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Note

A chromatographic method for the detection of LSD in biological liquids

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The exceptionally small amounts of LSD (lysergide) (Fig. 1) required for a "trip"[1] make its detection in biological specimens extremely difficult and we know of no method so far published which is specific enough to give an unequivocal identification suitable for forensic purposes[2]. High-pressure liquid chromatography (HPLC) using a fluorimetric detector has the necessary sensitivity and specificity to make detection possible[3–5]. A combination of HPLC and thin-layer chromatography (TLC) has been found to be satisfactory for the isolation and identification of LSD in biological liquids, especially urine. Identity of the compound has been further confirmed by mass spectrometry.

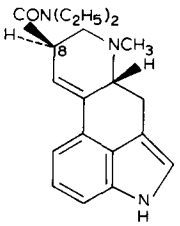

Fig. 1. Structural formula of LSD (iso-LSD has the opposite configuration at C-8).

EXPERIMENTAL

Materials and conditions

The extractions were carried out away from direct lighting. All glassware was silanised. All reagents used were of analytical grade; in addition a fresh bottle of ether was used each day and the 1,2-dichloroethane and methanol were redistilled. Evaporations and concentrations were performed on a water bath at 50° under a stream of nitrogen or compressed air. Specimens were deep frozen during storage.

HPLC conditions were as follows: column (25 cm × 4.6 mm), packed with Partisil of average particle size 6 μm; pump, Waters Assoc. 6000-Pump; pressure,

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2,000 p.s.i.; flow-rate, 1 ml/min; eluent, methanol–water containing 0.2% ammonium nitrate (11:9); detector, Perkin-Elmer MPF-2A fluorescence spectrometer, fitted with a flow-cell[6], emission wavelength set at 430 nm and excitation wavelength at 325 nm; syringes, 5 or 100 μl S.G.E.

TLC conditions were: plates, Merck silica gel F-254 pre-coated, pre-washed by eluting with 1,2-dichloroethane and then methanol; eluents, for LSD: (a) 1,2-dichloroethane, (b) acetone-chloroform–methanol (15:4:1); for iso-LSD: acetone–chloroform–methanol (4:1:5).

p-Dimethylaminobenzaldehyde reagent was prepared by dissolving 1 g of *p*-dimethylaminobenzaldehyde in 100 ml of ethanol and adding 10 ml of concentrated hydrochloric acid.

A Vacuum Generators Micromass 12F single focussing mass spectrometer was used. Conditions were: ionising potential, 70 eV; ionising current, approx. 800 μA; accelerating potential, 4,000 V; source temperature, after 45 sec of maximum heating.

Methods

HPLC. Urine (40 ml) was acidified to pH 3 with concentrated hydrochloric acid. This was then extracted with three volumes of 40 ml of ether, the layers being separated by centrifugation if necessary. The ether was discarded. The aqueous layer was adjusted to pH 9 with ammonia (sp.gr. 0.88) and re-extracted with 2 × 40-ml aliquots of ether. The ether extracts were dried sequentially over anhydrous sodium sulphate, concentrated and transferred to an agglutination tube in which the total extract was evaporated to dryness. The residue was redissolved in 40 μl of the HPLC eluting solvent. 1 μl of this extract was injected on to the liquid chromatographic column and the resulting chromatogram obtained (Figs. 2 and 3).

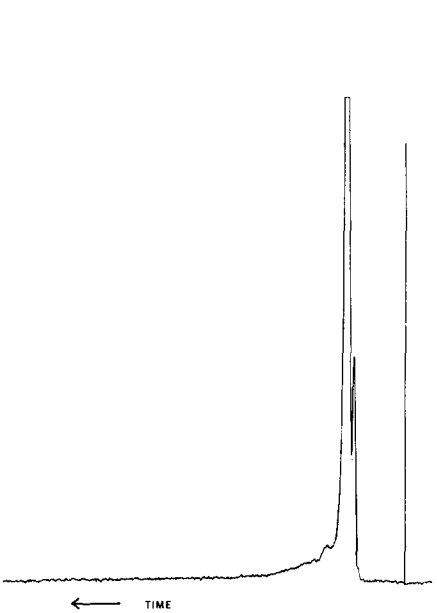


Fig. 2. HPLC trace of a control urine extract.

