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Validation of an LC-MS/MS method for the quantitative analysis of 1P-LSD and its tentative metabolite LSD in fortified urine and serum samples including stability tests for 1P-LSD under different storage conditions

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Abstract

A variety of hallucinogens of the lysergamide type has emerged on the drug market in recent years and one such uncontrolled derivative of lysergic acid diethylamide (LSD) is 1-propionyl-LSD (1P-LSD). Due to the high potency of LSD and some of its derivatives (common doses: 50-200 µg), sensitive methods are required for the analysis of biological samples such as serum and urine. The occurrence of an intoxication case required the development of a fully validated, highly sensitive method for the quantification of 1P-LSD and LSD in urine and serum using LC-MS/MS. Given that LSD is unstable in biological samples when exposed to light or elevated temperatures, we also conducted stability tests for 1P-LSD in urine and serum under different storage conditions. The validation results revealed that the analysis method was accurate and precise with good linearity over a wide calibration range (0.015-0.4 ng mL^{-1}). The limit of detection (LOD) and the lower limit of quantification (LLOQ) of 1P-LSD and LSD in serum and urine were 0.005 ng mL^{-1} and 0.015 ng mL^{-1}, respectively. The stability tests showed no major degradation of 1P-LSD in urine and serum stored at -20 °C, 5 °C or at room temperature for up to five days, regardless of protection from light. However, LSD was detected in all samples stored at room temperature showing a temperature-dependent hydrolysis of 1P-LSD to LSD to some extent (up to 21% in serum). Serum samples were particularly prone to hydrolysis possibly due to enzymatically catalyzed reactions. The addition of sodium fluoride prevented the enzymatic formation of LSD. The method was applied to samples obtained from the intoxication case involving 1P-LSD. The analysis uncovered 0.51 ng mL^{-1} LSD in urine and 3.4 ng mL^{-1} LSD in serum, whereas 1P-LSD remained undetected. So far pharmacokinetic data of 1P-LSD is missing, but with respect to the results of our stability tests and the investigated case rapid hydrolysis to LSD in-vivo seems more likely than instabilities of 1PLSD in urine and serum samples.

Keywords

Lysergamides; biological samples; LSD derivatives; forensic toxicology

Abbreviations: 1P-ETH-LAD, 1-propionyl-ETH-LAD; 1P-LSD, 1-propionyl-LSD; 3-FPM, 3-fluorophenmetrazine; ACN, acetonitrile; AL-LAD, *N*-allyl-nor-LSD; ECPLA, *N*-ethyl-*N*-cyclopropyllysergamide; EMCDDA, European Monitoring Centre for Drugs and Drug Addiction; ETH-LAD, *N*-ethyl-nor-LSD; GTFCh, German Society of Toxicological and Forensic Chemistry IS, internal standard; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLOQ, lower limit of quantification; LOD, limit of detection; LSD, lysergic acid diethylamide; LSZ, lysergic acid 2,4-dimethylazetidide; LSM-775, lysergic acid morpholide, MRM, multiple-reaction-monitoring; NaF, sodium fluoride; NPS, new psychoactive substances; QC, quality control; RSD, relative standard deviation;

1. Introduction

In recent years, a number of hallucinogens of the lysergamide type emerged on the drug market. Besides lysergic acid diethylamide (LSD) itself, several new lysergamide derivatives were encountered on the new psychoactive

substances (NPS) market with examples including: 1-propionyl-LSD (1P-LSD), lysergic acid 2,4-dimethylazetidide (LSZ), *N*-allyl-nor-LSD (AL-LAD), *N*-ethyl-nor-LSD (ETH-LAD), lysergic acid morpholide (LSM-775), *N*-ethyl-*N*-cyclopropyllysergamide (ECPLA), and 1-propionyl-ETH-LAD (1P-ETH-LAD) (Figure 1) [1-5]. In Germany and most European countries, these LSD derivatives are uncontrolled substances. They are often sold as research chemicals via the Internet and are mainly available as powdered material or on paper trips ('blotters'). Among the above mentioned LSD derivatives, 1P-LSD was first reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2015 by the United Kingdom. From this date on, the number of seizures with 1P-LSD in Europe has been increasing. In 2017, more than 100,000 paper trips were confiscated in the state of Baden-Wuerttemberg (Germany), which was the largest seizure of 1P-LSD in Germany until now. Although comprehensive analytical characterization of 1P-LSD has been conducted by Brandt et al. [1], reliable and sensitive analysis methods for the quantitative detection of 1P-LSD in biological samples (such as blood and urine), especially in the context of forensic toxicology are still missing. In general, highly sensitive methods are needed for the analysis of LSD and drugs with similar potency such as 1P-LSD (common doses: 50 to 200 µg [6]) in biological matrices. Furthermore, stability tests with LSD suggested the substance to be susceptible to degradation especially when exposed to light or elevated temperatures [7-11]. Similar instabilities might be expected for 1P-LSD but information about this appears to be unavailable in the literature.

