Solid Phase Extraction of Lysergides from Biological Samples

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1 INTRODUCTION

Lysergic acid diethylamide (LSD) is one of the most potent hallucinogenic drugs. Detection of LSD in biological samples can be an analytical challenge due to the low doses consumed and extensive metabolism. LSD's major metabolite 2-oxo-3-hydroxy LSD (OH-LSD) is detectable in urine at 16-43 times the concentration of LSD. The only analogue of LSD to have received widespread interest is the N-methylpropylamide of lysergic acid (LAMPA), and any analytical technique should be capable of separating LAMPA from LSD.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☐ Quantitation
Matrices	Blood, urine
Analytes	Lysergic acid diethylamide (LSD), 2-oxo-3-hydroxy LSD (OH-LSD) (LAMPA included in validation)
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

Blood and urine specimens are fortified with internal standard (d_3-LSD), buffered, and extracted with Cerex® PolyChrom™ CLIN II solid phase columns. the dried eluent is reconstituted in 20% acetonitrile and analyzed by liquid chromatography-electrospray ionization-high resolution mass spectrometry (LC-ESI-HRMS/MS) for both LSD and OH-LSD.

4 SPECIMEN CRITERIA

This procedure uses 2 mL of blood or urine.

5 EQUIPMENT

5.1 Equipment

A. Routine laboratory supplies and equipment, including disposable pipets, graduated cylinders, volumetric flasks, pH paper, vortex mixer, centrifuge, etc.
B. Volumetric flasks (10 and 25 mL)
C. Positive pressure solid-phase extraction manifold with nitrogen supply
D. Evaporator with nitrogen supply

5.1.1 Column

A. Grace Alltima® C18, 150x2.1 mm, 5 µm d_p, or equivalent
B. Guard/Filter

5.2 Consumables

A. 16x100 mm screw-top tubes with caps
B. 12x75 mm and 16x100 mm culture tubes with snap-tops

C. Cerex® PolyChrom™ CLIN II solid phase columns (50mg) 6mL

5.3 Instruments

A. Thermo LTQ Orbitrap XL Hybrid Ion Trap/Fourier Transform Mass Spectrometer
B. Shimadzu HPLC

5.4 Software

Component	Software	Version
Operating System	Microsoft Windows	7 Pro SP 1 / XP Professional
Mass Spectrometer	Foundation	1.0.2 or higher
	Xcalibur	2.1.0 SP1 / 2.0.7
	LTQ Tune Plus	2.5.5
	Shimadzu LC Controller	5.4 / 6.5

5.5 Chemicals/Reagents

Storage/stability determined by manufacturer unless otherwise noted.

5.5.1 Purchased

Chemical or Reagent	Minimum Grade or Purity
Acetic Acid (concentrated)	Certified ACS
Acetonitrile	Optima
Ammonium Hydroxide (concentrated)	Certified ACS
Ammonium Formate	99.99 %
Ethyl Acetate	HPLC
Formic Acid (concentrated)	Optima
Hydrochloric Acid (concentrated)	Certified ACS
Methanol	Optima
Potassium Bicarbonate (anhydrous)	Certified ACS
Potassium Carbonate (anhydrous)	Certified ACS
Sodium Phosphate (monobasic, monohydrate)	Certified ACS
Sodium Phosphate (dibasic, heptahydrate)	Certified ACS
Water	Deionized (DI) and Optima

5.5.2 Prepared

A. Mobile Phase 1 (aqueous) 20 mM ammonium formate, pH 4.4

Add 1.26 g ammonium formate to 1 L of water (Optima grade). Mix well to dissolve, and filter. Add 70 µL concentrated formic acid and verify pH ~4.4, adjusting with concentrated formic acid or concentrated ammonium hydroxide as necessary. Store in glass at room temperature. Stable for 2 weeks.

