LC–MS Analysis of Human Urine Specimens for 2-Oxo-3-Hydroxy LSD: Method Validation for Potential Interferants and Stability Study of 2-Oxo-3-Hydroxy LSD Under Various Storage Conditions*

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Abstract

2-Oxo-3-hydroxy lysergic acid diethylamide (O-H-LSD), a major LSD metabolite, has previously been demonstrated to be a superior marker for identifying LSD use compared with the parent drug, LSD. Specifically, O-H-LSD analyzed using liquid chromatography–mass spectrometry has been reported to be present in urine at concentrations 16 to 43 times greater than LSD. To further support forensic application of this procedure, the specificity of the assay was assessed using compounds that have structural and chemical properties similar to O-H-LSD, common over-the-counter products, prescription drugs and some of their metabolites, and other drugs of abuse. Of the wide range of compounds studied, none were found to interfere with the detection of O-H-LSD or the internal standard 2-oxo-3-hydroxy lysergic acid methyl propylamide. The stability of O-H-LSD was investigated from 0 to 9 days at various temperatures, pH conditions, and exposures to fluorescent light. Additionally, the effect of long-term frozen storage and pH was investigated from 0 to 60 days. There was no significant loss of O-H-LSD under both refrigerated and frozen conditions within the normal human physiological pH range of urine (4.6–8.4). However, significant loss of O-H-LSD was observed in samples prepared at pH 4.6–8.4 and stored at room temperature or higher (24–50°C).

Introduction

The analysis of lysergic acid diethylamide (LSD) in urine is particularly challenging because of the low doses consumed (typically 40 to 150 µg) (1), its extensive metabolism with less than 1% excreted unchanged in urine (2) and its relatively short elimination half-life (3.6 h) (3). Collectively, these factors contribute to an extremely short window of detection (12–22 h) when using the parent drug as the target analyte (4). Gas chromatography–mass spectrometry (GC–MS) analysis is also complicated as LSD is thermally unstable at GC temperatures, relatively involatile, photosensitive and undergoes considerable absorptive losses during extraction and GC–MS analysis (5).

Based on the sensitivity limitations of GC–MS for the detection of LSD, current research efforts have focused on identifying and characterizing LSD metabolites present in concentrations higher than the parent drug. Specifically, several research groups have developed liquid chromatography–ion trap mass spectrometry (LC–MS–MS) (6,7), liquid chromatography–mass spectrometry (LC–MS) (7,8), and gas chromatography–tandem mass spectrometry (GC–MS–MS) (9,10) methods capable of identifying and quantitating LSD and 2-oxo-3-hydroxy lysergic acid diethylamide (O-H-LSD), a prevalent human LSD metabolite (11). O-H-LSD is found at concentrations 16–43 times greater than the parent compound, LSD (6–9). O-H-LSD is not generated during the specimen extraction and analytical processes, nor is its presence due to parent compound degradation in aqueous urine samples (6). Although the analytical integrity of these assays has been characterized in terms of accuracy, precision, linearity, and sensitivity, to the authors' knowledge, none of the assays have been evaluated regarding assay specificity. The purpose of this investigation was to assess the assay specificity by investigating the potential interference of compounds that are structurally similar to O-H-LSD, common over-the-counter (OTC) products, prescription drugs and some of their metabolites, and other drugs commonly tested for in workplace drug-testing

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laboratories. Additionally, the stability of O-H-LSD under various storage conditions that would be expected in typical laboratory or transportation conditions (light, temperature, and pH) was assessed.

Materials and Methods

Preparation of standards

All solvents used for the preparation of standard stock solutions were purchased from Aldrich (Milwaukee, WI) and were high-performance liquid chromatography (HPLC) grade. An O-H-LSD standard (100 µg/mL) purchased from Cerilliant, Inc. (Austin, TX) was diluted with acetonitrile (ACN) to give a final stock solution of 25 ng/mL. A stock solution of the internal standard 2-oxo-3-hydroxy lysergic acid methyl propylamide (O-H-LAMPA) obtained from Radian International (now Cerilliant, Inc., Austin, TX) was prepared in ACN at 200 ng/mL.

Interference standards

OTC drug and pharmaceutical standards consisting of the following drug categories: barbiturates, benzodiazepines, cocaine and cocaine metabolites, tricyclic antidepressants, stimulants, hallucinogens, opiates, and ergot and other alkaloids were obtained from the Armed Forces Institute of Pathology (AFIP, Rockville, MD), Alltech Associates, Inc. (Deerfield, IL), Sigma Chemical (St. Louis, MO), and International Chemical Nexus (Costa Mesa, CA). The multi-constituent standards were spiked into test tubes containing 1000 pg/mL of O-H-LSD and 4000 pg/mL of O-H-LAMPA in methanol at concentrations ranging from 4 to 800 µg/mL. Table I lists the final concentration of all compounds assessed.

