

UNIVERSIDADE ESTADUAL DE CAMPINAS
SISTEMA DE BIBLIOTECAS DA UNICAMP
REPOSITÓRIO DA PRODUÇÃO CIENTÍFICA E INTELLECTUAL DA UNICAMP

Versão do arquivo anexado / Version of attached file:

Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:

<https://link.springer.com/article/10.1007/s11419-022-00622-0>

DOI: 10.1007/s11419-022-00622-0

Direitos autorais / Publisher's copyright statement:

©2022 by Springer. All rights reserved.

DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo

CEP 13083-970 – Campinas SP

Fone: (19) 3521-6493

<http://www.repositorio.unicamp.br>



High-sensitivity method for the determination of LSD and 2-oxo-3-hydroxy-LSD in oral fluid by liquid chromatography—tandem mass spectrometry

Kelly Francisco da Cunha^{1,2}  · Julia Martinelli Magalhães Kahl^{1,3}  · Taís Regina Fiorentin^{1,2}  · Karina Diniz Oliveira²  · Jose Luiz Costa^{1,3} 

Received: 31 January 2022 / Accepted: 17 March 2022

© The Author(s), under exclusive licence to Japanese Association of Forensic Toxicology 2022

Abstract

Purpose We have developed and validated a high-sensitivity method to quantify lysergic acid diethylamide (LSD) and 2-oxo-3-hydroxy-LSD (OH-LSD) in oral fluid samples using liquid–liquid extraction and liquid chromatography—tandem mass spectrometry (LC–MS/MS). The method was applied to the quantification of both substances in 42 authentic oral fluid samples.

Methods A liquid–liquid extraction was performed using 500 µL each of samples (oral fluid samples collected using Quantisal™ device) and dichloromethane/isopropanol mixture (1:1, v/v). Enzymatic hydrolysis was evaluated to cleave glucuronide metabolites.

Results The limit of quantification was 0.01 ng/mL for both LSD and OH-LSD. The linearity was assessed between 0.01 and 5 ng/mL. Imprecision and bias were not higher than 10.2% for both analytes. Extraction recovery was higher than 69%. The analytes were stable in the autosampler at 10 °C for 24 h, and up to 30 days at 4 and -20 °C. The method was applied to the analysis of 42 oral fluid samples. LSD was detected in all samples (concentrations between 0.02 and 175 ng/mL), and OH-LSD was detected in 20 samples (concentrations between 0.01 and 1.53 ng/mL).

Conclusions A high-sensitive method was fully validated and applied to authentic samples. To our knowledge, this is the first work to report concentrations of LSD and OH-LSD in authentic oral fluid samples.

Keywords Lysergic acid diethylamide (LSD) · 2-Oxo-3-hydroxy-LSD (OH-LSD) · Oral fluid · LC–MS/MS · Authentic samples

Introduction

Lysergic acid diethylamide (LSD) is a semi-synthetic compound, known by its hallucinogenic properties due to its agonist action at 5-HT_{2A} receptors [1], and being used mainly for recreational purposes. Generally, it is used in small doses and is extensively metabolized, which requires

highly sensitive techniques to detect it. The LSD plasma concentrations reported are 7.4 and 9.7 ng/mL, falling below 5 pg/mL after 24 and 48 h [2].

LSD is majorly consumed by oral administration, with an oral bioavailability of around 71%, being metabolized mainly in liver tissue [1]. One of the last discovered LSD metabolites is the 2-oxo-3-hydroxy-LSD (OH-LSD), which is present in the organism at a mean ratio between 16- and 43 with LSD [3, 4]. Klette et al. [5] incubated LSD with human liver microsomes and human cryopreserved hepatocytes to demonstrate that OH-LSD is generated by human metabolism and not produced during extraction procedure and/or LSD degradation in a matrix. No OH-LSD was detected in either incubations not treated with LSD or in the presence of 1-aminobenzotriazole inhibitor. The use of the most predominant metabolite, OH-LSD, to extend the detection window of LSD seems to be a coherent tool in forensic analysis,

✉ Jose Luiz Costa
jose.jlc@fcf.unicamp.br

¹ Campinas Poison Control Center, University of Campinas, Campinas, São Paulo 13083-859, Brazil

² Faculty of Medical Sciences, University of Campinas, Campinas, São Paulo 13083-859, Brazil

³ Faculty of Pharmaceutical Sciences, University of Campinas, Campinas, São Paulo 13083-859, Brazil

as it is present in concentrations commonly greater than the parent drug [5, 6].

Dolder et al. [7] reported a 24 h LSD and OH-LSD pharmacokinetic study in plasma and urine samples after a single oral dose of 200 µg of LSD in 16 volunteers (8 male and 8 female). LSD plasma concentrations higher than 0.1 ng/mL were measured in all subjects up to 12 h. The maximum mean concentration of 4.3 ng/mL was reached 1.5 h (median) after administration. OH-LSD was measured in eight volunteers with a maximum mean concentration of 0.4 ng/mL after 4 h (median) of administration. The highest LSD and OH-LSD concentrations obtained in urine samples was between 8 and 16 h after the oral administration, with mean concentrations of 1.1 and 17.7 ng/mL, respectively.

The LSD instability under exposure to certain conditions as heat, sunlight and ultraviolet light irradiation was already described [1] and this issue increase the challenge to analyze it in biological matrix. In addition to greater metabolite stability observed in urine samples, simultaneous analysis of LSD and its metabolite is recommended to improve the drug detection time [6]. Despite being already detected in alternative matrices as vitreous humor [8] and hair [9], no data of LSD and OH-LSD in oral fluid samples have been reported.

Oral fluid sample is an alternative matrix that is gaining attention especially in onsite context [i.e., driving under the influence of drugs (DUID), workplace drug testing, and electronic music festivals]. The advantages include a non-invasive collection, without requiring a medical personnel for sampling [10]. Despite few controlled studies involving LSD, and none for oral fluid samples, some countries stated an LSD cutoff for oral fluid samples in a DUID context at 1 ng/mL, considering a zero tolerance approach [11, 12].

