Stability Study of LSD Under Various Storage Conditions

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Abstract

A controlled study was undertaken to determine the stability of LSD in pooled urine samples. The concentrations of LSD in urine samples were followed over time at various temperatures, in different types of storage containers, at various exposures to different wavelengths of light, and at varying pH values. LSD concentrations were measured quantitatively by the Abuscreen RIA and by HPLC using a fluorescence detection method. Good correlation was observed between the immunoassay and the fluorescent integrity of the LSD molecule. Thermostability studies were conducted in the dark with various containers. These studies demonstrated no significant loss in LSD concentration at 25°C for up to 4 weeks. After 4 weeks of incubation, a 30% loss in LSD concentration at 37°C and up to a 40% at 45°C were observed. Urine fortified with LSD and stored in amber glass or nontransparent polyethylene containers showed no change in concentration under any light conditions. Stability of LSD in transparent containers under light was dependent on the distance between the light source and the samples, the wavelength of light, exposure time, and the intensity of light. After prolonged exposure to heat in alkaline pH conditions, 10 to 15% of the parent LSD epimerized to iso-LSD. Under acidic conditions, less than 5% of the LSD was converted to iso-LSD. We also demonstrated that trace amounts of metal ions in buffer or urine could catalyze the decomposition of LSD and that this process can be avoided by the addition of EDTA. This study demonstrates the importance of proper storage conditions of LSD in urine in order to insure proper analytical testing results over time.

Introduction

(+)-Lysergic acid diethylamide (LSD) is a hallucinogenic drug that acts on the central nervous system. The hallucinatory effect of LSD can alter sensory perception, states of consciousness, and thought processes. All of these effects have contributed to the increase in LSD use (1–4). Detection of LSD and its metabolites in body fluids is still a challenge because of the small dosage (20–80 mg/dose) (4) of drug administered to humans, and the rapid and extensive metabolism of the drug. Limited knowledge has been accumulated regarding the chemical properties and stability of LSD. It is known that LSD is unstable under prolonged exposure to heat, alkaline conditions, and UV light irradiation (5–9). Therefore, it is necessary to explore the best storage and treatment conditions for LSD urine specimens to assure proper drug screening results.

In this paper, we describe a study on the stability of LSD under various storage conditions. Studies included the effects of light, temperature, pH, EDTA, metal ions, and type of storage container at LSD concentrations of 0.5 and 500 ng/mL. The Abuscreen® LSD RIA method was used to determine the immunochemical reactivity of 0.5-ng/mL LSD solution following various treatments. To avoid loss of LDS during sample extraction, urine fortified with 500-ng/mL LSD was directly injected for high-performance liquid chromatography analysis with fluorescence detection. LSD was well separated from other fluorescent chemicals present in normal urine. To ensure adequate buffering capacity when studying pH effects, buffer solutions with desired pH values were fortified with LSD to a final concentration of 500 ng/mL. In this study, the most suitable storage conditions for LSD clinical samples were determined by using two independent methods. The mechanism for the decomposition of LSD was also investigated.

Materials and Methods

Reagents

The Abuscreen radioimmunoassay (RIA) reagents for the detection of LSD, the Abuscreen RIA urine calibration standards (0, 0.25, 0.5, and 1.0 ng/mL) and the LSD were manufactured by Roche Diagnostic Systems (Somerville, NJ). The concentration of these LSD standards was confirmed by gas chromatography–tandem mass spectrometry (GC–MS–MS) methods. All other chemicals were purchased from Fisher (Pittsburgh, PA). Pooled human urine (pH 7.0) was collected and determined to be negative for LSD by RIA, and for the SAMHSA five panel (Canabinoids, Opiates, Cocaine metabolite, Amphetamine, and Phencyclidine) by OnLine™ method. The urine pool and various buffers were fortified with LSD to yield a concentration of 500 ng/mL.