Immunoassay screening

LSD immunoassay (IA) screening was accomplished on an

Table I. Compounds Investigated as Potential Interferants in Analysis of O-H-LSD

Compounds related to LSD (mg/L)					
D,L-Tryptophan ethyl ester	2.0	Dihydroergotamine	2.0	Lysergic acid	2.0
Dihydroergocornine	2.0	α-Ergocryptine	2.0	Lysergol	2.0
Dihydroergocristine	2.0	Ergotamine	2.0	*N*-Ethyl-*N*-(*N*-propyl) Lysergide	0.02
α-Dihydroergocryptine	2.0	Ergonovine	0.002	*N*-Ethyl-*N*-(*N*-isopropyl) Lysergide	0.02
β-Dihydroergocryptine	2.0	Iso-LSD	0.5	NorLSD	0.5
Known drugs and medications that interfere with LSD EMIT measurements (mg/L)					
Amitriptyline	0.5	Doxepin	2.0	Verapamil	2.5
Chlorpromazine	0.5	Fluoxetine	0.4		
Desipramine	0.5	Imipramine	0.5		
Compounds unrelated to LSD (mg/L)					
Acebutolol	0.0002	EDDP	0.5	Nalorphine	20.0
Alprazolam	0.2	Ephedrine	0.4	Nicotine	0.5
Amobarbital	0.5	Ethosuximide	0.5	*N*-normethsuximide	0.5
Amoxapine	2.0	Ethotoin	0.5	Nordiazepam	0.5
Amphetamine	0.8	Flurazepam	0.5	Nortriptyline	0.5
Azacyclonol	2.0	Glutethimide	0.5	Oxozepam	0.5
Barbital	0.5	Hydromorphone	20.0	Oxycodone	20.0
Bromazepam	0.5	Hydroxyzine	2.0	PEMA	0.5
Butabarbital	0.5	Lidocaine	0.5	Pentazocine	0.5
Brompheniramine	0.4	Lorazepam	0.5	Phencyclidine	0.4
Caffeine	2.0	α-Methyl-α-propylsuccinimide	0.5	Pentobarbital	0.5
Carbamazepine	2.0	4-Methylprimidone	0.5	Phenobarbital	0.5
Chloroquine	0.4	Medazepam	0.5	Phensuximide	0.5
Chlorpheniramine	0.4	Mefloquine	0.4	Phenylpropanolamine	0.8
Cocaine	0.5	Meperidine	0.5	Primidone	0.5
Codeine	0.5	Mephenytoin	0.5	Proadifen	2.0
Cotinine	2.5	Mephobarbital	0.5	Promethazine	0.5
5,5-Diphenylhydantoin	0.5	Methadone	0.5	Pseudoephedrine	0.8
10,11-Dihydrocarbamazepine	0.5	Methamphetamine	0.8	Quinidine	2.0
Dextromethorphan	0.5	Methaqualone	0.5	Secobarbital	0.5
Diacetylmorphine	20.0	Metharbital	0.5	Temazepam	0.5
Diazepam	0.5	Methsuximide	0.5	Thioridazine	0.5
Diphenhydramine	0.4	Methyl PEMA	0.5		
Doxylamine	2.0	Morphine	0.5		

Olympus AU800 automated chemistry analyzer (Olympus America, Inc., Melville, NY) using the LSD Enzyme-Multiplied Immunoassay Technique (EMIT II) IA kit purchased from Syva, (Palo Alto, CA). LSD was also detected in human urine using the Coat-a-Count® radioimmunoassay (RIA) kit purchased from Diagnostic Products Corp. (DPC, Los Angeles, CA). Calibrators and controls were either purchased from Syva or DPC or prepared in-house. All IA and RIA reagents, standards, and controls were used in accordance with the manufacturers' instructions for the analysis of urine specimens.

Extraction of human urine specimens for LC–MS analysis

An O-H-LSD stock solution of 25 ng/mL was spiked into 5 mL of drug-free urine (Roche Diagnostics Corp., Indianapolis, IN) to prepare O-H-LSD standards at concentrations of 500, 1000, and 1250 pg/mL. Negative controls consisted of drug-free urine. Internal standard (4.0 ng/mL in final solution), 0.25 g of NaCl, and 0.1 mL of concentrated NH_4OH were added to 5-mL aliquots of each O-H-LSD control or stability sample. The resulting solution was immediately extracted into 5 mL of a solution of methylene chloride/isopropanol (85:15). The tubes were sealed using Teflon-lined caps, shaken at 55 cycles/min for 30 min followed by centrifugation at 1350 × *g* for 20 min. The organic layer was removed, evaporated under nitrogen gas at 55°C, and reconstituted with 2 mL of 0.05M sodium phosphate buffer (pH 6.0) followed by solid-phase extraction (SPE) using Clean Screen® CSDAU203 (United Chemical Technologies, Bristol, PA). The columns were conditioned with 2 mL of methanol, 2 mL of deionized water, and 2 mL of 50mM phosphate buffer (pH 6.0). The specimens were applied, and the columns were washed with 2 mL of deionized water, 2 mL of 0.01M HCl, and 2 mL of methanol/water (60:40) and aspirated to dryness. O-H-LSD was eluted with 2 mL of methylene chloride/isopropanol/NH_4OH (70:20:5) and evaporated under a stream of nitrogen at 55°C. The specimens were reconstituted in 200 µL of the LC mobile phase (0.01M ammonium acetate buffer [pH 8.0]/ACN [80:20]).