B. Mobile Phase 2 (Organic): Methanol

	Refer to 5.5.1.
C. Phosphate Buffer (0.1 M, pH 6)	
	To a 500-mL volumetric flask, add 400 mL DI water. Add 6.1 g sodium phosphate, monobasic, monohydrate and 1.6 g sodium phosphate, dibasic, heptahydrate and mix well to dissolve. Verify pH ~6 and dilute to the mark with DI water. Store refrigerated in glass. Stable for 2 months.
D. Carbonate Buffer (0.27 M, pH 9):	
	To a 250-mL volumetric flask, add 200 mL DI water. Add 2.5 g anhydrous potassium carbonate and 5 g anhydrous potassium bicarbonate and mix well to dissolve. Adjust pH to ~9 with concentrated acetic acid or concentrated ammonium hydroxide and dilute to the mark with DI water. Store refrigerated in glass. Stable for 1 month.
E. 0.1 M Hydrochloric Acid	
	In a 100-mL graduated cylinder, dilute 0.8 mL of concentrated hydrochloric acid to 96 mL with DI water and mix well. Store in glass at room temperature. Stable for 6 months.
F. Elution Solvent (25:1 ethyl acetate:ammonium hydroxide)	
	Measure 75 mL of ethyl acetate into a 100-mL graduated cylinder. Add 3 mL of concentrated ammonium hydroxide and mix well. Prepare fresh daily.
G. Reconstitution Solvent (20:80 acetonitrile:water)	
	Combine 4 mL acetonitrile with 16 mL water (Optima grade) and mix well. Store in glass at room temperature. Stable for 6 months.

5.6 Standards/Controls

Storage/stability determined by manufacturer unless otherwise noted.

5.6.1 Purchased

Compound	Stock Concentration
d_3-LSD	100 µg/mL
LSD	1 mg/mL
Iso-LSD	100 µg/mL
LAMPA (lysergic acid methylpropylamide)	1 mg/mL
OH-LSD	100 µg/mL

Purchased from Cerilliant or another approved vendor.

5.6.2 Prepared

A. d_3-LSD Working Internal Standard (100 ng/mL)
To a 25-mL volumetric flask, add 25 µL of the d_3-LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.
B. LSD Intermediate Working Solution (2 µg/ml)

To a 10-mL volumetric flask, add 20 µL of the LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

C. OH-LSD Intermediate Working Solution (2 µg/ml)

To a 10-mL volumetric flask, add 200 µL of the OH-LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

D. Lysergide Control Working Solution (20 ng/ml LSD; 40 ng/ml OH-LSD):

To a 25-mL volumetric flask, add 250 µL of the LSD intermediate Working Solution and 500 µL of the OH-LSD Intermediate Working Solution. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

E. LAMPA Intermediate Working Solution (2 µg/ml)

To a 10-mL volumetric flask, add 20 µL of the LAMPA Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year.

F. iso-LSD Intermediate Working Solution (2 µg/ml)

To a 10-mL volumetric flask, add 200 µL of the iso-LSD Stock Standard. Dilute to the mark with acetonitrile and mix well. Store below 0ºC in glass. Stable for at least 1 year

G. Lysergide Testmix Stock Solution (25 ng/ml each component)

To a 10-mL volumetric flask, add 125 µL each of the OH-LSD, LAMPA, and iso-LSD Intermediate Working Solutions. Dilute to the mark with acetonitrile and mix Well. Store below 0ºC in glass. Stable for at least 1 year.

H. LC-MS Performance Standard (5 ng/ml per component)

Combine 30 µL of the Lysergide Testmix Stock Solution with 120 µL Optima-grade water and mix well. Prepare fresh daily.

I. Negative Control Blood and/or Urine

Purchased from Cliniqa, UTAK, Dynatek, or another approved vendor or prepared in-house from an appropriate blank specimen.

J. Positive Control Blood and/or Urine (0.5 ng/ml LSD; 1 ng/ml OH-LSD)

Prepared the day of extraction by adding 50 µL of Lysergide Control Working Solution to 2 mL of Negative Control Blood or Urine.