Recently, an acute intoxication case (17-year-old male) following the ingestion of a blotter allegedly containing 1P-LSD (labeled '1P-LSD 100 µg') was reported to the Poisons Information Center in Freiburg (Germany). Additionally, the ingestion of the psychostimulant 3-fluorophenmetrazine (3-FPM, ingested dose unknown) was in question. Within 1.5 hours after ingestion the patient displayed palpitation, restlessness, anxiety and hallucinations. When the Emergency Medical Service arrived, the patient was agitated. In addition, a pronounced sinus tachycardia (140 beats/min) and an elevated blood pressure (160/100 mmHg) were reported. The emergency physician applied midazolam. On arrival at the children's hospital the increased heart rate and blood pressure had normalized, and the patient was slightly somnolent. The clinical course was further characterized by recurrent restlessness and anxiety for another 12 hours resulting in sleeplessness. The next day all symptoms had reversed and the patient was discharged. A urine and a serum sample, presumably collected two hours post ingestion, were sent to the Institute of Forensic Medicine in Freiburg (Germany) together with the presumed 1P-LSD blotter and 3-FPM powder for analysis. While 3-FPM could be detected with sufficient sensitivity using in-house methods, a sensitive method for the detection of 1P-LSD in urine and serum was missing. Therefore, a highly sensitive method for the quantitative analysis of 1P-LSD and LSD in urine and serum using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and fully validated. Analysis of urine and serum samples confirmed the uptake of 3-FPM but surprisingly only LSD and no 1P-LSD was detected. To clarify whether 1P-LSD remained undetectable in the authentic urine and serum samples due to degradation, a stability test was conducted with spiked urine and serum samples stored under different conditions.

2. Materials and methods

2.1. Chemicals and reagents

Boric acid (H_3BO_3, for molecular biology, 99.8%) was purchased from AppliChem (Darmstadt, Germany). Potassium chloride (KCl, ≥ 99.5%, p.a.), formic acid (HCOOH, > 98%, p.a.), and propan-2-ol (Rotisolv®, ≥ 99.95%, LC-MS grade) were purchased from Carl Roth (Karlsruhe, Germany). Sodium carbonate (Na_2CO_3, anhydrous, for analysis) and 1-chlorobutane (C_4H_9Cl, LiChrosolv®, for liquid chromatography) were purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN, HPLC-Super gradient grade) and ammonium formate (10 M, 99,995%) were obtained from VWR International (Fontenay-sous-Bois, France) and Sigma Aldrich (Steinheim, Germany), respectively. Methanol (MeOH, Chromasolv™, LC-MS, ≥ 99.9%) was purchased from Honeywell Riedel-de Haën (Seelze, Germany). Deionized water (H_2O) was prepared using a Medica® Pro single high flow purification system from ELGA LabWater (Celle, Germany). LSD ((8β)-N,N-diethyl-6-methyl-9,10-didehydroergoline-8-carboxamide) and the deuterated internal standard (IS) LSD-D3 were obtained from LGC Standards (Wesel, Germany) and Cerilliant (Round Rock, TX, USA), respectively. 1P-LSD ((8β)-N,N-6-methyl-1-propanoyl-9,10-didehydroergoline-8-carboxamide) was provided by Synex Synthetics BV (Maastricht, The Netherlands) (> 98%, confirmed by NMR analysis). Drug free urine and serum samples were obtained from volunteers.

2.2. Solutions

Two solutions (A and B) were used for the preparation of the borate buffer (pH 9). Solution A consisted of 61.8 g H_3BO_3 and 74.6 g KCl in 1.0 L deionized water. For solution B, 106 g Na_2CO_3 was dissolved in 1.0 L deionized water. Solution A (630 mL) was mixed with 370 mL solution B. Solution B was subsequently added to adjust the pH value to 9. Mobile phase A consisted of deionized water with 1% ACN, 0.1% formic acid, and 2 mM ammonium formate. Mobile phase B consisted of 0.1% formic acid and 2 mM ammonium formate in ACN. One mg 1P-LSD was dissolved in 1 mL ACN and served as a reference solution. A stock solution of LSD and 1P-LSD (10 µg mL^{-1} each) was prepared in ACN. The stock solution was further diluted with ACN to yield working solutions with a concentration of 50 and 5 ng mL^{-1} for LSD and 1P-LSD. Quality control (QC) working solutions were prepared in the same way using the same stock solution since 1P-LSD reference material was only available in limited quantities. The IS working solution (500 ng mL^{-1}) was prepared by diluting the LSD-D3 standard solution with ACN.