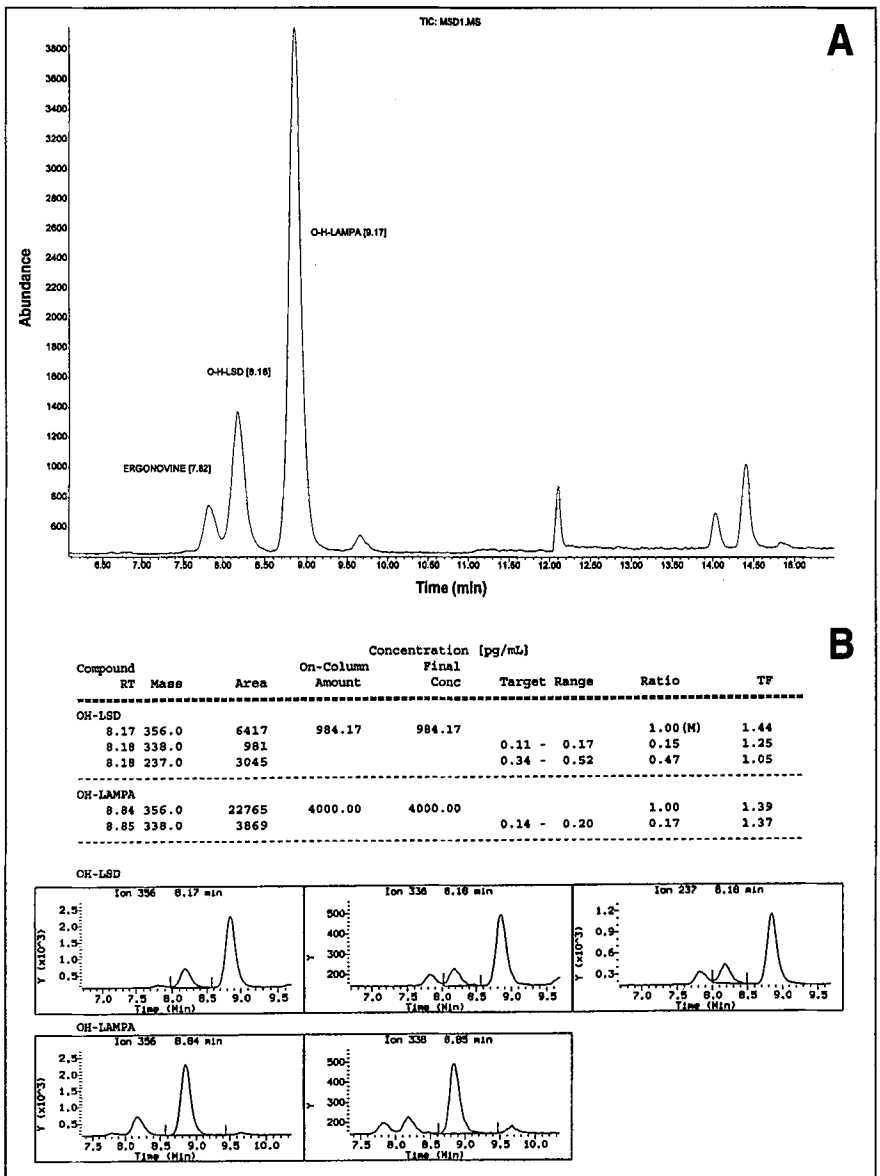

Figure 1. TIC of a sample containing 1000 pg/mL O-H-LSD and 10 ng/mL ergonovine (A) and SIM report of 1000 pg/mL O-H-LSD with 10 ng/mL ergonovine (B).

REMEDi HS (HPLC) analysis

Two-hundred microliters of the internal standard purchased from Bio-Rad Laboratories (Hercules, CA) consisting of *N*-ethyl nordiazepam (2 µg/mL in final solution) and chlorpheniramine (3 µg/mL in final solution) was added to 1.0-mL aliquots of human urine. The mixture was centrifuged and decanted into 2-mL polypropylene cuvettes provided by Bio-Rad Laboratories. The injection volume was 1.0 mL. The mobile phase, exchange buffer, transfer buffer, application buffer, and wash reagent were purchased from Bio-Rad Laboratories. All reagents, standards and controls were utilized in accordance with the manufacturer's instructions for the analysis of urine specimens. The REMEDi HS instrument, purchased from Bio-Rad Laboratories, was equipped with two polymeric cartridges for the purification (reversed phase) and extraction (anion exchange) of basic compounds, and two analytical cartridges (reversed phase and unmodified silica) for compound separation. A check mix purchased from Radian consisted of diazepam (1.5 µg/mL), amphetamine (2.2 µg/mL), imipramine (2.0 µg/mL), morphine (2.0 µg/mL), and hydrocodone (2.0 µg/mL) was used to calibrate the instrument every 50 specimens. Presumptive identification of the unknown compound was based on retention time match and UV_{max} match as compared to an identification library of 760 drugs and metabolites.

Stability of O-H-LSD in urine

One-liter aliquots of drug-free urine were prepared using 2M sodium phosphate buffer to adjust the urine pH to 4.6, 6.5, or 8.4. A sufficient amount of a 100-µg/mL O-H-LSD standard was added to obtain a final concentration of 5000 pg/mL. Five-milliliter aliquots of the resulting solution was pipetted into glass test tubes. A time-zero aliquot was assayed immediately and the remaining tubes were stored under the following conditions to investigate the combined effect of temperature (–20°C, 8°C, 24°C, and 50°C) and pH conditions (pH 4.6, 6.5, and 8.4). To investigate the potential effect of fluorescent laboratory light on O-H-LSD, samples prepared within the same pH range at 24°C were exposed to constant laboratory fluorescent lighting (ceiling fluorescent light (32-watt), at a distance of 0.5 m from the samples) and compared with a control set under dark conditions. The effect of pH and fluorescent light was investigated at 24°C to mimic exposure of samples to typical laboratory conditions.

On days 0, 1, 2, 4, 9, and 60, 5-mL aliquots (5 replicates) were spiked with O-H-LAMPA (4000 pg/mL), extracted, and analyzed by LC–MS as described in the Methods section. Newly prepared O-H-LSD calibrator (1000 pg/mL) and controls (500 and 1250 pg/mL) were used to determine and control for the concentration of O-H-LSD present under the various stability conditions.