6 PROCEDURE

	Step	Note	Reference/Lot
	A. Samples/Controls		
	1. To labeled 16 x 100 mm screw-top tubes add:		
☐	i. 2 mL of biological fluid		
	B. Controls		
☐	1. Negative Control(s) Source		
☐	2. Prepare Positive Control(s) i. Add 50 µL of Control Working Solution to Negative Control(s)		
	C. Internal Standard(s)		
☐	1. Add 20 µL of Internal Standard Working Solution		
	D. Buffer		
☐	1. Add 2 mL of Phosphate Buffer, pH 6		
☐	2. Vortex for 30 seconds		
	E. Centrifuge *(blood and turbid urine specimens only)*		
☐	1. ~3500 rpm for 15 minutes		
☐	2. Decant into a 16 x 100 mm tube		
	F. Extract		
☐	1. Load samples (1 mL/min) on SPE cartridges		
☐	2. Push samples through column using low vacuum setting		
	3. Wash cartridges (1 mL/min)		
☐	i. Add 1 mL Potassium Carbonate Buffer		
☐	ii. Add 2 mL 0.1M HCl		
☐	iii. Add 1 mL of methanol		
☐	iv. Add 3 mL of ethyl acetate		
	4. Elute (by gravity)		
☐	i. Add 4 mL Elution Solvent		
☐	ii. Collect eluent in 12 x 75 mm tubes		
	G. Concentrate		
☐	1. Evaporate to dryness at 55ºC		
	H. Reconstitute		

☐	1. Add 100 µL of Reconstitution Solvent		
☐	2. Vortex and transfer to ALS vial, cap		
☐	**I. Instrumental Analysis** 1. LC/MS: analyze 10 µL i. Analyze LC/MS Performance Standard prior to batch analysis		
	ii. Mobile Phase 1 (aqueous)		
	iii. Mobile Phase 2 (organic)		
	iv. LC Column		

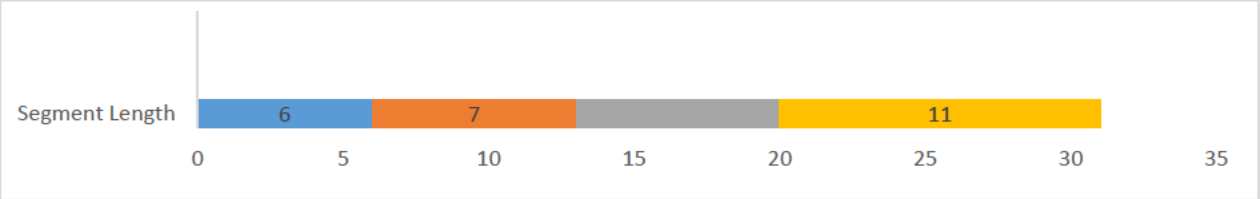
7 ANALYTICAL PARAMETERS

7.1 Shimadzu HPLC Gradient/Conditions

Time (min)	Mobile Phase %		Flow Rate		
	1-Aqueous	2-Organic	(mL/min)	Column Heater (°C)	35
0	80	20	0.30		
3	80	20	0.30	Run Time (min)	31
10	65	35	0.30		
19	52	48	0.30		
20	10	90	0.30		
25	10	90	0.30		
26	80	20	0.30		
31	80	20	0.30		

7.2 LTQ-XL Orbitrap

J. Source Mode: ESI (+)



Segment	Event	Mode	Precursor (m/z)	Isolation Width (m/z)	Collision Energy	Range (m/z)	Analyzer	Resolution
1	1	MS				200-400	FTMS	15000
2	1	MS				200-400	FTMS	15000
	2	MS/MS	356.2	2.0	20	100-400	FTMS	7500
3	1	MS				200-400	FTMS	15000
	2	MS/MS	324.2	2.0	40	100-380	FTMS	7500
4	1	MS				200-400	FTMS	15000

8 DATA ANALYSIS

8.1 Decision Criteria

8.1.1 LC-MS Performance Standard Decision Criteria

8.1.1.1 Chromatography

In order for the LC to be considered in good operating condition, full MS molecular ion traces for each analyte in the performance standard should generate reasonably symmetric chromatographic peaks. m/z 356.196 is traced for OH-LSD and m/z 324.206 for iso-LSD and LAMPA. The retention times of the 3 analytes should be within ± 5 % of the previous run of the performance standard. LAMPA and iso-LSD should be resolved with at least a 50% drop to baseline when displayed in an extracted ion chromatogram (EIC) with a ±0.005 Da tolerance.