The aim of this work was to develop and validate a sensitive method to quantify LSD and OH-LSD (Fig. 1) in oral fluid samples collected with Quantisal™ device using liquid-liquid extraction (LLE) by liquid chromatography—tandem mass spectrometry (LC–MS/MS). As a proof of the method reliability and understand the concentration relationship

between both analytes in this matrix, the method was applied to 42 authentic samples collected from onsite college parties and electronic music festivals.

Materials and method

Standards and chemicals

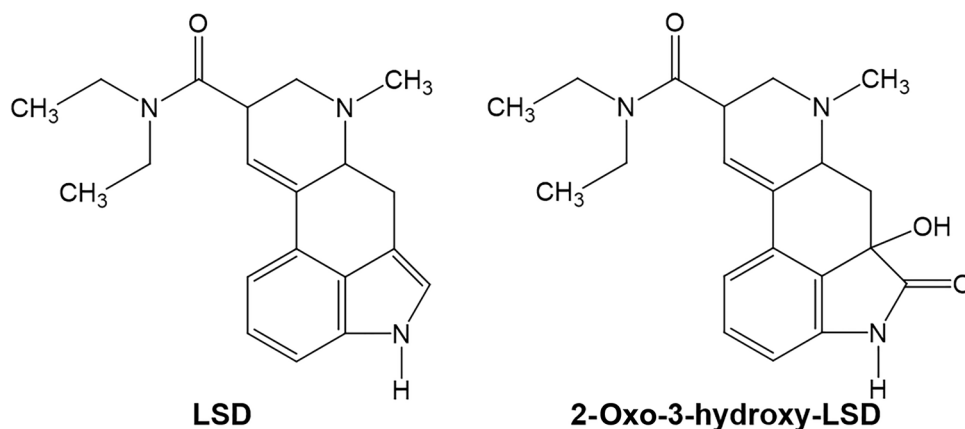
The LSD, OH-LSD and LSD-*d*₃ (internal standard, IS) standards were purchased from Cerilliant (Round Rock, TX, USA); isopropanol, dichloromethane, methanol, acetonitrile and formic acid (98–100%) from Merck (Darmstadt, Germany); sodium tetraborate from LS Chemicals (Ribeirao Preto, SP, Brazil). Ultrapure deionized water was supplied by a Milli-Q RG unit from Millipore (Billerica, MA, USA). *Helix pomatia* β-glucuronidase (Type HP-2, ≥ 100.000 units/mL) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents employed in the extraction were of HPLC grade. Quantisal™ oral fluid collection devices and its extraction buffer (phosphate buffer solution: PBS, 100 mmol/L) were purchased from Immunalysis (Pomona, CA, USA).

Calibrators, quality controls and internal standard

Stock solution containing LSD and OH-LSD at 500 ng/mL each was prepared by dilution of reference standards in acetonitrile. Dilutions of the stock solution were performed to generate working calibrators at 0.1, 0.5, 1, 2.5, 5, 10, 25 and 50 ng/mL in acetonitrile. Quality control (QC) solutions were prepared by a different analyst at concentrations of 0.3, 8 and 40 ng/mL in acetonitrile. Internal standard LSD-*d*₃ at 10 ng/mL was prepared by its appropriate dilution in acetonitrile. All solutions were stored in amber glass vials at – 20 °C.

Quantisal™ oral fluid devices were used for all authentic sample collections. To simulate the real device conditions,

Fig. 1 Structures for lysergic acid diethylamide (LSD) and its major metabolite, 2-oxo-3-hydroxy-LSD (OH-LSD)



all fortified samples were prepared by 1:10 dilution of the working or QC solutions in blank oral fluid samples and then mixed with Quantisal™ extraction buffer (1:3, v/v). From this mixture, 500 µL were used for the sample extraction.

Sample collection and screening

The Quantisal™ oral fluid collection device consists in a pad collector and a transport tube, containing 3 mL of PBS buffer solution (100 mmol/L). The oral fluid sample collection was performed as recommended by the manufacturer, placing the pad under the volunteer's tongue during 2–5 min, until the volume indicator turned blue (1 ± 0.1 mL of oral fluid collected). The pad was transferred to the transport tube, capped and transported to the laboratory in a biological sample transport box protected from the light containing dry ice.

The sample collection occurred at college parties and electronic music festivals in Brazil. The volunteers were informed about the project aim and invited to answer an anonymous questionnaire, followed by donation of oral fluid. All samples were extracted and analyzed using a screening method [13] to detect psychoactive substances. All samples ($n=42$) previously screened positive to LSD were submitted to this quantitative proposed method.

Oral fluid sample extraction

To 500 µL of sample, 20 µL of LSD- d_3 (10 ng/mL) and 500 µL of saturated sodium tetraborate aqueous solution were added. The LLE was performed with 1.5 mL of dichloromethane/isopropanol mixture (1:1, v/v). The samples were vortexed at 2500 rpm for 3 min, centrifuged at 4500 rpm for 5 min, and the organic phase was dried under nitrogen stream (5 psi, 40 °C for 15 min). The residue was reconstituted in 100 µL of mobile phase mixture (A/B, 50:50, v/v) and 5 µL injected into the LC–MS/MS system.

Instrumental

The analysis was performed on a Nexera UHPLC chromatographic system coupled to a LCMS8060 triple

quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The chromatographic separation was performed on a Raptor™ Biphenyl column (100 × 2.1 mm, 2.7 µm; Restek, Bellefonte, PA, USA), maintained at 40 °C. The mobile phase was set to 0.4 mL/min and it was composed by (A) water and (B) methanol, both containing 0.1% formic acid and 2 mmol/L ammonium formate. The gradient elution started with 5% B to 0.5 min, followed by a linear change to 95% B over 5.5 min, held at 95% B for 1 min and returned to initial conditions over 0.2 min (9.0 min run time).