LC–MS conditions

LC–MS analysis was accomplished using an HP 1100 HPLC with a 3.5-µm, 150 × 4.6-mm ZORBAX Eclipse® XDB-C18 column (Rockland Technologies, Inc., Newport, DE), HP 1100 autosampler, and HP1100 mass selective detector (MSD) using an atmospheric pressure chemical ionization (APCI) interface. Instrument control and signal processing were performed using HP LC–MSD Rev A.07.01 [682] software. Mixtures of 10mM ammonium acetate buffer, pH 8.0 and ACN were used as the mobile phase. The initial ratio of buffer to ACN was 79:21 using a flow rate of 0.8 mL/min held for 8 min. The ratio of buffer to ACN was decreased to 63:37 over 0.5 min and held for 7.5 min. The column was re-equilibrated for 5 min using the initial mobile phase. The entire gradient-equilibration cycle required 21 min for completion.

Positive APCI was achieved using nitrogen as the sheath and collision gas at 60 psi and a drying gas flow at 6.0 L/min. The API vaporizer and capillary temperatures were 450°C and 350°C, respectively. The discharge of the corona needle was 4.0 µA, and the interface capillary voltage was 4 kV. The fragmentor was ramped and set at 140 V for *m/z* 237, 130 for *m/z* 338, and 100 V for *m/z* 356. The gain was set at 1.

LC–MS analysis

The MS was operated in the selective ion monitoring (SIM) mode with the following ions being monitored: the parent ion, *m/z* 356 [M + H⁺], and the prominent ions at *m/z* 237 and *m/z* 338. The O-H-LSD/O-H-LAMPA quantitation ratio was *m/z* 356/356, and the identification qualifiers for each compound were the *m/z* 338/356 and *m/z* 237/356 ratios. O-H-LSD concentrations for specimens and controls (500 and 1250 pg/mL) were determined by single-point calibration at 1000 pg/mL. Identification of O-H-LSD was considered acceptable if the specimens and controls exhibited retention times within ± 1% of the calibration standard and identity ion abundance ratios within ± 20% of the calibration standard. Additionally, all O-H-LSD controls within each analytical set were required to quantitate within ± 20%

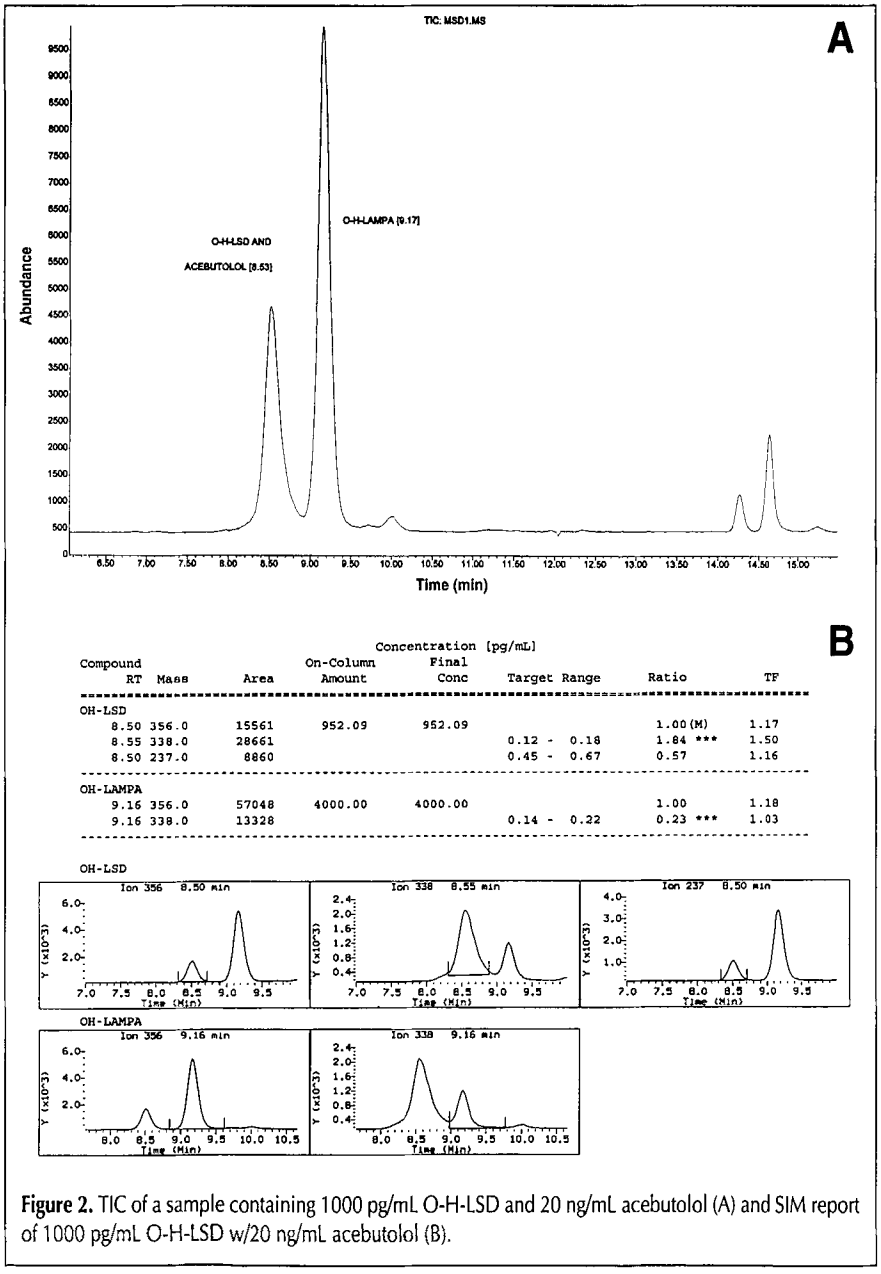

Figure 2. TIC of a sample containing 1000 pg/mL O-H-LSD and 20 ng/mL acebutolol (A) and SIM report of 1000 pg/mL O-H-LSD w/20 ng/mL acebutolol (B).

of the expected theoretical concentration.