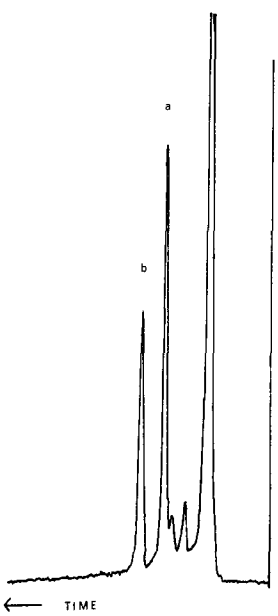


Fig. 3. HPLC trace of a suspect urine extract. a = LSD; b = iso-LSD.

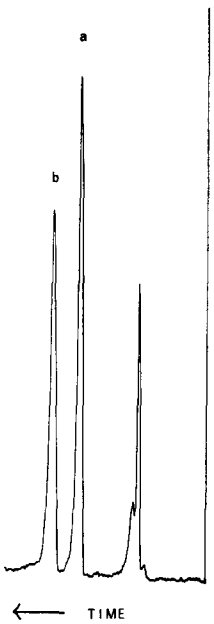


Fig. 4. HLPC chromatogram of LSD (a) and iso-LSD (b).

The remainder of the extract was then consigned to the column, the sensitivity lowered and the fractions having the same retention times as LSD and iso-LSD (Fig. 4), respectively, collected individually, the excitation shutter on the fluorimeter being closed during collection to prevent photodecomposition[7]. The resulting fractions each comprised approx. 2 ml of solution.

TLC. The fraction having the shorter retention time (approx. 9 min) was treated with 3 drops of an aqueous solution containing 1% acetic acid and the resulting solution evaporated to approx. 1 ml. 40–50 mg of solid sodium bicarbonate was added to render the solution basic and this was then extracted with 2 × 2 ml of 1,2-dichloroethane. The extracts were combined, dried by passage through anhydrous sodium sulphate, concentrated to a small volume and applied to a TLC plate as a discrete spot. Similar spots containing, respectively, 18 and 24 ng of lysergide tartrate were also applied to the plate. The plate was eluted first with 1,2-dichloroethane to remove any fatty material and then with a mixture of acetone–chloroform–methanol (15:4:1). The LSD was identified first as a blue fluorescent spot under irradiation with long-wave (360 nm) ultraviolet light (Fig. 5) and then by spraying with *p*-dimethylaminobenzaldehyde reagent followed by warming (Fig. 6).

The fraction having the longer retention time on HPLC (approx. 13 min) was processed in a similar fashion but with the omission of the acetic acid. The eluting solvents used on this extract were acetone–chloroform–methanol (4:1:5) and the controls used were iso-LSD and a mixture of LSD and iso-LSD. The plates were visualised as described above (Figs. 7 and 8).

Mass spectrometry. For confirmation by mass spectrometry the spot having the same retention time as the LSD controls was located under long-wave ultraviolet light, scraped off, moistened with 25 μl of 1 *N* ammonium hydroxide solution and

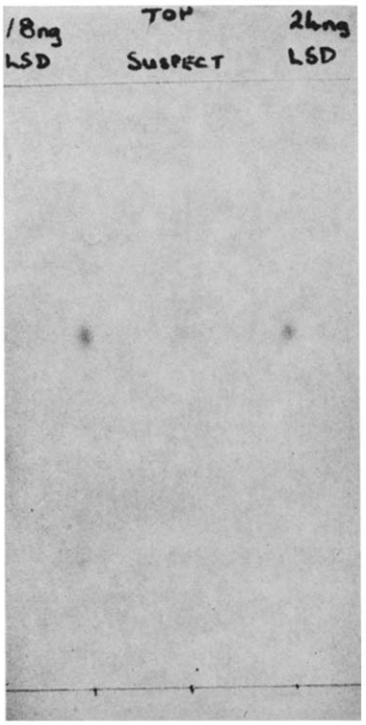


Fig. 5. TLC plate showing suspect LSD fraction viewed under UV light.