8.1.1.2 Mass Spectrometry

In order for the MS to be considered in good operating condition, the correct mass assignments for each of the three analytes in the performance standard should be present. The following ions should be present for each analyte. Each observed mass should be within ±0.005 Da of the theoretical value.

OH-LSD:	MS – 356.196	MS/MS – 338.185 (base peak), 265.096, 237.102
Iso-LSD:	MS – 324.206	MS/MS – 281.164 (base peak), 251.117, 223.122, 208.075
LAMPA:	MS – 324.206	MS/MS – 281.164, 251.117, 223.122 (base peak), 208.075

8.1.2 Batch Acceptance Criteria

d_3-LSD (m/z 327.226) should be detectable in an EIC for every extracted sample. None of the targeted lysergides (LSD, OH-LSD, LAMPA, iso-LSD) should be detected in the Negative Control Sample(s). Both LSD and OH-LSD should be detectable, based upon criteria given in 8.1.2, using the mass spectral parameters of LAMPA for LSD, in the Positive Control Sample(s).

8.1.3 Analyte Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In most cases, all of the below should be met in order to identify LSD or OH-LSD within a biological specimen:

8.1.3.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape,width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs.

Additionally, the following two criteria should be met:

8.1.3.1.1 Retention Time

The retention time of the peak should be within ±5% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard or positive control.

8.1.3.1.2 Signal-to-Noise

To justify the existence of a peak, its signal-to-noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10-fold greater than that for any observed peak at a similar retention time in a Negative Control or blank sample injected just prior to that sample.

8.1.3.2 Mass Spectrometry

The MS/MS fragmentation spectra should meet the criteria given in the *Guidelines for Comparison of Mass Spectra* standard operating procedure (TOX-104) with comparison to a contemporaneously analyzed reference standard or positive control.

a. LSD: (fragments of m/z 324.206) The base peak should be m/z 223.122. m/z 281.164 and either 251.117 or 208.075 will should be used for the calculation of ion ratios.

b. OH-LSD: (fragments of m/z 356.196) The base peak should be m/z 338.185. m/z 265.096 and 237.102 will should be used for the calculation of ion ratios.

8.2 Calculations

8.2.1 Software

Qualitative calculations may be performed by one or more of the following software packages:

A. Thermo Xcalibur
    1. QualBrowser
    2. Tracefinder
B. Microsoft
    1. Excel

9 REPORTING

Refer to CHEM-100, TOX-100 and TOX-101.

10 CORRECTIVE MEASURES

Refer to TOX-101 for guidance on action steps in the event of a quality control failure.

11 Performance Characteristics

11.1 LOD

LSD: at least 150 pg/mL in blood and urine

OH-LSD: at least 300 pg/mL in blood and urine

11.2 Carryover

None identified.

12 Limitations

12.1 Sample Storage

LSD is known to be subject to photodegradation. Standards and processed samples should be stored in darkness whenever possible.

12.2 Interferences

Grossly decomposed or putrefied samples may adversely affect limits of detection. Iso-LSD is an isomer of LSD, and is not chromatographically resolved from LSD. The two compounds yield the same MS/MS fragment ions, but with radically different ion ratios. The presence of significant levels of iso-LSD in a sample will adversely affect the limit of detection for LSD. The current version of TOX-203 does provide a baseline resolution of LSD and Iso-LSD and a reinject under those chromatographic conditions can provide additional information.

13 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 Revision History

Revision	Issued	Changes
01	02/11/2022	Reformat of document. Minor changes to wording in several sections. 1-Added LAMPA description.