Interference samples were analyzed by operating the MS in SIM and full scan mode, simultaneously using the multisignal acquisition mode available on the instrument (12). SIM data were collected as described, and full scans were performed from *m/z* 50 to 500 with the fragmentor set at 125 V and the gain set at 1.

Results and Discussion

The analytical capability of the LC–MS method for the analysis of human urine regarding accuracy, precision, linearity, and sensitivity, for the major LSD metabolite, O-H-LSD has been previously reported (7). Additionally, in that same study, a lack of chromatographic interference from endogenous urine components in 100 drug-free human urine specimens was clearly demonstrated. However, good laboratory practices suggest that the analytical method also be evaluated for interferences from both endogenous urine matrix components as well as compounds that have chemical properties similar to the target analyte at concentrations equivalent to or exceeding the assays cutoff concentration (13). Therefore, the purpose of this investigation was to assess the specificity of the assay by investigating the potential interference of compounds that are structurally similar to O-H-LSD, common OTC products, prescription drugs and some of their metabolites, and other drugs commonly tested for in workplace drug-testing laboratories.

Unextracted interference standards

Chromatographic interference was investigated by assessing the potential for other compounds to coelute with and possess ions that are common to the target analyte or the internal standard thus interfering with the ability to successfully identify and quantitate O-H-LSD. Of the 93 standards examined, only ergonovine (Figure 1A) and acebutolol (Figure 2A) produced a chromatographic peak near the retention time of O-H-LSD and displayed ions in common with O-H-LSD (*m/z* 237, *m/z* 338, and *m/z* 356) (Figures 3A–C). Importantly, these ions occurred in low abundance. The only ion common to both compounds is the qualifier ion at

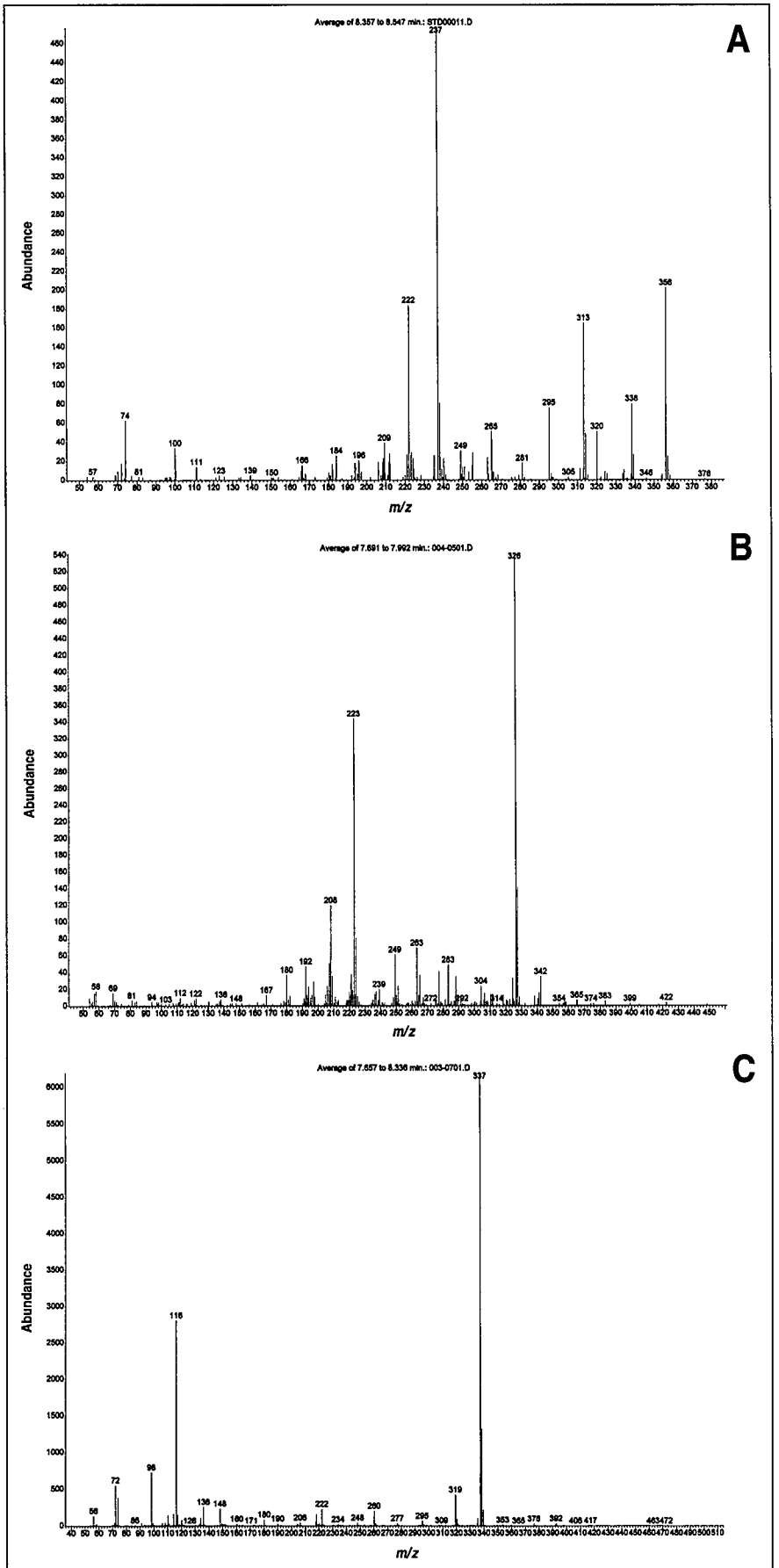

Figure 3. Full scan spectrum of O-H-LSD (A), ergonovine (B) and acebutolol (C).

m/z 237. A possible rationale for the occurrence of the ions at *m/z* 338 and 356 for this ergot alkaloid, which occurs in extremely low abundance, is that the ergonovine sample analyzed may contain minor impurities of O-H-LSD.

