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Determination of Lysergic Acid Diethylamide in Body Fluids by High-Performance Liquid Chromatography and Fluorescence Detection—A More Sensitive Method Suitable for Routine Use

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ABSTRACT: A new method for determination of lysergic acid diethylamide (LSD) in body fluids by high-performance liquid chromatography and fluorescence detection was developed based on previously published methods. The new method is suitable for confirmation of samples tested positive by immunoassay, avoiding loss of LSD by absorption to surfaces. The reduced loss of LSD results in improved sensitivity. This is achieved by adding ethylene glycol to the samples, which cover glass surfaces. This principle can similarly be used to improve analysis of other drugs.

Body fluids for analysis included urine and whole blood. An internal standard was applied for quantification of LSD.

The new method offers satisfying precision data and has a detection limit of less than 0.05 ng/nL.

KEYWORDS: forensic science, forensic toxicology, lysergic acid diethylamide, high-performance liquid chromatography, fluorescence detection, ethylene glycol

Soon after its discovery by Hofmann in 1938 lysergic acid diethylamide (LSD) changed from a legally sold drug used in psychiatry to a drug that was consumed illegally by many people. The peak of its illegal consumption was reached around 1965, followed by a steady decline (14,28).

Being out of the focus of public perception for many years, LSD seems to play a more important role today. There is evidence that its use has been increasing again since about 1988 (3,9,30). Thus it is more and more necessary to have effective methods for identification of LSD in body fluids.

Determination of LSD (Fig. 1) in body fluids is a challenging task; usually, only very low doses of LSD are orally consumed, about 0.5 to 2.0 μg/kg body weight (4,5,31,33). The reported plasma clearance half-life is about 2.9 h to 5.1 h (1,4,26). Most of the drug is metabolized, but not all of the metabolites are identified yet (9). As a consequence, the determination concentrates on LSD excreted unchanged—1 to 2% (4,25) in human urine, for example.

LSD can be determined from body fluids with several different methods, including immunoassay (2,5,7,9–11,22,24,31,32,36), gas chromatography/mass spectrometry (12,16,20,21,25–27,29) and liquid chromatography (4,6,8,10,11,13,15,17–19,23,34,35).

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A common method is high-performance liquid chromatography (HPLC). Compared with other methods, HPLC offers good specificity and sensitivity with less demand of instrumentation and thus lower costs (9).

But even though previously released methods based on HPLC offer fairly good results (4,10,11,15,34), there seemed to be possibilities of further improvement, especially as far as sensitivity is concerned. The aim of this investigation was the development of a more sensitive method for determination of LSD in body fluids by HPLC and fluorescence detection, suitable for routine examination of body fluids. The new method is based on the method described by Besserer in 1993 (4).

Materials and Methods

Chemicals

Methanol, triethylammonium phosphate buffer 1.0 M, acetonitrile, ethylene glycol and water.

Solutions used for solid-phase extraction and HPLC were prepared as follows:

Buffer-solution 1:	25 mL triethylammonium phosphate buffer 1.0 M in 1 L water, pH 2.90.
Buffer-solution 2:	buffer-solution 1 with acetonitrile (80:20), pH 3.30, used for solid-phase extraction and as mobile phase of HPLC.
Lavage-solution:	acetonitrile with water (90:10).

Pharmaceuticals

Crystalline LSD was purchased from Sigma (Deisenhofen, Germany). Methysergide (Fig. 2), sold as ''Deseril retard'' by Sandoz, was used as the internal standard.

Extraction Columns

DetectAbuse Type R (content: Amberlite XAD2 200 mg) (Biochemical Diagnostics, NY).

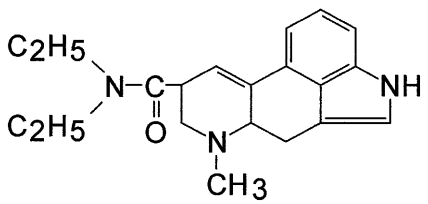

FIG. 1—*Lysergic acid diethylamide (6).*

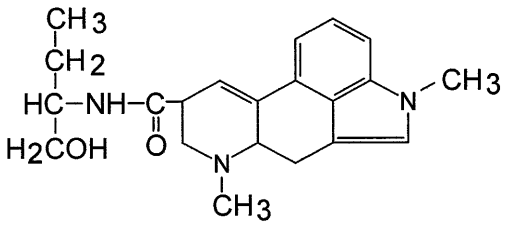

FIG. 2—*Methysergide (37).*

Calibrators and Samples

Human urine, to which LSD was added, was used for calibration and controls. Forensic urine samples positive for LSD were obtained from the following institutions: Institut für Rechtsmedizin der Ludwig-Maximilians-Universität München, Institut für Rechtsmedizin der Universität Ulm, Der Polizeipräsident in Berlin.

Forensic blood samples positive for LSD were obtained from the Institut für Rechtsmedizin der Ludwig-Maximilians-Universität München.

Instrumentation and Chromatographic Parameters

For the solid-phase extraction the extraction columns were placed on a 12-place vacuum manifold. A rotational volatilizer was used at 65°C. HPLC was performed with a LiChroSphere 60 RP-Select B (5 μm) column (E. Merck, Darmstadt, Germany); the injection volume was 20 μL.

Wavelengths for fluorescence detection were 320 nm (excitation) and 420 nm (emission).