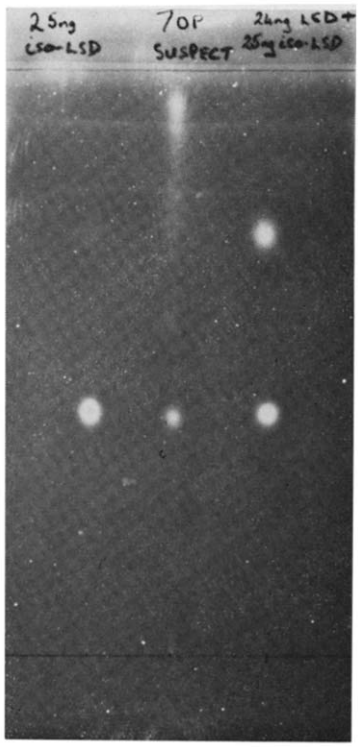
Fig. 6. TLC plate showing suspect LSD fraction after spraying with *p*-dimethylaminobenzaldehyde reagent.

extracted with 1,2-dichloroethane (2 × ½ ml). The extracts were combined and concentrated to a volume of 1–2 μl. Approximately 5 μl of a solution of tartaric acid in methanol (134 ng/μl) was added and the resulting solution transferred to a probe tube, evaporated to dryness and inserted into the mass spectrometer.

RESULTS AND DISCUSSION

The method described has been applied successfully to samples from persons believed to have taken LSD. Urine specimens from people known not to have taken LSD have also been examined and no peaks having the same retention time as either LSD or iso-LSD have appeared on the chromatogram. Additionally we know of no fluorescent compound that has the same retention time as LSD on HPLC under the conditions described. Specimens to which known amounts of LSD tartrate have been added have also been processed. In all cases, including spiked samples, chromatograms displayed peaks with retention times corresponding to LSD and iso-LSD. This indicates that LSD is partially isomerised to iso-LSD in the extraction procedure. Both peaks have also been present in the limited number of blood, plasma and bile samples that have also been examined.

In order to estimate the amounts of LSD recovered, 1 μl of the extract was



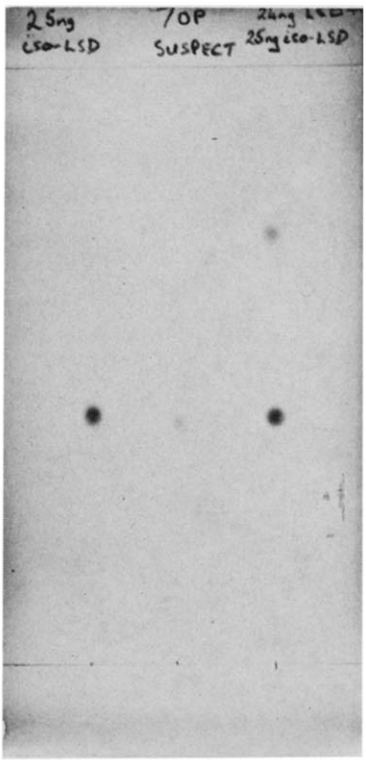


Fig. 7. TLC plate showing suspect iso-LSD fraction viewed under UV light.

Fig. 8. TLC plate showing suspect iso-LSD fraction after spraying with *p*-dimethylaminobenzaldehyde reagent.

injected on to the HPLC column and the area of the resulting peak compared with that obtained from 1 μl of a freshly prepared solution of LSD tartrate of known concentration. The amounts of LSD (calculated as the tartrate) isolated from the specimens so far examined range from 0.3–19.5 ng/ml. The efficiency of the extraction procedure is approx. 70%.

The extraneous fluorescent spot on the TLC plates (R_F *ca.* 0.9) appears to originate in the HPLC eluent.

The identity of LSD extracted from a case specimen has been confirmed by electron impact mass spectrometry: *m/e* 323 (100); 221 (92); 181 (64); 222 (60); 223 (50); 196 (39); 324 (28)[8].

ACKNOWLEDGEMENT

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