Ergonovine at a sample concentration of 10 ng/mL was resolved within 10% of baseline from O-H-LSD (Figure 1A). At higher concentrations ergonovine was not resolved to 10% of the chromatographic baseline. However, the O-H-LSD identity ion ratios exceeded the ± 20% limit established by the O-H-LSD calibrator (i.e., 4.45 and 0.94 for the *m/z* 237/356 and *m/z* 338/356 ratios, respectively). The expected O-H-LSD identity ion ratio *m/z* 237/356 and *m/z* 338/356 ratio were 0.37–0.55 and 0.13–0.19, respectively. Thus, the presence of ergonovine in an unknown sample or the concomitant use of LSD and ergonovine would not result in a false-positive result because of the difference in retention times and the O-H-LSD confirmation identity ion ratios would exceed the ± 20% range of the O-H-LSD calibrator. Importantly, ergonovine produced very limited cross-reactivity to the DPC Coat-A-Count and Syva EMIT II LSD IA screening kits. Ergonovine showed a response approximately equivalent to the response from the cutoff calibrator (500 pg/mL) only at concentrations greater than 3850 ng/mL.

Table II. REMEDi Results

Compounds identified	Number of samples
Alverine	1
Amitriptylin and metabolites	1
Amphetamine/Phentermine/Phenylamine	13
Bupivacaine	1
Bupropion metabolite	2
Chlorpheniramine, *N* – demethyl	1
Dextrorphan and metabolites	2
Diazepam	1
Dihydroergotamine	3
Diphenhydramine	1
Doxylamine	1
Ephedrine/Psuedoephedrine/Phenmetrazine	18
Hydrocodone and metabolites	1
Isometheptene	4
Lamotrigine	9
Lidocaine	1
Lincomycin	1
Methylphenidate	1
Metoclopramide	1
Morphine	1
Nadolol	2
Nyridrin	1
Panoxetine	1
Pentazocine	1
Pentoxifylline	2
Phenobarbital [-s-]	1
Promethazine and metabolites	1
Quinine and metabolites	3
Ranitidine and metabolites	1
Sertraline	4
Tocainidine	1
Trazodone and metabolites	1
Trimethoprim	3
Verapamil	17
Not determined	20

Acebutolol, at a sample concentration of 18,000 pg/mL, coeluted with O-H-LSD (Figure 2A). The only ion common to both of these compounds is *m/z* 338 (Figure 3C). A false positive would not be reported if acebutolol alone were present in an unknown sample because acebutolol lacks the *m/z* 237 and *m/z* 356 O-H-LSD identity and quantitation ions, respectively. Notably, there was no interference of the *m/z* 338 ion when the concentration of acebutolol was less than 180 pg/mL (i.e., at 180 pg/mL the *m/z* 338/356 ion ratio was 0.19). The typical 338/356 O-H-LSD identity ion ratio range was 0.13–0.19. However, acebutolol when present at concentrations exceeding 180 pg/mL, interfered with the O-H-LSD *m/z* 338/356-identity ratio (i.e., acebutolol at 18,000 pg/mL produced a *m/z* 338/356 ion ratio of 2.25) (Figure 2B). Nonetheless, cross-reactivity studies utilizing the DPC Coat-A-Count and Syva EMIT II LSD immunoassays were unable to detect the presence of acebutolol at 1.0 mg/mL (i.e., acebutolol at 1.0 mg/mL produced a response equivalent to the response of a drug-free control at 1.0 mg/mL).

None of the 93 standards interfered with the ability of the assay to quantitate O-H-LSD. The mean O-H-LSD concentration was 992 ± 60.3 (N = 24) for the 1000 pg/mL target concentration.

Extracted human urine interference specimens

Although immunoassays are known to exclude thousands of natural substances as possible interfering compounds in laboratory tests, they also are known to cross-react with some common substances. Therefore, it is critical that the LC–MS analysis of O-H-LSD produce a negative result when compounds/drugs that cross-react in the LSD immunoassay are present. Currently there are no commercial IA kits available that screen for O-H-LSD. Therefore, IA kits that target the parent compound (LSD) were used to identify potential interfering compounds/drugs and their metabolites believed to be cross-reactive with the LSD assay antibody and therefore may have chemical properties similar to O-H-LSD.

In order to establish that human urinary metabolites of these compounds/drugs do not interfere in the analysis of O-H-LSD, 300 human urine specimens that screened positive by the Syva EMIT II LSD IA at a cutoff concentration of 500 pg/mL were used in the drug metabolite interference study. These samples were subsequently analyzed for LSD using the DPC Coat-A-Count RIA kits, applying the same LSD cutoff concentration. Specimens that failed to screen positive by RIA were analyzed using the REMEDi to identify potential EMIT II cross-reacting metabolites. One-hundred specimens were prepared by pooling from the original 300 samples in order to obtain the necessary volume of urine required for REMEDi and subsequent LC–MS analysis using a previously reported LC–MS method (7).