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LSD under various storage conditions

LSD exposed to light

Ultraviolet light. Aliquots (3 mL) of 0.5 ng/mL or 500 ng/mL LSD in urine were placed in a 15-mL transparent polypropylene tube (Corning), a 3.5-mL nontransparent high density polyethylene tube (HDPE) (Roche), a 4-dram amber glass vial (Kimble), or a 4-mL quartz cuvette. A CC-80 ultraviolet fluorescence analysis cabinet (Spectronics Corp.) was used to conduct the ultraviolet light irradiation experiments using wavelengths peaked at 254 nm or 365 nm with 345 W of power. The distance between the light source and the samples was approximately 20 cm. The LSD samples were subjected to UV irradiation for various times up to 8 h at 25°C. Samples kept in the dark at 25°C were used as controls.

Fluorescent light. Aliquots urine containing 0.5 or 500 ng/mL LSD were placed in various containers as described and exposed to normal room light (ceiling fluorescent light, at a distance of 2 m from samples), or placed 15 cm (light source to the top of the samples) directly under a 15 W fluorescent light. The LSD samples were subjected to fluorescent light for different time periods for up to 4 weeks. Experimental temperature was controlled at 25°C.

LSD exposed to elevated temperatures

In urine. Aliquots of urine containing 0.5 or 500 ng/mL LSD were placed in appropriate containers, protected from light, and incubated at 25, 37, and 45°C for various times up to 4 weeks. Samples kept at 4°C and in the dark were used as controls.

In buffer of varying pH containing EDTA and metal ions. LSD was dissolved in 10mM sodium citrate buffer (pH 5.5), 10mM potassium phosphate buffer (KPi, pH 7.0), 10mM MOPS buffer (pH 7.0), or 10mM Bicine (pH 8.5) to a final concentration of 500 ng/mL. All buffers contained 0.1% Tween 20 and 150mM sodium chloride. All buffers were prepared with or without the addition of 5mM of EDTA. Ferric chloride (0.5mM final concentration) was also added to LSD-fortified citrate, MOPS, and Bicine buffers. No EDTA was added to these solutions. Three milliliters of each LSD solution was stored in a polyethylene container and incubated at 45°C for 2 or 4 weeks in the dark. Samples kept in the dark at 4°C were used as controls.

Analysis of LSD samples stored under various conditions

Analysis of LSD samples by the RIA method. All LSD samples with a starting concentration of 0.5 ng/mL were analyzed in triplicate by RIA. The Abuscreen LSD RIA was conducted as specified by the manufacturer. Briefly, 200 µL of ^{125}I-LSD reagent and 500 µL of secondary antibody solution were added to a 100-µL sample. After vortex mixing, 200 µL of anti-LSD serum reagent was added. The solution was vortex mixed again, incubated at 25°C for 1 h, then centrifuged for 10 min at 2000 $\times g$ in a swing bucket rotor. Supernatants were decanted, and the pellets were counted in a Micromedic 4/200 automated gamma counter. LSD standards with concentrations of 0, 0.25 0.5, and 1.0 ng/mL were used to generate a calibration curve. Using this curve, the concentration of LSD in the samples was determined.

Analysis of LSD samples by HPLC–fluorescence method. All LSD samples with a starting concentration of 500 ng/mL were analyzed in duplicate by directed injection using HPLC with fluorescence detection. The HPLC-fluorescence analysis of LSD was conducted with a Waters 600 multisolvent delivery system equipped with a Waters 420 fluorescence detector (Waters, Milford, MA). Separation of the LSD from other fluorescent compounds in urine was accomplished on a reversed-phase µBondapak C18 column (Waters, Milford, MA) using a mobile phase of 0.05M potassium phosphate (pH 7.5) with acetonitrile. Acetonitrile concentrations were increased from 10 to 40% in 20 min at a flow rate of 2 mL/min. The eluent was monitored using an excitation wavelength of 340 nm and an emission wavelength of 425 nm. The degree of decomposition was determined by the reduction of the LSD peak intensity. LSD in urine at concentrations of 0, 100, 300, and 500 ng/mL was used to generate a calibration curve using a linear regression method. The concentration of LSD in the samples was then estimated from this curve.