The mass spectrometer was equipped with an electrospray ionization (ESI) source, operated in positive mode. The source parameters were set as follows: heat block temperature 400 °C; interface temperature 300 °C; ion spray voltage 4.0 kV; nebulizer gas (N_2) flow 3 L/min; desolvation line temperature 250 °C; drying gas (N_2) flow 10 L/min; heating gas (air) flow 10 L/min; and collision induced dissociation gas pressure (Ar) 270 kPa. The analyses were performed in multiple reaction monitoring (MRM) mode. For each compound, two MRM transitions were selected (Table 1), one for quantification and one qualifier for confirmation/identification using the MRM ratio as identification criteria. Data were acquired and processed with the LabSolutions 5.97 software (Shimadzu).

Method validation

The present method was validated based on the recommendations of the ANSI/ASB Standard 036 guideline for quantitative analyses in forensic toxicology [14]. Analytes identification criteria were symmetrical chromatographic peaks with retention times within $\pm 2\%$ of the average calibrator retention time, signal-to-noise ratio of at least 3 for both qualifier and quantifier ions, and the ratio between quantifying and qualifier MRM within $\pm 20\%$ (for LSD) and $\pm 25\%$ (for OH-LSD). The following validation parameters were evaluated after method development: limit of detection (LOD), limit of quantification (LOQ), linearity, bias, imprecision, carryover, recovery, matrix effect, selectivity, specificity, stability and dilution integrity.

Table 1 Mass spectrometer parameters for analysis of lysergic acid diethylamide (LSD) and 2-oxo-3-hydroxy-LSD (OH-LSD) in oral fluid samples by liquid chromatography–tandem mass spectrometry (LC–MS/MS)

Analyte	MRM transitions (m/z)	Q1 Pre bias (V)	CE (V)	Q3 Pre bias (V)	RT (min)
LSD	324.1 → 223.0	–17	–25	–23	4.67
	324.1 → 208.0	–10	–31	–21	
OH-LSD	356.1 → 222.0	–18	–34	–23	3.69
	356.1 → 237.0	–19	–25	–15	
LSD- d_3 (IS)	327.3 → 226.2	–17	–26	–14	4.67
	327.3 → 210.2	–17	–45	–21	

MRM multiple reaction monitoring, CE collision energy, RT retention time, IS internal standard

LOD, LOQ and linearity

The LOD was determined as the lowest analyte concentration that met all the identification criteria with a signal-to-noise (S/N) ratio of at least 3, while the LOQ was defined as the lowest concentration of linearity with an $S/N \geq 10$ and quantifying within $\pm 20\%$ of the target concentration. The linearity was evaluated over 5 days, with calibrators prepared at concentrations of 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5 and 5 ng/mL. All calibrators should quantify within $\pm 15\%$ of each target concentration, and the correlation coefficient (r) should be greater than 0.99.

Bias and imprecision

Fortified oral fluid samples were analyzed in triplicate over 5 days ($n = 15$) at three different concentrations—0.03 (low), 0.8 (medium) and 4 ng/mL (high) QCs to assess bias and imprecision (intraday and interday). Maximum acceptable value for bias was $\pm 15\%$ for each nominal concentration. For imprecision, LOQ (0.01 ng/mL) was also evaluated in triplicate, but in this case over 3 days ($n = 9$). One-way analysis of variance approach (ANOVA, $p < 0.05$) was used to calculate the imprecision on each QC and LOQ concentration, and the highest acceptable variation was 15% in all cases.

Carryover

A negative oral fluid sample was analyzed immediately after the highest point calibration over 5 days. It was considered carryover absent if the analyte's peak did not reach the LOD criteria.

Recovery and matrix effect

For recoveries, two sets of blank oral fluid samples were prepared in sextuplicate at low and high QC concentrations. One of them was fortified with the QC and IS solutions and extracted following the above described method; the other one (matrix only) was extracted and, before the evaporation step, fortified with the same QC and IS standard solutions. The average peak area of each set was compared, considering the second set correspondent to a 100% of analyte recovery. To verify the suppression or enhancement on the analyte signals due to the matrix influence, a batch composed of six blank oral fluid samples was extracted and fortified with the QC and IS solutions at low and high concentrations prior to the evaporation step. The average absolute peak area was compared with the average absolute peak area of a neat

solution containing the QC and IS solutions at the same concentration levels and injected six times.

Selectivity and specificity

Selectivity was evaluated by analyzing blank oral fluid samples from 10 healthy non-drug-consuming volunteers. Specificity was performed fortifying negative oral fluid samples with 31 different pharmaceuticals or drug of abuse mixture at concentrations of 100, 1000 or 5000 ng/mL. The samples were analyzed for the presence of peaks that could interfere with the substances of interest satisfying the LOD adopted criteria.

Stability

Long-term stability was evaluated for 7, 15 and 30 days at 4 and -20°C . Blank oral fluid samples were fortified at low and high QC concentrations and aliquoted in several batches (triplicate). A batch of each concentration was extracted and analyzed immediately as day zero. At each designed day, batches stored under determined conditions were extracted and analyzed against a freshly prepared calibration curve. The mean concentration of each day was compared with the mean concentration on day zero.

Autosampler stability was evaluated at three concentration levels (low, medium and high) by analysis of the extracts ($n = 3$) storage in the autosampler at 10°C for 24 h. Samples were analyzed right after extraction protocol and reinjected after 24 h against a freshly prepared calibration curve.

In both studies the results are presented as %difference concentration between the two studied periods. The analyte was considered stable when the %difference was within $\pm 20\%$.