2.3. Instrumentation and methods

The LC system from Shimadzu consisted of two NexeraX2 LC-30AD pumps, a Prominence LC-20AD pump (for post-column addition of propan-2-ol), a DGU-$20A_{5R}$ degasser unit, a Nexera X2 SIL-30AC autosampler (set to 10 °C), a Prominence CTO-20AC column oven (set to 30 °C) and a CMB-20AC communications BUS module. A Sciex QTRAP®5500 mass spectrometer was coupled to the LC system using positive electrospray ionization (ESI+). The injection volume was 10 µL. MS source parameters were as follows: curtain gas 30 psi, ion source gas (1) 40 psi, ion source gas (2) 65 psi, ion spray voltage +4,000 V. The source temperature was set to 550 °C. The transitions used in full scan multiple-reaction-monitoring (MRM) mode and the respective MS parameters for all analytes are shown in Table 1. LC-MS/MS operation and data analysis was performed using Analyst® software (version 1.6.2, Sciex, Darmstadt, Germany). For chromatographic separation a biphenyl column (100 x 2.1 mm, 2.6 µm particle size, Phenomenex, Aschaffenburg, Germany) with a corresponding guard column (SecurityGuard™ ULTRA Cartridges UHPLC Biphenyl for 2.1 mm ID columns, Phenomenex, Aschaffenburg, Germany) was used. The chromatographic gradient (total flow rate: 0.3 mL min^{-1}) started at 10%

mobile phase B and was linearly increased to 30% mobile phase B within 3 min. The percentage of mobile phase B was further increased and reached 50% after 4 min and 75% after 6 min. For column cleaning, mobile phase B was increased to 95% within 0.5 min and kept for 1 min. Subsequently, starting conditions were restored within 0.5 min and kept for 7 min for column re-equilibration. To enhance signal intensities propan-2-ol was added post-column (0.1 mL min^{-1}). Metabolites of LSD such as 2-oxo-3-hydroxy-LSD were not included in the method development as the simultaneous extraction of metabolites with considerably higher hydrophilicity would have reduced the sensitivity for 1P-LSD and LSD.

2.4. Sample preparation

Serum or urine (0.5 mL), 10 µL IS working solution, and 1 mL borate buffer (pH 9) were fortified with 2 mL 1-chlorobutane. Subsequently, the samples were shaken in an overhead shaker for 5 min. After centrifugation (5 min, 2879 g), the organic top layer was transferred into a glass vial and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The dry residue was then reconstituted in 100 µL mobile phase A-mobile phase B (99:1, *v/v*).

2.5. Method validation

Validation of 1P-LSD and LSD in serum and urine was carried out according to the protocol of the German Society of Toxicological and Forensic Chemistry (GTFCh) [12]. Statistical evaluation was performed using Valistat 2.0 (Arvecon GmbH, Walldorf, Germany) and Excel 2010 (Microsoft Corporation, Redmond, WA, USA). For assessment of selectivity and sensitivity six drug-free blank samples and two zero samples (blank matrix spiked with IS) were analyzed. For the determination of the limits of detection (LOD) serum and urine samples were spiked to yield the following concentrations: 0.005, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ng mL^{-1}. Estimation of LOD was then performed using the signal-to-noise ratio (S/N; S/N ≥ 3 for both ion transitions). To determine the lower limits of quantification (LLOQ), six urine and serum samples were spiked with 0.015 ng mL^{-1} 1P-LSD and LSD (concentration of the lowest calibrator, acceptance criteria: bias and relative standard deviation (RSD) ≤ 20%). The calibration model was evaluated by the analysis of six calibration curves in serum and urine (0.015, 0.03, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4 ng mL^{-1}). Quality control (QC) samples at low (0.05 ng mL^{-1}) and high (0.3 ng mL^{-1}) concentration levels were spiked in serum and urine, pooled, aliquoted and analyzed in duplicates at eight different days to gather precision data. Since no certified reference material was available to determine the precision, the QC working solutions which were based on our reference material were used for the preparation of the QC samples. To determine the stability of processed samples stored in the autosampler eight QC samples were prepared at 0.05 and 0.3 ng mL^{-1} in serum and urine. The reconstituted samples were pooled, aliquoted and injected at a regular basis for an overall time period of 30 h. Matrix effects, extraction recoveries and overall process efficiencies were determined using the protocol suggested by Matuszewski *et al*. [13]. For this experiment three sample sets at low and high QC concentrations were prepared. Set 1 consisted of working solution in mobile phase A-mobile phase B (99:1, *v/v*). For set 2 six different blank matrix samples were spiked with working solution after extraction of the samples. For set 3 the same donors of blank matrix as in set 2 were used, but the samples were extracted after fortification with working solution. Comparison of absolute peak areas of the different sets yielded matrix effects (set 2/set 1), recoveries (set 2/set 3), and process efficiencies (set 3/set 1).