Results and Discussion

Analysis of LSD samples by the RIA method

The standard deviation (SD) for the RIA method, as expressed in percent loss in RIA reactivity, was found to be about 2.5% ($n = 6$). We estimated that >7.5% loss (or 3 SD of percent loss) represented significant decomposition of the LSD compound.

Separation of LSD in urine using the HPLC method

In order to reduce the interference from other fluorescent compounds in urine, sample pretreatment is generally performed before a urine sample containing LSD is applied to a HPLC column. However, multiple preparation steps and the use of alkaline extraction conditions during sample extraction could potentially result in LSD decomposition (6,9). Therefore, for HPLC analysis, a 500-ng/mL LSD concentration was used instead of 0.5 ng/mL, and the samples were assayed by direct injection. Conditions were established such that LSD was well separated from other fluorescent compounds (Figure 1). These conditions were also found to be suitable for the detection of iso-LSD, which eluted 1.1 min later than LSD. The response for iso-LSD was slightly less than that of LSD under the same excitation and emission conditions. The standard deviation for the method, expressed in percent loss, was found to be about 3.0% ($n = 6$). We estimated that >9.0% loss (or 3 SD of percent loss) in fluorescent intensity represented significant decomposition of the LSD compound.

LSD exposed to light

It is well known that LSD is sensitive to UV light exposure, and it is believed that the C-9,10 double bond of LSD undergoes photocatalytic hydration (5,6,10,11). A potential structural change at this position is indicated if a change in fluorescence intensity occurs when compared to a control. The Abuscreen RIA can also be used to detect these types of changes. The LSD antibodies used in the Abuscreen RIA were generated using an LSD analogue derivatized through the indole nitrogen. It can be predicted that this type of antibody could preferentially

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recognize the part of the LSD molecule that is distal to the indole nitrogen (12,13). The changes in the C-9,10 bond caused by exposure to UV light should lead to a loss in antibody-binding activity.

Table I indicates that amber glass or nontransparent polyethylene containers protected LSD better than quartz or transparent polypropylene containers following exposure to light at 365 nm. All but the quartz container were capable of blocking light at 254-nm. Because a 365-nm wavelength is closer to the excitation wavelength of LSD (320 nm), exposure to that wavelength resulted in greater LSD decomposition than exposure to the 254-nm wavelength source under the same conditions despite the greater energy of 254-nm light. Normal fluorescent light consists of a wide range of wavelengths, including UV light. Table II illustrates that fluorescent light can cause decomposition of LSD in transparent containers when they are placed in close proximity to the light source. Under these conditions, the half-life of LSD was approximately 4 weeks. As the distance between the source of fluorescent light and the samples increased, the percent of LSD decomposition decreased. The results demonstrate that LSD can withstand normal room light conditions at a constant temperature of 25°C for 1 week without noticeable structural change. In addition, excellent correlation between fluorescent integrity and immunoreactivity was observed under

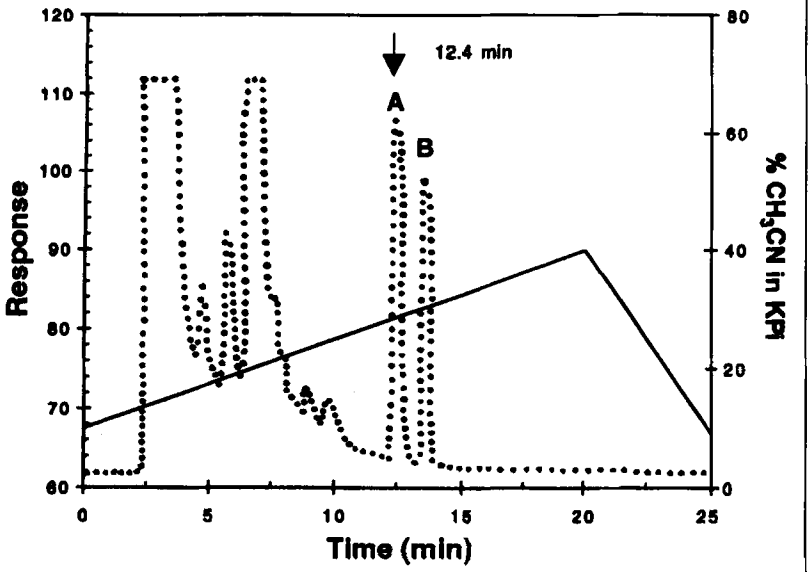

Figure 1. HPLC elution profile for 500 mL of 500 ng/mL LSD in normal human urine. A, LSD and B, iso-LSD.

