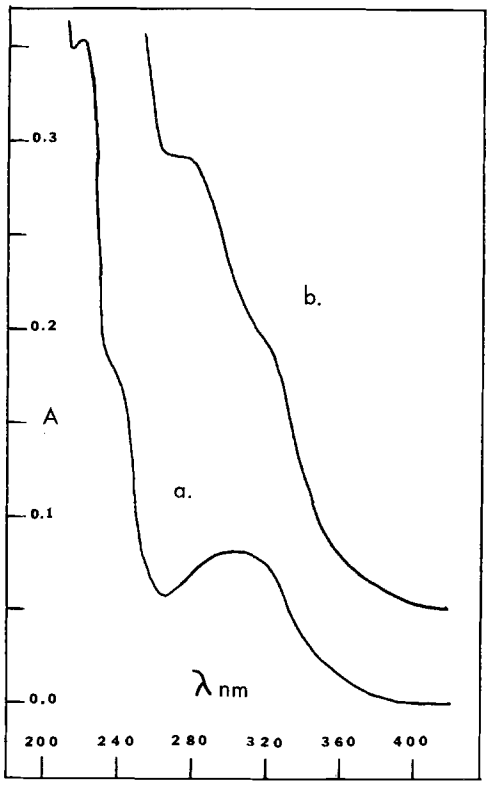

FIG. 2—*The UV absorption spectrum in 1*M *hydrochloric acid of* (a) *1.09* × 10^{-5}M *standard* D-*LSD and* (b) *pink microdot Specimen 4.*

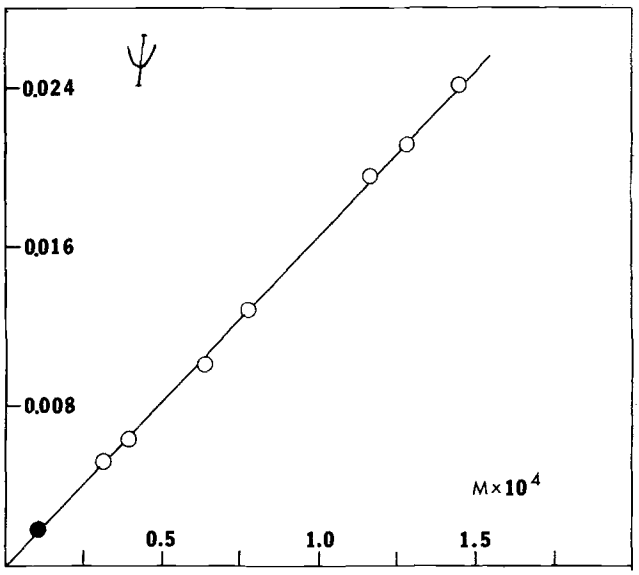

FIG. 3—*Calibration curve of experimental ellipticity versus molar concentration of* D-*LSD. The solid circle is Specimen 4 in 10 mL of* 1M *hydrochloric acid. Data are measured at 317 nm. Slope is equal to [θ], calculated to be +165.*

Excessive noise is an inherent instrument problem at the shorter wavelengths because of the absorption intensity. For D-LSD solutions a signal-to-noise (S/N) ratio of two in the CD spectrum is obtained at a concentration of 3.2×10^{-5} M for measurements made at 236 nm and at a concentration of 4.8×10^{-6} M from 317-nm data. In confiscated samples or specimens, the estimated amount of D-LSD present normally varies from 3 to 60 μg. For high relative concentrations, quantitation from the 317-nm band is preferred because of the superior S/N ratio. Theoretically, use of either band maximum is equally effectively at lower relative concentrations.

However, prior to distribution, intensely colored dyestuffs are frequently added to the drug solution; these compounds absorb strongly over a spectral range that is usually wide enough to obliterate the qualitatively recognizable LSD absorption band, which has a maximum at 310 nm (Fig. 2*b*). Quantitation of LSD by UV absorption is not possible without first separating the tablet into its components. On the other hand, neither the colored additions nor any sugars added as fillers to the tablets are CD active, so the presence of LSD is quickly and easily detectable from its CD spectrum. Another feature that contributes to the successful identification of LSD is the zero ellipticity at 250 nm, the wavelength of a weak shoulder in the absorption spectrum. The minimum quantity detectable with the present instrumentation is 1.6 μg/mL.

Results from the analysis of seven confiscated specimens are given in Table 1. No independent determinations had been made with which we could compare our results, and all of the CD determinations were made directly. There seemed to be no need for a comparative study in which the drug was first separated from the mixture because the interferences at 317 nm were minimal. Repeated measurements made over extended time intervals were in agreement to within ±0.1% of the calculated weight of the free base in the complete specimen. The lack of control in the preparation of the specimens and the uncertainty that multiple samples were taken from the same batch make the comparison statistically meaningless. Furthermore the parent population was very limited in every case, never exceeding six individual samples [*9*].

TABLE 1—*Composition of confiscated samples containing LSD.*

Specimen	Description	Average Sample, mg	LSD Content, μg
1	red blotter	61.6	17
2	white blotter	2.5	19
3	blue blotter	11.2	45
4	pink microdot	10.2	46
5	purple microdot	29.2	<3
6	brown tablet	143.8	42
7	tan tablet	61.8	18

These results for D-LSD are a further illustration of the application of a new and important analytical method for the easy identification and rapid quantitation of anonymous drugs and toxic substances that are CD active. It is becoming more and more apparent as data are collected that CD spectra are unique and are not replicas of UV absorption spectra [*10*]. As a general rule CD spectra show more features than conventional absorption spectra.

In earlier work [*4–7*] the noninterference of added sugars was described. This is again true for D-LSD samples, where the only interference from added dyestuffs is the loss in S/N ratio. The particular dyes were not identified. Presumably, their excessive absorption is a consequence of extended conjugation in the molecular structure, which will tend to be planar and therefore nonchiral.

In summary, CD spectropolarimetry as an analytical technique is proving to be distinctive, specific, quick, straightforward, quantitative, and free from a number of the usual interferences encountered in the analysis of drugs.

Acknowledgments

We thank the National Institute for Drug Abuse and the Research Triangle Institute for providing the standard samples and the Oklahoma State Bureau of Investigation and the Oklahoma City Police Department for providing the confiscated samples. This work was supported by the National Science Foundation, under Grant NSF-CHE-7909388.

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