REMEDi analysis of the 100 specimens revealed 37 identifiable drugs/drug metabolites (Table II). Twenty of the specimens contained compounds that could not be identified. The most prevalent compounds identified were ephedrine/pseudoephedrine, verapamil (and metabolites), phenethylamine/

phentermine, and lamotrigine. Importantly, the compounds listed in Table II contain either a α-hydroxyamine (i.e. pseudoephedrine, ephedrine, and other α-hydroxyamines) or a primary amine (e.g., isometheptene, amphetamine, etc). This cross-reactivity, also reported by other researchers (14), may be due to chemical moieties similar in structure to LSD and/or O-H-LSD.

Only 1 of the 100 specimens when analyzed by LC–MS produced a chromatographic peak near the retention time of O-H-LSD and displayed ions in common with O-H-LSD (*m/z* 237, *m/z* 338, and *m/z* 356). This compound could not be identified by REMEDi analysis. Nonetheless, the unknown compound produced identity ion ratios that exceeded the ± 20% limit established by the O-H-LSD calibrator.

O-H-LSD stability

The stability of O-H-LSD was also assessed as part of the method validation study. Previous reports on the stability of LSD in urine specimens have shown LSD to be labile to temperature, pH, and light (15,16). In the present study, the effect of both moderate and extreme conditions encountered during the shipment of specimens from the collection site to the processing and storage of the specimens at the testing facility was investigated. Data were evaluated using a three-way, crossed, fixed-effect ANOVA with replication and followed by least significant difference means testing. The levels used were time, temperature, and pH. For this analysis, all samples had the same light treatment. Additionally, a three way ANOVA was used to evaluate the effect of time, pH, and light on samples all run at 24°C. Significance was assigned at the 95% confidence level.

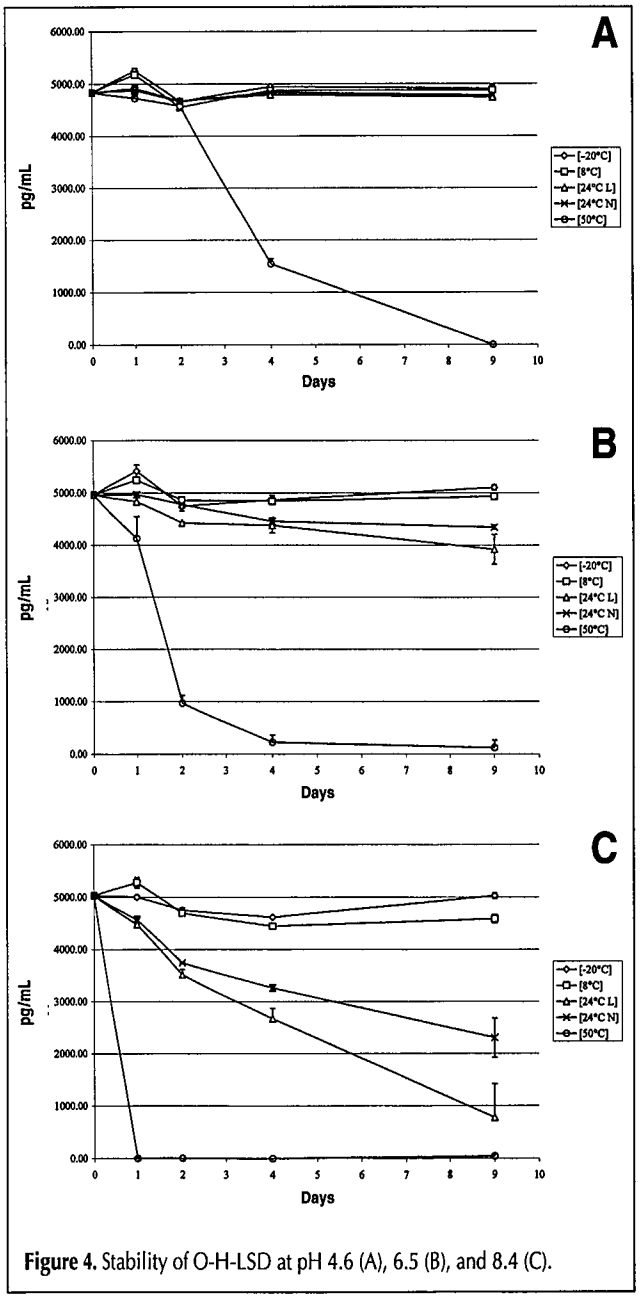

Figure 4. Stability of O-H-LSD at pH 4.6 (A), 6.5 (B), and 8.4 (C).

Within the pH range investigated, no significant loss of O-H-LSD was demonstrated under frozen (–20°C) and refrigerated conditions (8°C) (Figures 4A–C). Even after 60 days at –20°C, no significant loss of O-H-LSD was detected (Table III). A significant loss ($p < 0.05$) was observed in samples maintained at 50°C and pH 8.4. Complete loss of O-H-LSD was found after 1 day at pH 8.4, after 4 days at pH 6.5, and after 9 days at pH 4.6. (Figure 4C).

A significant ($p < 0.05$) pH × time × light effect was observed. Constant exposure to fluorescent light at 24°C showed no significant effect at pH 4.6 and 6.5 (Figures 4A and 4B). At pH 8.4 the combined effect of fluorescent light and 24°C significantly ($p < 0.05$) reduced the concentration of O-H-LSD when compared with samples not exposed to light after 9 days (Figure 4C). An 87% loss of O-H-LSD after 9 days occurred with exposure to fluorescent light. Samples without light exposure only had a 50% reduction in O-H-LSD concentrations.