Extraction Procedure

Preparation of the samples: 2 mL of each urine sample was mixed with 2 mL of buffer-solution 2 and 1 mL of internal standard. Blood samples were homogenized by adding distilled water (1:1) and centrifuged at 15 000 rpm for 5 min. Two milliliters of the supernatant were mixed with 2 mL of buffer-solution 2 and 1 mL of internal standard.

The extraction column was placed on the vacuum manifold. It was conditioned by washing with 2 × 2 mL methanol and activated by washing with 1 × 2 mL buffer-solution 2. The sample (2 mL, prepared as described above) was applied to the column and completely drawn through it by a vacuum. For the washing step 2 × 2 mL buffer-solution 2 was used, followed by 1 × 1 mL lavage-solution. LSD was eluted with 2 × 1 mL methanol and kept in glass tubes.

In the next step, 25 μL ethylene glycol was added to the eluate. The eluate was then evaporated carefully in a rotational volatizer at 65°C. After evaporation of the methanol, 300 μL of methanol was added to clean the wall of the glass tube from adhering LSD. The evaporation was then repeated.

Twenty-five μL of buffer-solution 2 was added, followed by centrifugation at 5000 rpm for 3 min to keep the whole fluid on the bottom of the glass tube. Twenty μL of this fluid was used for HPLC.

Results

With the new method as described above the precision data as shown in Tables 1 and 2 were found. The detection limit of the new method is 0.05 ng/mL (obtained by analysis of spiked urine samples) (Fig. 3).

Urine as well as blood samples could be analyzed with the described method. To meet forensic standards, positive results for LSD with the new method should be confirmed with another method, because interfering substances from biologic material might imitate false positive samples.

TABLE 1—*Precision in series (*n = *10).*

Concentration of LSD in Urine	Standard Deviation	LSD/Methysergid-Ratio Mean Values	LSD/Methysergid-Ratio Range of Values
1 ng/mL	36.33%	0.57	0.27–0.87
10 ng/mL	9.83%	1.77	1.55–2.04

TABLE 2—*Precision from day to day (*n = *10).*

Concentration of LSD in Urine	Standard Deviation	LSD/Methysergid-Ratio Mean Values	LSD/Methysergid-Ratio Range of Values
1 ng/mL	37.70%	0.43	0.20–0.82
10 ng/mL	13.55%	1.04	0.88–1.26

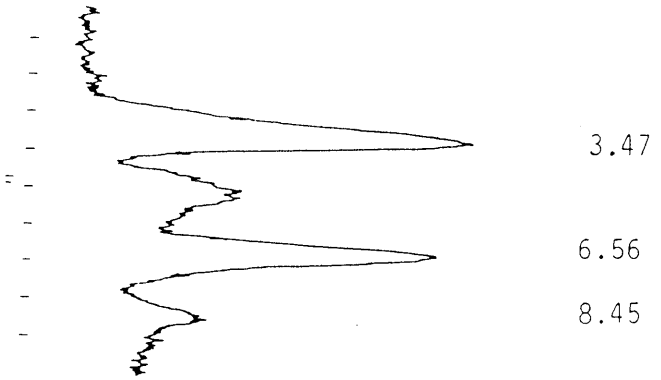

FIG. 3—*Chromatogram at a concentration of LSD of 0.05 ng/mL urine: peak of methysergide at Rt 6.56, peak of LSD at Rt 8.45 (Rt = retention time in minutes).*

Discussion

The abuse of LSD has been increasing again for some years (3,9,30). Although there are several different methods for its determination from body fluids, these methods are either not sensitive enough for routine examination of biologic forensic material, or are more expensive.

The aim of this investigation was the development of a more sensitive method for determination of LSD in body fluids suitable for routine use, good reproducibility of a high sensitivity was very important.

HPLC and fluorescence detection were used because of their advantages compared with other methods for routine determination of LSD; HPLC offers high sensitivity with less demand of instrumentation and thus lower costs (9). Compared with the method of Besserer (4), on which the described method was based, improved sensitivity and the possibility of quantitative statements were demanded.

To improve sensitivity, several changes had to be introduced:

—For solid-phase extraction, Amberlite XAD2 200 mg extraction columns were used (Biochemical Diagnostics, NY). These columns contain artificial resin; the variations of natural material are avoided.

—A very important problem with the determination of LSD is loss of substance by adhesion to glass tubes during volatilization of the eluate (9). To reduce this loss in the new method, ethylene glycol was added to the eluate after solid-phase extraction. Ethylene glycol is a less volatile alcohol, covering and protecting the glass surface.

With these changes it was possible to improve the detection limit ten times, to 0.05 ng/mL, compared with the method of Besserer.

Quantitative statements were made possible by the introduction of an internal standard. Methysergide was used as the internal standard, a substance with a molecular structure similar to LSD.

The described method can be used alternatively for routine examination of body fluids suspicious of drugs, offering sufficient precision data and high sensitivity. The principle of reduction of loss of substance by adhesion to glass surfaces can similarily be used to improve analysis of other drugs. The described method is in use at the Institut für Rechtsmedizin der Universität München.

It is necessary for positive results to be confirmed with another method, because interfering substances from biologic material might imitate false positive samples.

Recently it has been shown that methods based on immunoaffinity chromatography in combination with HPLC offer possibilities of further improvements in specificity (6). Immunoaffinity extraction columns will be used for extraction with the addition of ethylene glycol as described above. In this way, the risk of extracting interfering substances from biologic material during the extraction procedure will be minimized.

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