Dilution integrity

Two dilution ratios and concentrations were evaluated. Fortified blank samples ($n = 3$) at 50 and 100 ng/mL were diluted in blank samples at ratios of 1/25 or 1/50, respectively (v/v), extracted following the proposed protocol and injected into LC–MS/MS. If the concentration after the dilution with a fixed factor is within $\pm 15\%$ of the target concentration, the integrity of the dilution is established.

Results

The linearity was obtained between 0.01 and 5 ng/mL ($r > 0.996$, $1/x^2$ weighted). The LOD and LOQ were 0.01 ng/mL for LSD and its metabolite (Fig. 2). The LOD and LOQ for LSD were administratively set as the lowest point of the calibration curve (0.01 ng/mL) as there is no biological need

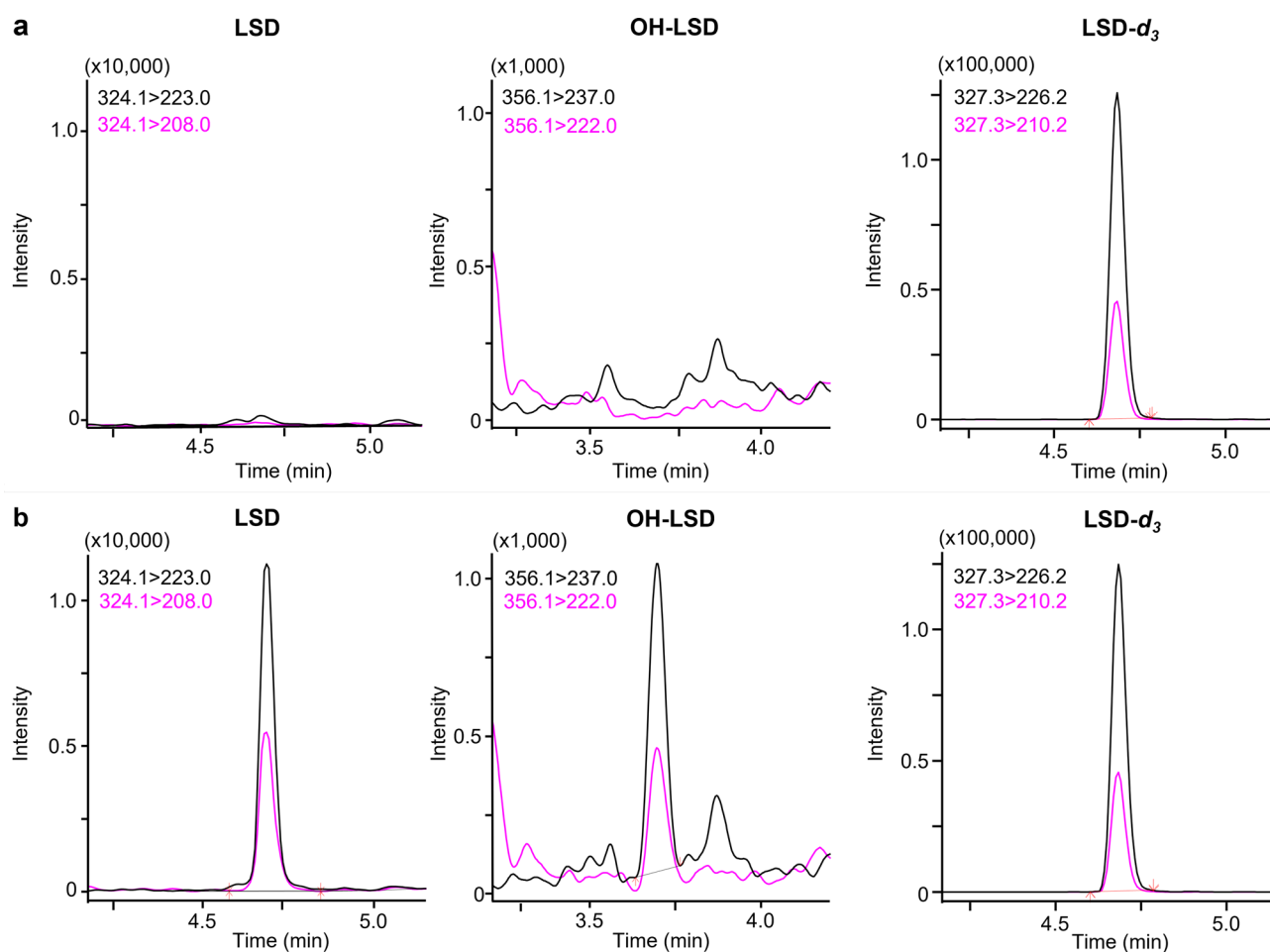


Fig. 2 Multiple reaction monitoring (MRM) chromatograms for **a** blank oral fluid sample and **b** blank oral fluid sample fortified with LSD and OH-LSD at 0.01 ng/mL (limit of quantification)

to go lower in these analytical limits for the purpose of the proposed method.

Intra- and interday imprecisions showed values of %RSD not higher than 4.3 and 10.2% for QC and LOQ concentrations, respectively (one-way ANOVA, $p < 0.05$). Bias values were not higher than 6.6% for all samples tested. Matrix effect showed low values for LSD, but expressive results for OH-LSD. Even so, these values did not compromise the quantitative validation results as presented here. Extraction recovery was higher than 69% for both analytes at low and high QC concentrations. A compilation of the results can be seen in Table 2.

Dilution integrity was performed in blank samples spiked at 50 ng/mL and diluted in a proportion of 1/25; and 100 ng/mL diluted in a proportion of 1/50. Biases were 3.8% and -7.2% for LSD and OH-LSD, respectively, at 1/25 ratio dilution; and 10.9% and -0.4% at 1/50 ratio dilution.

The extracted samples were stable when kept in the autosampler (10 °C) for 24 h. The % difference were not higher than 10.9% for both analytes. Spiked oral fluid

samples were also stable up to 30 days at 4 and -20 °C (concentration differences not higher than 15.4%). The stability results are shown in Table 3.