2.6. Stability tests

Blank serum and urine samples were spiked with 1P-LSD to yield a concentration of 4.0 ng mL^{-1}. The samples were pooled and aliquoted to triplicates for each test condition (Table 2). The samples were either stored at room temperature, +5 °C or -20 °C for three or five days either with or without light protection. Additional sets (sets 2, 9) stored at the presumed most unfavourable conditions (room temperature, without light protection) were prepared with addition of NaF to investigate whether this would provide stabilizing effects. Four additional sets (sets 15-18) for the investigation of the freeze-thaw stability were stored at -80 °C for 5 days. Each freeze-thaw cycle comprised at least 20 h at -80 °C followed by 1 h thawing at room temperature. Set 15 (light protection) and 16 (without light protection) underwent two freeze-thaw cycles, while sets 17 (light protection) and 18 (without light protection) were only defrosted once prior to analysis. Four triplicates were stored at -80 °C as reference (sets 19-22) until joint analysis of all samples. To verify stability of the reference triplicates, four triplicates of samples (control samples, sets 23-26) were fortified directly before sample preparation of all stability and reference samples and were compared with the respective set of reference samples. Sets 1-7 (storage time 3 days) were stored at -80 °C for two days to enable joint analysis of all samples. Light protection was realized by utilizing brown glass vials and covering tubes and glass ware with aluminium foil. The IS was added to all samples directly before sample preparation. The results were evaluated by comparison of the mean values of the test set triplicates using the ratio of 1P-LSD or LSD and the IS.

2.7. Intoxication case

The research chemicals (3-FPM powder and 1P-LSD blotter) were analyzed using routine screening methods (LC-MS/MS, GC-MS). The urine and serum samples were analyzed using the here presented method as well as a semi-quantitative LC-MS/MS method for designer stimulants (including 3-FPM). Prior to analysis, urine and serum samples were stored at -20 °C for two weeks. Written consent was obtained from the patient for toxicological analysis and publication of clinical data.

3. Results and discussion

3.1. Method validation

The method proved selective and sensitive. A linear relationship between analyte/IS area ratios and concentration was shown over the whole calibration range (Mandel test, 99% significance). A weighted least squares model (weighting factor: $1/x^2$) was used to compensate for heteroscedasticity. Precision values are given in Table 3. RSD values for interday and intraday precision for all concentration levels, matrices and analytes were below 10%. LOD and LLOQ of 1P-LSD and LSD in serum and urine were 0.005 ng mL^{-1} and 0.015 ng mL^{-1}, respectively (RSD for LLOQ < 19% for all analytes and matrices). The developed method therefore showed lower limits of detection compared to recently published LC-MS/MS based methods for the quantification of LSD in biological matrices [14-16]. Hence, the method meets the demand for high sensitivity considering the low urine and serum concentrations that have to be expected [14-17]. Processed sample stability was given for serum and urine for at least 30 h (deviation of absolute peak areas < 25% compared to first injection). Data on matrix effects, recoveries and process efficiency are given in Table 4. Matrix effects ranged

from 84 to 102% and from 81 to 95% for LSD and 1P-LSD, respectively. RSD was < 24% and < 32% for serum and urine, respectively, showing higher variations in matrix effects for urine than for serum. Overall process efficiency for serum and urine samples was above 50% for both analytes at low and high concentrations (RSD < 27%).