The potential decomposition of O-H-LSD to LSD was investigated by analyzing an aliquot of urine from each stability condition for cross-reactivity to the Syva EMIT II LSD IA. None of the stability samples under the conditions described above cross-reacted with LSD. All of the IA responses were equivalent to the response of a drug-free control. Additionally, LSD was not detected in any of the stability samples when analyzed by LC–MS in the SIM mode. These data indicate no significant production of LSD as an O-H-LSD degradation product.

The results of this study demonstrate that the stability of O-H-LSD is similar to the reported stability of LSD in human urine (15,16). The presented data shows that O-H-LSD is stable in specimens exhibiting pH readings from 4.6-6.5 when maintained at temperatures from –20°C to 24°C. However, the combination of an alkaline pH environment (pH 8.4) and exposure to heat (24°C to 50°C) contributes significantly to the degradation of O-H-LSD. The described assay is also reliably able to distinguish O-H-LSD from a wide range of potential interfering compounds.

Table III. O-H-LSD Stability (–20°C) at Day 60

pH	Mean	SD	% CV
4.6	5241.7	110.1	2.1
6.5	5346.2	71.3	1.3
8.4	5258.4	47.1	0.9

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References

1. T. Greiner, N.R. Burch, and R. Edelbert. Psychopathology and psychophysiology of minimal LSD-25 dosage. A preliminary dosage-response spectrum. *Arch. Neurol. Psychiatr.* **79:** 208 (1958).
2. Z.H. Siddik, R.D. Barnes, L.G. Dring, R.L. Smith, and R.T. Williams. The fate of lysergic acid di[14C] ethylamide in rat, guinea pig and rhesus monkey and of [14C] iso-LSD in rat. *Biochem. Pharmacol.* **28:** 3093–3101 (1979).
3. H.K. Lim, D. Andrenyak, P. Francom, R. Foltz, and R.T. Jones. Quantification of LSD and N-desmethyl-LSD in urine by gas chromatography/resonance electron capture ionization mass spectrometry. *Anal Chem.* **60:** 1420–1425 (1988).
4. C.C. Nelson and R.L. Foltz. Chromatographic and mass spectrometric methods for determination of lysergic acid diethylamide (LSD) and metabolites in body fluids. *J. Chromatogr.* **580:** 97–109 (1992).
5. C. Radeca and I.C. Nigam. Gas chromatography of LSD. *J. Pharm. Sci.* **55:** 861–865 (1966).
6. G.K. Poch, K.L. Klette, D.A. Hallare, M.G. Manglicmot, R.J. Czarny, L.K. McWhorter, and C.J. Anderson. The detection of metabolites of LSD in human urine specimens: 2-oxo-3-hydroxy-LSD, a prevalent metabolite of LSD. *J. Chromatogr.* **724(1):** 23–33 (1999).
7. G.K. Poch, K.L. Klette, and C.J. Anderson. The quantitation of 2-oxo-3-hydroxy LSD in human urine specimens, a metabolite of LSD: comparative analysis using liquid chromatography–selected ion monitoring mass spectrometry and liquid chromatography–ion trap mass spectrometry. *J. Anal. Toxicol.* **24:** 170–179 (2000).
8. J.H. Sklerov, J. Magluilo, K.K. Shannon, and M.L. Smith. Liquid chromatography–electrospray ionization mass spectrometry for the detection of lysergide and a major metabolite, 2-oxo-3-hydroxy-LSD, in urine and blood. *J. Anal. Toxicol.* **24(7):** 543–549 (2000).
9. S.A. Reuschel, S.E. Percey, S. Liu, D.M. Eades, and R.L. Foltz. Quantitative determination of LSD and a major metabolite, 2-oxo-3-hydroxy-LSD, in human urine by solid-phase extraction and gas chromatography–tandem mass spectrometry. *J. Anal. Toxicol.* **23:** 306–312 (1999).
10. A.G. Verstraete and E.J. Van de Velde. 2-Oxo-3-hydroxy-LSD: an important LSD metabolite? *Acta Clin. Belg. Suppl.* **1:** 94–96 (1999).
11. K.L. Klette, C.J. Anderson, G.K. Poch, A.C. Nimrod, and M.A. ElSohly. Metabolism of lysergic acid diethylamide (LSD) to 2-oxo-3-hydroxy LSD (O-H-LSD) in human liver microsomes and cryopreserved human hepatocytes. *J. Anal. Toxicol.* **24:** 550–556 (2000).
12. C. Miller. Multisignal LC/MS Analysis for Compound Screening. *Agilent Application Brief,* publication number (23) 5968-8658E, (2000).
13. B.A. Goldberger, M.A. Huestis, and D.G. Wilkins. Commonly practiced quality control and quality assurance procedures for gas chromatography/mass spectrometry analysis in forensic urine drug-testing laboratories. *Forensic Sci. Rev.* **9:** 59–80 (1997).
14. D. Ritter, C.M. Cortese, L.C. Edwards, J.L. Barr, H.D. Chung, and C. Long. Interference with testing for lysergic acid diethylamide. *Clin. Chem.* **43(4):** 635–637 (1997).
15. P. Francom, D. Andrenyak, H.K. Lim, R.R. Bridges, R.L. Foltz, and R.T. Jones. Determination of LSD in urine by capillary column gas chromatography and electron impact mass spectrometry. *J. Anal. Toxicol.* **12:** 1–8 (1988).
16. Z. Li, A.J. McNally, and S.J. Salamone. Stability study of LSD under various storage conditions. *J. Anal. Toxicol.* **22:** 520–522 (1998).

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