Ten blank oral fluid samples from different sources were extracted and analyzed under this method. No peak that satisfied the identification criteria were observed in the window acquisitions for LSD, OH-LSD and LSD- d_3 . Specificity was evaluated in negative oral fluid samples spiked with 31 drugs of abuse, metabolites and pharmaceuticals, with concentrations of 100, 1000 or 5000 ng/mL. The studied analytes and respective concentrations are found in the supplementary material (Table S1).

The 329 authentic oral fluid samples collected from attendees of college and electronic music parties were analyzed in a screening method [13] and, from those, 42 samples were positive to LSD (12.8%) and quantified by the proposed method. LSD was detected and quantified in all samples, with concentrations ranging between 0.02 and 175 ng/mL, with a mean concentration of 10.6 ng/mL and median of 0.40 ng/mL (Fig. S3a). OH-LSD was detected

Table 2 Method validation results for imprecisions, biases, matrix effects and recovery rates for LSD and OH-LSD in oral fluid samples by LC–MS/MS

Parameter	Analyte	
	LSD	OH-LSD
Intraday imprecision (%RSD) (<i>n</i> = 3)		
LOQ	2.1	5.2
Low	3.7	3.6
Medium	3.0	2.8
High	1.4	3.2
Interday imprecision (%RSD) (<i>n</i> = 15)		
LOQ ^a	5.3	10.2
Low	4.3	3.8
Medium	4.0	4.0
High	2.7	4.3
Bias (%) (<i>n</i> = 15)		
Low	6.6	5.1
Medium	4.0	3.5
High	1.2	0.8
Matrix effect (%) (<i>n</i> = 6)		
Low	−3.2	−43.7
High	−9.4	−59.3
Recovery (%) (<i>n</i> = 6)		
Low	79.1	69.3
High	85.7	74.1

LOQ limit of quantification, RSD relative standard deviation

^a*n* = 9**Table 3** Long-term and autosampler stability results for LSD and OH-LSD in oral fluid by LC–MS/MS

Stability study (%)	Temperature (°C)	Analyte	
		LSD	OH-LSD
Long term (<i>n</i> = 4)			
30 days			
Low	4	− 10.9 (2.3)	− 15.4 (3.1)
	− 20	− 3.3 (1.7)	0.6 (2.2)
High	4	− 12.1 (2.7)	− 7.9 (4.3)
	− 20	1.2 (1.5)	− 0.3 (1.9)
Autosampler (<i>n</i> = 5)			
24 h			
Low	10	3.1 (2.0)	10.9 (6.1)
High	10	2.1 (1.1)	7.9 (1.5)

Results are presented as %difference (%RSD)

and quantified in 20 samples (47.6%), with concentrations ranging between 0.01 and 1.53 ng/mL (mean = 0.05 ng/mL, excluding sample 36 due to high values). All samples' results were shown in Table 4, and the MRM chromatograms of two samples are depicted in Fig. 3.

Discussion

As part of the method development, three different extraction solvents were tested: dichloromethane/isopropanol mixture (1:1, v/v), MTBE (*t*-butyl methyl ether) and chloroform/ethanol mixture (80:20, v/v). Spiked oral fluid samples at 0.02 ng/mL were extracted in duplicate using these solvents and the analyte peak area was evaluated with main focus on OH-LSD. MTBE, as the less polar solvent within the evaluated solvents, showed lower response for OH-LSD and a noisier baseline in LC–MS/MS chromatogram. Dichloromethane/isopropanol and chloroform/ethanol mixture showed similar results, without significant difference according to a post-hoc Tukey test (Past 4.01 software, Natural History Museum, Oslo). As chloroform/ethanol mixture tended to present a lower response and a noisier baseline for the second MRM transition (356.1 → 222.0), the authors decided to proceed with the use of dichloromethane/isopropanol mixture to perform the extraction. For comparison, extracted MRM chromatograms showing the three different solvents tested can be found in the supplementary material (Fig. S1).

Another aspect that we had to work on was in respect to an interferent from the matrix in the 356.1 → 222.0 MRM transition of OH-LSD. Using 5.5 min of the chromatographic run time, a sample interferent and the metabolite eluted almost together. By decreasing the gradient ramp slope and, consequently, prolonging the run time, the authors were able to increase the baseline resolution between both analytes (Supplementary Material, Fig. S2).

Enzymatic hydrolysis was performed to exclude the possibility of a conjugated metabolite. One milliliter of an authentic positive oral fluid sample collected with Quantisal™ device received 20 µL of IS and 200 µL ammonium acetate 1 mol/L, containing acetic acid (2%, v/v) and was incubated at 55 °C for 60 min in duplicate with and without β-glucuronidase from *Helix pomatia* (> 100,000 units/mL) at pH = 5.0. The replicates were extracted and the area ratio of OH-LSD analyzed using a one-tailed paired sample *t*-test (*p* = 0.05). No significant difference was observed between the conditions [*t*(2) = 6.3, *p* = 0.07]. Sklerov et al. [15] had tested the hydrolysis of six urine samples positive for LSD with β-glucuronidase from *Helix pomatia* and four of them showed no significant differences, suggesting no glucuronide conjugate formation for OH-LSD.