3.2. Stability tests

Comparison of the reference with the control samples revealed no degradation of 1P-LSD, which allowed the reference samples to be used for further evaluation of stability. The results of the stability experiments for 1P-LSD are shown in Figure 2 and for LSD in Figure 3 grouping together all sets without (Figure 2a, 2c, 3a, 3c) and with light protection (Figure 2b, 2d, 3b, 3d). In urine (Figure 2 a, b), the degradation of 1P-LSD was below 12% for all samples (based on the area ratios of 1P-LSD/LSD-D3). However, LSD (Figure 3 a, b) could be detected in all samples stored at room temperature (sets 1-3, 8-10) showing that 1P-LSD was hydrolyzed to LSD to a minor extent (up to 4% after five days when referring to the spiked amount of 1P-LSD). Similar results were observed for the serum samples (Figure 2 c, d): Here, the degradation of 1P-LSD was below 14% for all samples (based on the area ratios of 1P-LSD/LSD-D3). Once again, LSD (Figure 3 c, d) was detected in all samples stored at room temperature (sets 1-3, 8-10). Serum samples without the addition of NaF were prone to this effect (sets 1, 3, 8 and 10) yielding nearly ten times the LSD/LSD-D3 area ratios compared to the respective urine samples. Addition of NaF (sets 2 and 9) stabilized 1P-LSD in serum samples (approx. 21% LSD in set 10 vs. approx. 3% LSD in set 9 when referring to the spiked amount of 1P-LSD), probably due to inhibition of serum enzymes. Hydrolysis of the N_1-propionyl moiety has already been reported by Brandt et al. [1] for serum samples spiked with 1P-LSD and incubated at 37 °C. However, in the present investigation, degradation of 1P-LSD was also observed for serum samples with addition of NaF yielding LSD/LSD-D3 area ratios similar to their urine counterparts. This raises the possibility that 1P-LSD might degrade in serum via two different mechanisms: a) via enzymatic reactions that can be inhibited by the addition of NaF; b) via a temperature dependent hydrolysis of the propionyl moiety. The latter was also observed in urine samples and could not be prevented by addition of NaF. Serum samples stored at 5 °C (sets 4-5, 11-12) showed only small LSD/LSD-D3 area ratios (approx. 1% LSD when referring to the spiked amount of 1P-LSD). This probably represents a decelerated enzymatic degradation of 1P-LSD since the corresponding urine samples showed no signs of LSD formation. For urine and serum, no difference was observed between the samples with and without light protection, which was surprising given the fact that LSD is often referred to being light sensitive [7-9, 11]. The degradation of 1P-LSD in the freeze-thaw stability serum samples was 7% and 13% after one and two cycles, respectively (based on the area ratios of 1P-LSD/LSD-D3). LSD was detected but with only small LSD/LSD-D3 area ratios comparable to those of serum samples stored at 5 °C, most probably due to enzymatic degradation.

3.3. Intoxication case

The identity of 3-FPM and 1P-LSD as ingredients of the powder and the blotter, respectively, was confirmed by routine screening. 3-FPM concentrations were approx. 200 ng mL^{-1} in urine and approx. 53 ng mL^{-1} in serum. Compared to other cases analyzed in our laboratory the 3-FPM serum concentration is in the lower range where acute effects are not expected. This is in line with the patient stating that he did not consume 3-FPM the same day. 1P-LSD could not be detected in the urine and the serum sample of the patient analyzed with the developed method, which supports earlier suggestions that 1P-LSD might serve as a precursor of LSD *in vivo* [1]. However,

due to missing long-term stability data and the time lag between sampling and analysis of two weeks, this needs further confirmation. LSD was found in the following concentrations: 0.51 ng mL^{-1} in urine and 3.4 ng mL^{-1} in serum, which would be in accordance with a usual recreational dose of LSD.

4. Conclusions

The stability study demonstrated that serum and urine samples with 1P-LSD can be stored under the tested conditions and can undergo at least two freeze-thaw cycles without major degradation of the analyte. However, hydrolysis of 1P-LSD to LSD was observed to some extent (up to 21% and 4% in serum and urine, respectively) after three and five days when the samples were stored at room temperature or 5 °C, regardless of protection from light. Urine and serum samples should therefore be stored at -20 °C, but it appeared that no precautionary measures need to be undertaken to protect samples from light. Addition of NaF to serum samples prevents enzymatic hydrolysis to LSD and is therefore recommended. The method validation showed satisfactory linearity over a wide concentration range with good accuracy and precision data and sufficient LODs and LLOQs which is important for the analysis of authentic cases. In the investigated intoxication case, urine and serum samples were stored at -20 °C until analysis and thus, a complete degradation of 1P-LSD and in particular hydrolysis to LSD after sampling seems unlikely. However, only LSD and not 1P-LSD was detected in the urine and the serum sample. Therefore, the question remains open if 1P-LSD is rapidly hydrolyzed to LSD during stomach passage and/or via first pass effect which could explain these findings. This would be of special interest in countries like Germany where 1P-LSD, in contrast to LSD, is not controlled by the narcotics law so far. Hence, data on pharmacokinetics of 1P-LSD are urgently needed to reliably differentiate between uptake of LSD and 1P-LSD.