Table I. LSD Stability: The Effect of UV Light*

	% LSD Decomposed†															
	HPLC–Fluorescence (1SD = 3.0%)								RIA (1SD = 2.5%)							
	254 nm				365 nm				254 nm				365 nm			
Container type	1h	2h	4h	8h	1h	2h	4h	8h	1h	2h	4h	8h	1h	2h	4h	8h
Amber glass	0	3	1	1	2	2	1	1	8	4	2	0	4	2	2	6
Nontransparent polyethylene	0	2	4	0	3	2	7	2	5	3	4	1	3	1	4	6
Transparent polypropylene	10	10	4	3	14	22	35	53	6	4	6	0	4	10	24	52
Quartz	10	13	17	28	8	28	38	53	10	18	14	20	10	26	36	58

* 345-W light, 20 cm from light, 25°C.
† Decompositions of less than 9.0% for HPLC–fluorescence and less than 7.5% for RIA are not statistically significant.

Table II. LSD Stability: The Effect of Fluorescent Light (25°C)

	% LSD Decomposed															
	HPLC–Fluorescence (1SD = 3.0%)								RIA (1SD = 2.5%)							
	15 cm from light				2 meters from light				15 cm from light				2 meters from light			
Container type	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks
Amber glass	0	0	8	10	4	0	4	15	0	4	10	12	6	4	12	14
Nontransparent polyethylene	2	3	7	11	5	2	5	12	3	3	8	11	4	3	10	12
Transparent polypropylene	6	15	31	64	0	2	12	0	0	14	26	52	0	12	14	8

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these conditions. This was demonstrated and confirmed by the loss of fluorescent activity and low binding activity with the Abuscreen RIA LSD antibodies. The binding activity of the On-Line LSD reagents to the light-decomposed LSD was also found to be low (data not shown). Therefore, it is necessary to protect LSD from light, especially near UV light.

LSD exposed to elevated temperature

LSD is unstable under prolonged heat exposure, although the mechanism of this thermal decomposition is not yet fully understood (6,10). In buffers at a pH of 7.0 or higher, approximately 10% of LSD was converted to iso-LSD after 1 week at 45°C or 2 weeks at 37°C. Longer incubations did not significantly change the ratio between LSD and iso-LSD (14). Table III summarizes the stability studies of LSD in urine under elevated temperatures using HPLC–fluorescence and RIA methods. Results obtained from both methods agreed well with each other. Thermal decomposition of LSD appeared to be independent of the containers used. Urine samples may be stored at 25°C for up to 4 weeks with less than 15% loss, and can be exposed to 37°C and 45°C for up to 3 days with less than 10% loss. Approximately 40% of the LSD was decomposed after 4 weeks at 45°C.

Figure 2 shows a typical HPLC elution profile for 500 ng/mL LSD in urine after exposure to 45°C for 4 weeks. The intact LSD eluted at 12.4 min, with another peak appearing at 13.5 min. Separate experiments using mixtures of LSD and iso-LSD demonstrated the second peak to have the same elution time as that of iso-LSD. We therefore have assigned the peak at 13.5 min in the elution profiles to the iso-LSD. The ratio between the emission intensities of LSD and iso-LSD was approximately 85 to 15 in Figure 2. The cross-reactivity of the Abscreen LSD assay to iso-LSD is low (2.2%) (14). The epimerization of LSD did not account for all fluorescence intensity changes measured by the HPLC-fluorescence method or for the reduction in immunoreactivity as indicated by the RIA method. Approximately 40% of LSD tested decomposed after 4 weeks at 45°C. Of this amount, only 7% was converted to iso-LSD. The remaining 33% was converted to nonfluorescent compound(s) or to more hydrophilic compound(s) which were eluted at an earlier time with other fluorescent compounds in urine. These compounds were also undetected by the RIA method.