To the best of the authors' knowledge, there are no published studies evaluating LSD and OH-LSD in authentic oral fluid samples. Lund et al. [16] reported a quantitative method for 32 drugs, including LSD, in oral fluid samples collected with Intercept® device and analyzed by ultra-performance liquid chromatography—tandem mass spectrometry (UPLC–MS/MS). The LOQ achieved for LSD

Table 4 LSD and OH-LSD concentrations in oral fluid samples collected in college and electronic music parties

Sample number	LSD (ng/mL)	OH-LSD (ng/mL)	Time (h) ^a	Other detected substances
1	0.02	<LOQ	1.7	MDMA, THC
2	0.02	<LOQ	12.0	MDMA, MDA, THC, ketamine
3	0.03	<LOQ	12.0	MDMA, THC
4	0.05	<LOQ	10.0	MDMA, THC
5	0.05	<LOQ	0.1	THC
6	0.05	<LOQ	4.0	MDMA
7	0.08	0.02	12.0	MDMA, MDA, MDEA, THC, ketamine
8	0.08	<LOQ	0.5	MDMA, MDA, THC, <i>N</i> -ethylpentylone
9	0.09	<LOQ	0.1	MDMA, MDA, MDEA, THC, ketamine
10	0.10	0.02	0.2	MDMA, MDA, MDEA, ketamine
11	0.14	0.01	3.0	MDMA, MDA
12	0.14	<LOQ	3.0	MDMA, MDA, THC
13	0.15	<LOQ	2.0	THC
14	0.16	<LOQ	0.7	MDMA, MDA, THC, ketamine
15	0.18	<LOQ	1.5	MDMA, THC
16	0.19	<LOQ	2.0	MDMA, paroxetine
17	0.20	<LOQ	1.5	nd
18	0.20	<LOQ	1.0	MDMA, THC, <i>N</i> -ethylpentylone
19	0.23	<LOQ	2.5	THC
20	0.24	0.01	3.0	MDMA, THC
21	0.24	<LOQ	1.0	nd
22	0.49	<LOQ	2.0	MDMA, THC
23	0.52	<LOQ	2.0	THC
24	0.58	<LOQ	1.0	Venlafaxine
25	0.88	0.01	1.0	MDMA, MDA, ketamine
26	0.99	<LOQ	1.0	MDMA, MDEA, THC
27	1.19	0.02	0.7	MDMA, THC
28	1.38	<LOQ	0.1	MDMA, MDEA, THC, ketamine
29	2.60	0.01	0.5	MDMA, THC
30	2.99	0.01	2.0	MDMA, THC
31	3.77	0.02	10.0	MDMA, MDA, MDEA, THC, <i>N</i> -ethylpentylone, ketamine
32	4.30	0.05	5.0	MDMA, MDA, MDEA, THC, ketamine
33	4.38	0.02	1.5	25I-NBOMe
34	8.50	0.03	1.5	MDMA
35	9.82	0.04	0.5	MDMA, MDA, THC
36	11.0	1.53	1.0	MDMA, THC
37	13.2	0.02	0.7	MDMA, MDA, MDEA, THC, methylone
38	27.9	0.13	0.5	THC
39	28.5	0.20	2.0	MDMA, MDEA, THC, <i>N</i> -ethylpentylone, cocaine, levamisole, mirtazapine
40	33.8	0.04	0.7	THC, sertraline
41	113	0.09	0.7	MDMA, MDEA, THC, ketamine
42	175	0.12	0.5	MDMA, MDA, THC, cocaine, levamisole

25I-NBOMe 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine, MDA 3,4-methylenedioxyamphetamine, MDEA 3,4-methylenedioxy-*N*-ethylamphetamine, MDMA 3,4-methylenedioxymethamphetamine, nd not detected, THC Δ^9 -tetrahydrocannabinol