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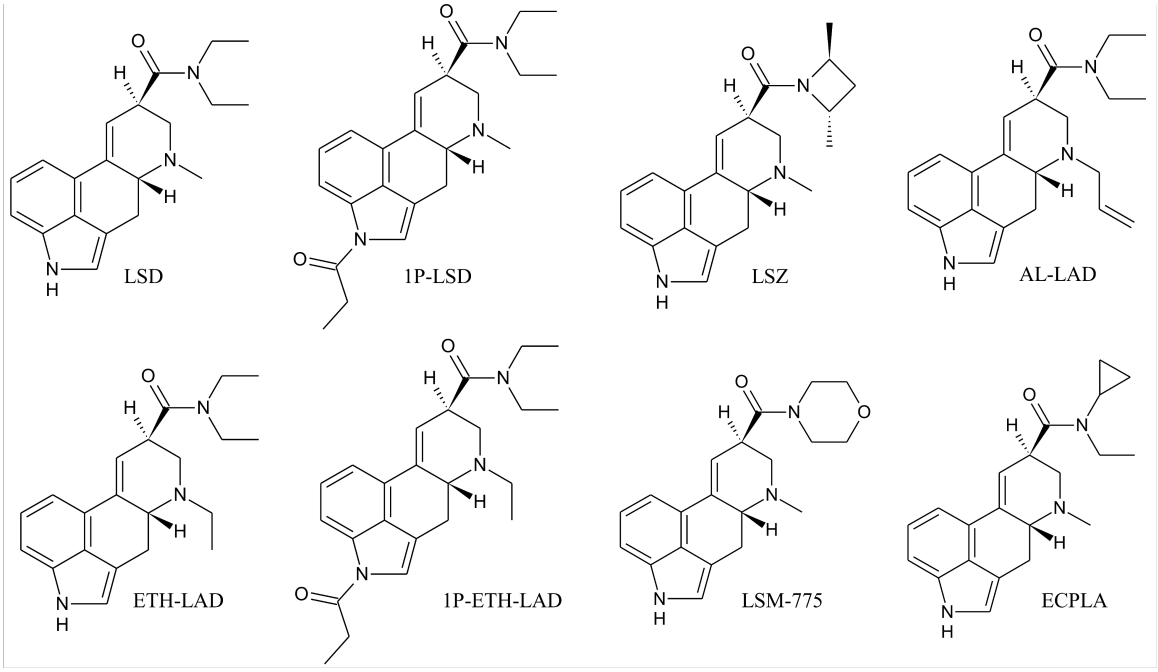

Figure 1: Chemical structures of LSD and LSD derivatives. LSD, lysergic acid diethylamide; 1P-LSD, 1-propionyl-LSD; LSZ, lysergic acid 2,4-dimethylazetidide; AL-LAD, *N*-allyl-nor-LSD; ETH-LAD, *N*-ethyl-nor-LSD; 1P-ETH-LAD, 1-propionyl-ETH-LAD; LSM-775, lysergic acid morpholide; ECPLA, *N*-ethyl-*N*-cyclopropyllysergamide