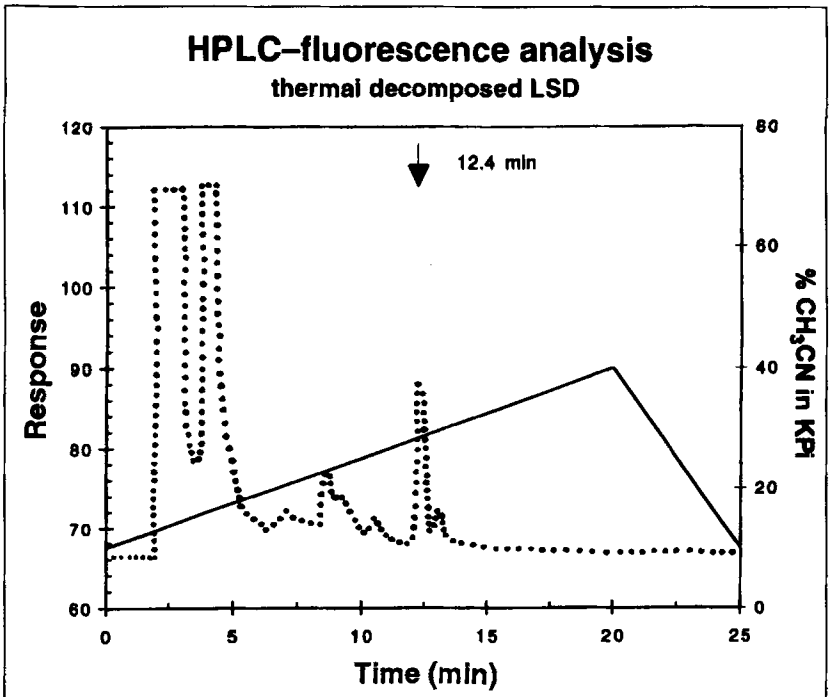

Figure 2. HPLC profile for 500 mL of 500-ng/mL LSD in normal human urine after incubating at 45°C for 4 weeks in a polyethylene container.

The effect of pH, EDTA, and metal ions

Table IV summarizes the effects of pH and EDTA on the stability of LSD. Decomposition mechanisms of LSD seemed to be buffer dependent. Epimerization was determined to be pH dependent. This observation is supported by our previous NMR studies where approximately 10% of iso-LSD was obtained at temperatures of 37°C or higher and at pH levels above 7.0 (14). In Bicine buffer at pH 8.5, the only decomposition of LSD was through epimerization. Ten to fifteen percent of LSD was epimerized to iso-LSD after 4 weeks at 45°C. The addition of EDTA did not prevent LSD from epimerization. In the citrate buffer at pH 5.5, 20 to 25% of the LSD was decomposed after 4 weeks at 45°C. As predicted by the low pH, less than 5% was epimerized to

Table III. LSD Stability: The Effect of Heat (In the Dark)

	% LSD Decomposed																							
	HPLC–Fluorescence (1SD = 3.0%)												RIA (1SD = 2.5%)											
	25°C				37°C				45°C				25°C				37°C				45°C			
Container type	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks	3 days	1 wk	2 wks	4 wks
Amber glass	4	0	3	10	0	16	26	34	8	16	29	43	0	4	12	10	8	18	16	30	6	18	18	36
Nontransparent polyethylene	3	2	2	10	2	12	23	30	6	18	28	40	1	6	10	8	6	15	20	28	6	18	18	42
Transparent polypropylene	0	1	2	12	6	13	21	33	0	20	31	46	0	8	6	8	0	14	22	26	4	16	24	44

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iso-LSD. The remaining decomposition products were not identified. However, as decomposition could not be avoided by the addition of EDTA, the remaining loss was determined to be independent of metal ion catalysis. An 80% loss of LSD was observed in the potassium phosphate buffer (pH 7.0) in the absence of EDTA after 4 weeks at 45°C. Approximately 10% of this loss was, as predicted, due to conversion to iso-LSD. The remaining loss could be reduced to 40% by the addition of EDTA. Finally, in the MOPS buffer at pH 7.0, under the same thermal conditions, much less decomposition of LSD was observed. The decomposition products, other than those formed as a result of epimerization, were eliminated by the addition of EDTA.