^aEstimated time between consumption and sample collection

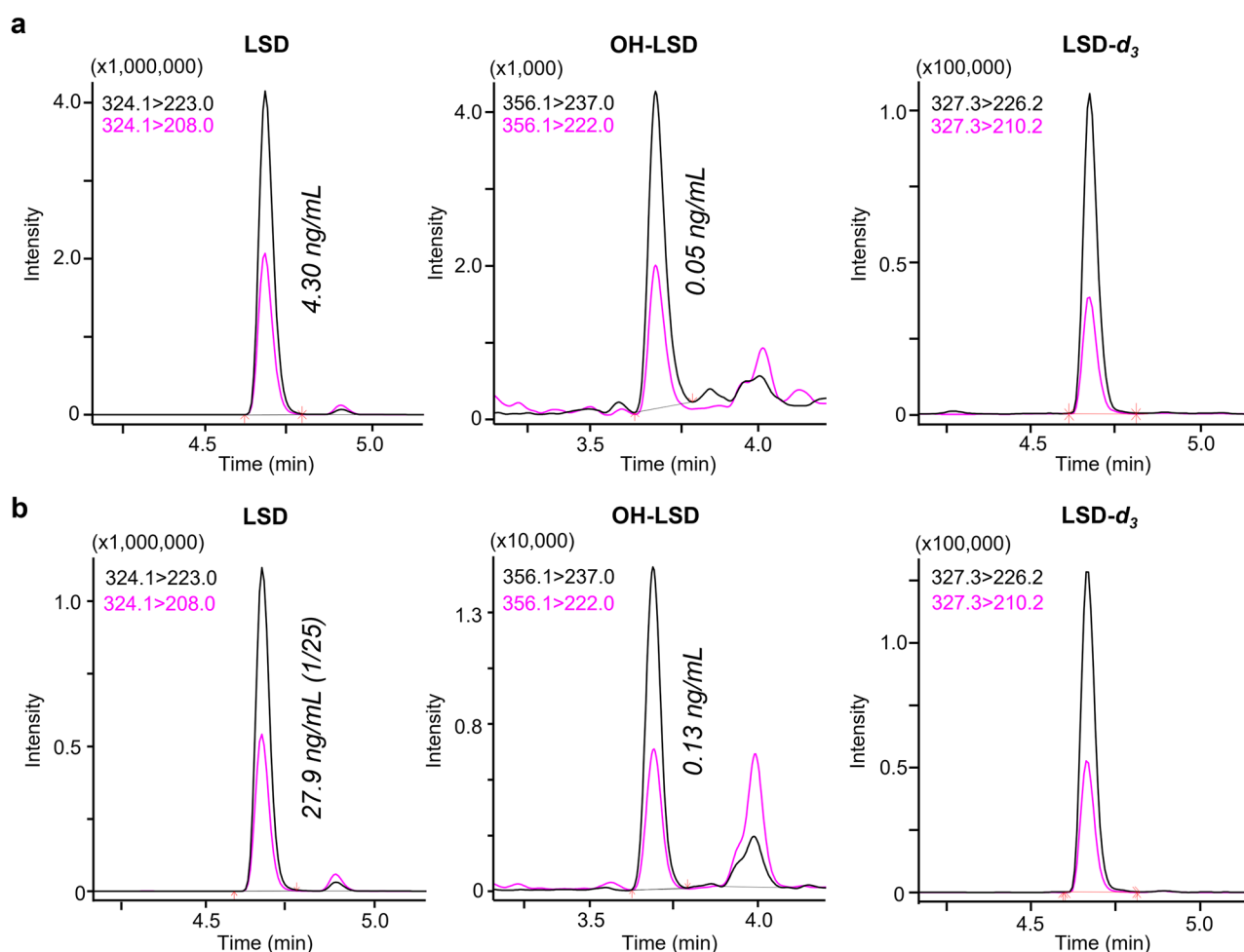


Fig. 3 MRM chromatograms of authentic oral fluid samples with positive results to LSD and OH-LSD. **a** Sample #32 and **b** sample #38 (LSD from sample 38 was diluted 25 times to fit to the calibration range)

was 0.01 ng/mL, using one MRM transition and same initial volume sample (0.5 mL). A matrix effect of 52% (30%RSD) was observed, and a higher result was observed when using a StatSure Saliva Sampler™ device 204% (38%RSD). However, in their study, selectivity, stability, recovery rate and presentation of authentic case(s) were lacking for LSD. Almost no metabolites of the 32 drugs were dealt with in their report.

Øiestad et al. [17] also reported a quantitative method for the 32 drugs as shown in the above paper including LSD in oral fluid samples collected with Intercept™ device with recovery higher than 68%. Previous results reported by our group [13] showed a Quantisal™ recovery result for LSD higher than 87%, and extraction recovery rates reaching 85.7% for LSD and 74.1% for OH-LSD at higher concentrations. However, in this paper by Øiestad et al. [17], selectivity, stability and the presentation of authentic cases for LSD and OH-LSD were not given.

LSD was found in much higher concentrations than its metabolite in the authentic samples (Table 4). No direct relationship was observed between the concentration of LSD and OH-LSD. The ratios obtained from the 19 OH-LSD positive samples are shown in the supplementary material (Fig. S3b). Eleven samples presented ratios between 79 and 285. Chung et al. [18] was the first to report OH-LSD in an authentic blood sample, with LOQ of 0.02 ng/mL for LSD and 0.05 ng/mL for OH-LSD, using 1 mL of sample for the extraction. The LSD and OH-LSD concentrations were 0.6 and 0.1 ng/mL in femoral blood, respectively. Dolder et al. [19] reported four LSD-positive cases with available serum samples. With LOQ of 0.1 ng/mL, the concentrations found for LSD and OH-LSD were 14.7 and 1.0 ng/mL for the first sample, and 6.1 and 0.5 ng/mL for the second sample, respectively. As observed in our samples, LSD was present in higher concentrations.

Six out of seven samples with LSD concentrations above 10 ng/mL (Table 4) had the consumption reported to be within 1 h before the collection. As a non-controlled study and occurring during a party/festival, the volunteers were not asked to perform mouth rinsing with water or other liquid. Knowing that, depending on the route of consumption, the drug may contaminate the oral mucosa [10], we should not disregard this possibility in these cases.

Sample 36 was the only one with an OH-LSD concentration pattern different from the other samples. While the concentrations were between 0.01 and 0.2 ng/mL overall, in sample 36 the concentration was as high as 1.5 ng/mL; this is the reason why this sample was excluded from the mean concentration calculations. Specificity and selectivity tests did not show any endogenous or exogenous interferents, considering common pharmaceuticals and drugs of abuse. As well as observed for most of the samples, only 3,4-methylenedioxymethamphetamine and Δ^9 -tetrahydrocannabinol were found together with the LSD when the sample was analyzed using the screening method (Table 4). Multiple LSD consumption in a short period of time prior to the sample collection is considered as a hypothesis for such a discrepant concentration found. The absence of sufficient information is one of the limitations in non-controlled collections.

It is also important to observe that the time consumption described in Table 4 is self-reported and, in most cases, the donor was under the influence of some substance, which can change their time–space orientation. Based on the information provided by the volunteers, LSD could be detected in oral fluid samples up to 12 h after its consumption.

Conclusions

A sensitive and reliable method to quantify LSD and its major metabolite, OH-LSD, in oral fluid samples collected with Quantisal™ device and analyzed by LC–MS/MS was fully validated. Both analytes achieved a limit of quantification of 0.01 ng/mL using LLE.

This is the first work to detect and quantify OH-LSD in oral fluid samples. In addition, only two preceding reports describing quantification of LSD in oral fluids are available [16, 17], but the authentic cases of LSD in oral fluid could not be found. Forty-two authentic samples obtained on-site were quantified by this method and OH-LSD was detectable in almost 48% of the samples. LSD appears to be quantified in oral fluid samples up to 12 h after the self-reported consumption. Despite evidence of concentrations of the metabolite being greater in urine than the parent drug itself [5], in this study, OH-LSD was found present in oral fluid samples in much lower concentrations than its parent drug. A controlled study may better evaluate the kinetics pattern of these

analytes in oral fluid samples and avoid any possible higher LSD concentrations coming from oral cavity contamination.

Even though LSD is used recreationally at low doses, the method proved to be efficient to detect LSD and its main metabolite in a recreational context.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11419-022-00622-0>.