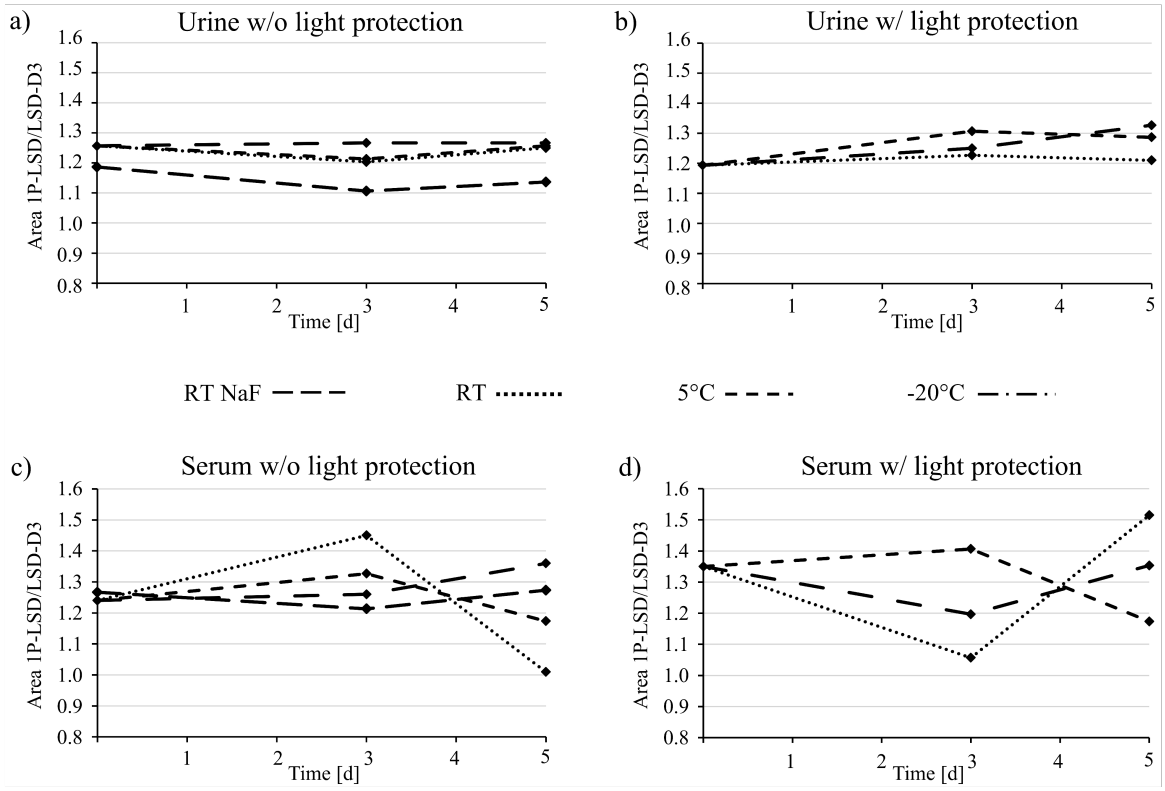

Figure 2: Results of the stability test. Area ratios of 1P-LSD/LSD-D3 in urine and serum samples fortified with 1P-LSD and stored at room temperature (RT) with addition of NaF, at RT, at 5 °C, at -20 °C for up to 5 days. a) urine samples without light protection; b) urine samples with light protection; c) serum samples without light protection; d) serum samples with light protection