To further investigate the catalytic effects of metal ions on the decomposition of LSD, we added 0.5mM ferric chloride to the LSD-fortified citrate, MOPS, and Bicine buffers described. Table V illustrates that in all cases, increasing the Fe^{3+} concentration resulted in increased LSD loss. Fe^{3+} may not be the only or the most efficient metal ion in buffer or urine which catalyzes the decomposition of LSD. Metal ions, such as Fe^{2+}, Mn^{2+}, or Cu^{2+}, may play similar or greater roles. However, epimerization and metal ion catalyzed hydration can not account for all LSD decomposition in KPi buffer. Therefore, there are other mechanisms for the decomposition of LSD in KPi buffer that have yet to be investigated.

Conclusion

We conducted a thorough investigation on the stability of LSD in pooled urine and in several buffers under various storage conditions. The concentrations of intact LSD in samples were measured over time at various temperatures and pH, under different wavelengths and intensities of light, and in different storage containers. We found good correlation between the HPLC–fluorescence method and the RIA method used to monitor the decomposition of LSD. Although LSD is sensitive to light, decomposition attributable to light under normal laboratory conditions should be minimal. Fluorescent light, when in close proximity to the samples, can cause considerable decomposition under unprotected conditions (i.e., transparent containers). To prevent the photo decomposition of LSD, containers that can effectively block near UV light, such as amber-glass bottles or high-density polyethylene bottles, should be used. At elevated temperatures, LSD is unstable in urine; however, the thermal stability of LSD seems to be independent of the type of container used (amber glass, non-transparent polyethylene and transparent polypropylene). Urine specimens may be stored at 25°C for up to 4 weeks under normal laboratory conditions without noticeable decomposition. However, at 37 or 45°C, greater than 15% decomposition will be observed after 3 days. We have also confirmed that under alkaline conditions, approximately 10% of LSD epimerized to iso-LSD after prolonged heat exposure. Under acidic conditions, less than 5% of LSD was converted to iso-LSD. Therefore, epimerization is not a major thermal decomposition product of LSD. Finally, we have demonstrated that trace amounts of metal ions in buffer or urine cause the decomposition of LSD by catalyzing the hydration of the C-9,10 double bond, and that this process can be avoided by using a saturating concentration of EDTA to chelate the metal ions. Examples of this type of metal ion catalyzed hydration reaction has been documented (15).

This study is the first controlled study that demonstrates the importance of proper storage conditions for urine samples containing LSD in order to assure proper analytical results over time. In general, if LSD is stored in amber glass, nontransparent polyethylene, or transparent polypropylene containers under normal laboratory conditions (fluorescent light, 25°C), greater than 90% of the LSD can be maintained safely for up to 2 weeks before freezing. At 37°C or higher temperatures, samples can be stored up to 3 days only. LSD urine samples should be protected from strong sun light or other UV conditions.

Table IV. LSD Stability: The Effect of pH and EDTA*

	% LSD Decomposed			
	pH 5.5 Citrate buffer	pH 7.0 KPi buffer	pH 7.0 MOPS buffer	pH 8.5 Bicine buffer
HPLC–Fluorescence method (1SD = 3.0%)				
Without EDTA				
2 weeks	10	65	24	10
4 weeks	25	80	–	11
With EDTA				
2 weeks	6	27	8	10
4 weeks	20	38	–	13

* In the dark, 45°C, polyethylene container.

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Table V. LSD Stability: The Effect of Metal Ions*

	% LSD Decomposed		
	pH 5.5 Citrate buffer	pH 7.0 MOPS buffer	pH 8.5 Bicine buffer
HPLC–Fluorescence method (1SD = 3.0%)			
Without Fe^{3+}	10	24	10
With Fe^{3+}	47	70	29

* In the dark, 45°C, 2 weeks, polyethylene container.

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