Acknowledgements The authors would like to thank the São Paulo Research Foundation—FAPESP (Process Number 2018/00432-1, 2018/11849-0, 2020/07470-6 and 2020/10809-5) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES (Finance Code 001 and Projeto INSPEQT, Edital No. 16/2020, Process Number 88887.516176/2020-00) for the financial support.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Oral fluid sample collection was conducted under a protocol reviewed and approved by the Research Ethics Committee (CEP) from the University of Campinas (CAAE 88770318.0.0000.5404) and it was in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Copies of the informed consent form were available to the volunteers at all events.

References

1. Marta RFLO (2019) Metabolism of lysergic acid diethylamide (LSD): an update. *Drug Metab Rev* 51:378–387. <https://doi.org/10.1080/03602532.2019.1638931>
2. Verstraete AG (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. *Ther Drug Monit* 26:200–205. <https://doi.org/10.1097/00007691-200404000-00020>
3. Poch GK, Klette KL, Hallare DA, Manglicmot MG, Czarny RJ, McWhorter LK, Anderson CJ (1999) Detection of metabolites of lysergic acid diethylamide (LSD) in human urine specimens: 2-oxo-3-hydroxy-LSD, a prevalent metabolite of LSD. *J Chromatogr B* 724:23–33. [https://doi.org/10.1016/S0378-4347\(98\)00574-X](https://doi.org/10.1016/S0378-4347(98)00574-X)
4. Poch GK, Klette KL, Anderson C (2000) The quantitation of 2-oxo-3-hydroxy lysergic acid diethylamide (O-H-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. <https://doi.org/10.1093/jat/24.3.170> (open access article)
5. Klette KL, Anderson CJ, Poch GK, Nimrod AC, ElSohly MA (2000) 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. <https://doi.org/10.1093/jat/24.7.550> (open access article)
6. Skopp G, Pötsch L, Mattern R, Aderjan R (2002) Short-term stability of lysergic acid diethylamide (LSD), *N*-desmethyl-LSD, and 2-oxo-3-hydroxy-LSD in urine, assessed by liquid chromatography–tandem mass spectrometry. *Clin Chem* 48:1615–1618. <https://doi.org/10.1093/clinchem/48.9.1615> (open access article)

7. Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME (2016) Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *Int J Neuropsychopharmacol* 19:pyv072. <https://doi.org/10.1093/ijnp/pyv072> (open access article)
8. Favretto D, Frison G, Maietti S, Ferrara SD (2007) LC-ESI-MS/MS on an ion trap for the determination of LSD, *iso*-LSD, *nor*-LSD and 2-oxo-3-hydroxy-LSD in blood, urine and vitreous humor. *Int J Legal Med* 121:259–265. <https://doi.org/10.1007/s00414-006-0078-x>
9. Jang M, Kim J, Han I, Yang W (2015) Simultaneous determination of LSD and 2-oxo-3-hydroxy LSD in hair and urine by LC–MS/MS and its application to forensic cases. *J Pharm Biomed Anal* 115:138–143. <https://doi.org/10.1016/j.jpba.2015.07.001>
10. Bosker WM, Huestis MA (2009) Oral fluid testing for drugs of abuse. *Clin Chem* 55:1910–1931. <https://doi.org/10.1373/clinchem.2008.108670> (open access article)
11. Wolff K, Agombar R, Clatworthy A, Cowan D, Forrest R, Osseltson D, Scott-Ham M, Johnston A (2017) Expert panel review of alternative biological matrices for use as an evidential sample for drug driving. <https://www.gov.uk/government/publications/review-of-oral-fluid-alternative-biological-matrices-for-drug-driving>. Accessed 15 Jan 2022
12. Vindenes V, Jordbru D, Knapskog A-B, Kvan E, Mathisrud G, Slørdal L, Mørland J (2012) Impairment based legislative limits for driving under the influence of non-alcohol drugs in Norway. *Forensic Sci Int* 219:1–11. <https://doi.org/10.1016/j.forsciint.2011.11.004>
13. da Cunha KF, Oliveira KD, Huestis MA, Costa JL (2020) Screening of 104 new psychoactive substances (NPS) and other drugs of abuse in oral fluid by LC–MS-MS. *J Anal Toxicol* 44:697–707. <https://doi.org/10.1093/jat/bkaa089> (open access article)
14. ANSI/ASB (2019) Standard practices for method validation in forensic toxicology. <https://www.aafs.org/asb-standard/standard-practices-method-validation-forensic-toxicology>. Accessed 16 Mar 2022
15. Sklerov JH, Magluilo J Jr, Shannon KK, Smith ML (2000) 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* 24:543–549. <https://doi.org/10.1093/jat/24.7.543> (open access article)
16. Lund HME, Øiestad EL, Gjerde H, Christophersen AS (2011) Drugs of abuse in oral fluid collected by two different sample kits—stability testing and validation using ultra performance tandem mass spectrometry analysis. *J Chromatogr B* 879:3367–3377. <https://doi.org/10.1016/j.jchromb.2011.09.002>
17. Øiestad EL, Johansen U, Christophersen AS (2007) Drug screening of preserved oral fluid by liquid chromatography-tandem mass spectrometry. *Clin Chem* 53:300–309. <https://doi.org/10.1373/clinchem.2006.074237> (open access article)
18. Chung A, Hudson J, McKay G (2009) Validated ultra-performance liquid chromatography-tandem mass spectrometry method for analyzing LSD, *iso*-LSD, *nor*-LSD, and O-H-LSD in blood and urine. *J Anal Toxicol* 33:253–259. <https://doi.org/10.1093/jat/33.5.253> (open access article)
19. Dolder PC, Liechti ME, Rentsch KM (2014) Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem* 407:1577–1584. <https://doi.org/10.1007/s00216-014-8388-1>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.