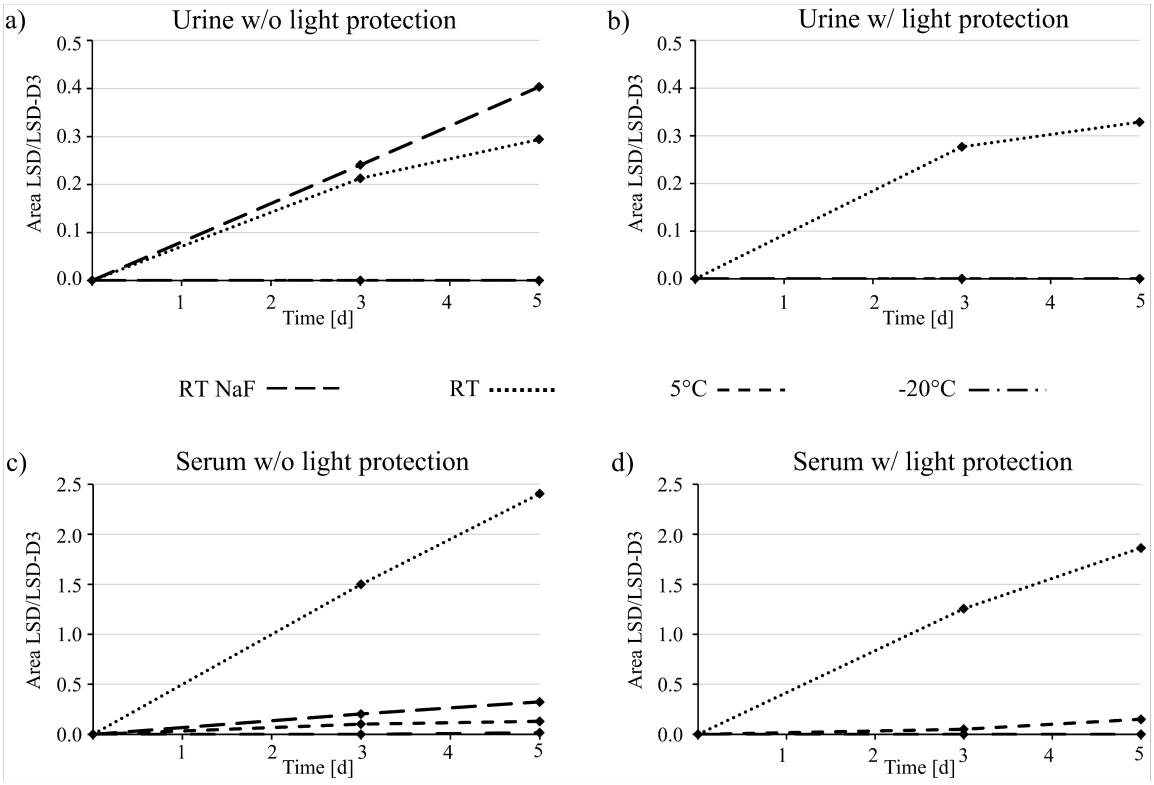

Figure 3: Results of the stability test. Area ratios of LSD/LSD-D3 in urine and serum samples fortified with 1P-LSD and stored at room temperature (RT) with addition of NaF, at RT, at 5 °C, at -20 °C for up to 5 days. a) urine samples without light protection; b) urine samples with light protection; c) serum samples without light protection; d) serum samples with light protection

Table 1: Summary of the mass spectrometry parameters used in this study. Retention time (RT) in min, m/z values in Da for the analytes in quadrupole 1 (Q1) and 3 (Q3), declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) (all potentials given in V).

Analyte	RT [min]	Q1/Q3 [m/z]	DP [V]	EP [V]	CE [V]	CXP [V]
LSD 1	4.5	324.2/223.2	50	10	32	13
LSD 2	4.5	324.2/208.2	50	10	50	11
1P-LSD 1	5.0	380.2/249.2	50	11	35	11
1P-LSD 2	5.0	380.2/208.1	50	11	50	11
LSD-D3	4.4	327.2/226.2	50	10	32	13

Table 2: Summary of the sample sets and their respective conditions including storage time, temperature, light protection, and addition of NaF.

Set	Storage time [d]	Temperature	Light protection	Addition of NaF	
1	3	RT	y	n	Stability samples
2	3	RT	n	y	
3	3	RT	n	n	
4	3	+5 °C	y	n	
5	3	+5 °C	n	n	
6	3	-20 °C	y	n	
7	3	-20 °C	n	n	
8	5	RT	y	n	
9	5	RT	n	y	
10	5	RT	n	n	
11	5	+5 °C	y	n	
12	5	+5 °C	n	n	
13	5	-20 °C	y	n	
14	5	-20 °C	n	n	
15	5	-80 °C	y	n	
16	5	-80 °C	n	n	
17	5	-80 °C	y	n	
18	5	-80 °C	n	n	
19	5	-80 °C	y	n	Reference samples
20	5	-80 °C	n	n	
21	5	-80 °C	y	y	
22	5	-80 °C	n	y	
23	0	-	y	n	Control samples
24	0	-	n	n	
25	0	-	y	y	
26	0	-	n	y	

y = yes, n = no, RT = room temperature (25 °C), storage time 0 = samples spiked directly before sample preparation

Table 3: Summary of precision data (relative standard deviation = RSD) for LSD and 1P-LSD in serum and urine samples at low (0.05 ng mL^{-1}) and high (0.3 ng mL^{-1}) quality control (QC) concentrations.

[%]	Serum				Urine			
	intraday		interday		intraday		interday	
	low	high	low	high	low	high	low	high
LSD	7.3	3.2	7.9	5.4	7.6	4.8	9.0	5.6
1P-LSD	9.8	3.1	9.8	5.1	5.3	9.2	6.5	9.7

Table 4: Summary of matrix effects, recoveries, and process efficiencies for LSD and 1P-LSD in serum and urine samples at low (0.05 ng mL^{-1}) and high (0.3 ng mL^{-1}) quality control (QC) concentrations as well as their respective relative standard deviations (RSD).

[%]	Serum												Urine											
	Matrix effect				Recovery				Process efficiency				Matrix effect				Recovery				Process efficiency			
	low	RSD	high	RSD	low	RSD	high	RSD	low	RSD	high	RSD	low	RSD	high	RSD	low	RSD	high	RSD	low	RSD	high	RSD
LSD	94	23	84	17	72	7	70	14	68	21	58	10	102	31	85	25	68	23	70	25	66	23	57	14
1P-LSD	92	22	82	13	83	6	76	12	77	20	61	12	95	29	81	24	69	28	66	26	63	26	51	17

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