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RESEARCH PAPER

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

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Abstract Lysergic acid diethylamide (LSD) is a widely used recreational drug. The aim of the present study is to develop a quantitative turboflow LC-MS/MS method that can be used for rapid quantification of LSD and its main metabolite 2-oxo-3-hydroxy LSD (O-H-LSD) in serum and urine in emergency toxicological cases without time-consuming extraction steps. The method was developed on an ion-trap LC-MS/MS instrument coupled to a turbulent-flow extraction system. The validation data showed no significant matrix effects and no ion suppression has been observed in serum and urine. Mean intraday accuracy and precision for LSD were 101 and 6.84 %, in urine samples and 97.40 and 5.89 % in serum, respectively. For O-H-LSD, the respective values were 97.50 and 4.99 % in urine and 107 and 4.70 % in serum. Mean interday accuracy and precision for LSD were 100 and 8.26 % in urine and 101 and 6.56 % in serum, respectively. For O-H-LSD, the respective values were 101 and 8.11 % in urine and 99.8 and 8.35 % in serum, respectively. The lower limit of quantification for LSD was determined to be 0.1 ng/ml. LSD concentrations in serum were expected to be up to 8 ng/ml. 2-Oxo-3-hydroxy LSD concentrations in urine up to 250 ng/ml. The new method was accurate and precise in the range of expected serum and urine concentrations in patients with a suspected LSD intoxication. Until now, the method has been applied in five cases with suspected LSD intoxication where the intake of the drug has been verified four times with LSD concentrations in serum in the range of 1.80–14.70 ng/ml and once with a LSD concentration of 1.25 ng/ml in urine. In serum of two patients, the O-H-LSD concentration was determined to be 0.99 and 0.45 ng/ml. In the urine of a third patient, the O-H-LSD concentration was 9.70 ng/ml.

Keywords LSD · O-H-LSD · LC-MS · Lysergic acid diethylamide · 2-Oxo-3-hydroxy LSD · Blood · Urine

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Introduction

Lysergic acid diethylamide (LSD) is a psychoactive substance changing the state of consciousness and perception. Its psychedelic effects made it popular as a recreational drug, especially in the early 1970s, but still today LSD is widely used [1]. Additionally, LSD (200 μg) has also recently been used in a clinical study as adjunct to psychotherapy [2]. LSD is one of the most potent psychotropic drugs and is used in low doses. Typical recreational doses of LSD range from only 25 to 200 μg with long-lasting, dose-dependent psychotropic effects [1]. Hence, low blood and urine concentrations are posing a challenge to all analytical methods.

LSD can only be detected in blood up to 8 h after administration due to serum concentrations in the low nanogram per milliliter range. 2-Oxo-3-hydroxy LSD (O-H-LSD) is the main metabolite present in urine at concentrations 16–34 times higher than LSD [3, 4]. To our knowledge, O-H-LSD has only been detected once in blood in a postmortem case [5]. According to Li et al. and Klette et al. LSD and O-H-LSD were regarded as stable under storage conditions of −20 °C [6, 7].

Most published methods for LSD detection use either GC-MS or LC-MS/MS with a single-stage quadrupole [4, 5, 8–12]. The aim of the present study was to develop a turboflow LC-MS/MS method with the purpose of rapid quantification of LSD and its main metabolite in serum and

urine in emergency toxicological cases without time-consuming extraction steps.

The method was developed using an ion-trap LC-MS/MS instrument in selected reaction monitoring (SRM) mode after atmospheric pressure ionization (APCI) for the quantification of LSD and O-H-LSD in urine and serum samples. Poch et al. used a similar APCI LC-MS/MS ion-trap instrument, but mainly for the detection of O-H-LSD [3].

Favretto et al. improved the method, but switched to electrospray ionization for suitable analysis of LSD and O-H-LSD in blood, urine, and vitreous humor [13]. Our method was established and successfully applied in five emergency toxicological cases with a suspected LSD intoxication. Additionally, the method will be used for the analysis of both blood and urine samples from a double-blind, placebo-controlled clinical trial.

Materials and methods

Chemicals and reagents

HPLC-grade purity acetonitrile, acetone, methanol, 2-propanol, formic acid, and acetic acid were all purchased from Merck (Darmstadt, Germany). Ammonium acetate and ammonium carbonate were obtained in HPLC grade from Merck (Darmstadt, Germany). Distilled water was obtained from an in-house installed purifier (ELGA, Bucks, UK).

Drug-free serum lyophilisate and urine negative control as blank matrices were obtained from Bio Rad Laboratories (Irvine, CA, USA). LSD and LSD-d_3 were obtained from Lipomed (Arlesheim, Switzerland) and 2-oxo-3-hydroxy LSD (O-H-LSD) from Cerilliant (Round Rock, TX, USA).

LC-MS analysis

Equipment

The sample extraction system (Transcend TLX1 HPLC, Thermo Scientific, Basel, Switzerland) consisted of a Thermo PAL autosampler and two Accela 600 pumps as eluting and loading pumps. The autosampler and the sample extraction system were all controlled by Aria software (version 1.6.3) from Thermo Scientific (Basel, Switzerland). A cyclone and a C18XL turboflow column (Thermo Scientific, Basel Switzerland) for extraction, and a 3 μm Betasil Phenyl/Hexyl column (Thermo Scientific, Basel, Switzerland) for chromatographic separation were used.

The online extraction system was coupled to a LTQ XL mass spectrometer from Thermo Scientific (Basel, Switzerland) using atmospheric pressure ionization (APCI), due to its performance regarding matrix effects [14, 15].

For the instrument control, the corresponding software package consisting of LTQ (v.2.6) for ion detection, Xcalibur (v.2.1.0) for method programming, and LC-Quan (v.2.6.1) for quantification (all Thermo Scientific, Basel, Switzerland) was used.

LC method

The method was based on a previously published method [16]. Four mobile phases were used in gradient mode. For extraction, loading B consisted of 10 mM ammonium carbonate in water; eluting A was 5 mM ammonium acetate in water containing 0.10 % formic acid and eluting B 5 mM ammonium acetate in methanol containing 0.50 % formic acid, respectively.

Loading B was used as alkaline loading buffer, eluting A and B for chromatographic separation. Loading and Eluting C (acetonitrile /acetone/2-propanol, 1:1:1 (*V*/*V*/*V*)) were used to clean the extracting and the analytical columns.

The gradient system with a total run time of 12 min is depicted in Table 1. Under the following gradient conditions, LSD and LSD-d_3 showed a retention time of 7.63 min, while O-H-LSD had a retention time of 6.34 min.

MS conditions

For the quantification of LSD and its metabolite, APCI was used as the ionization source in positive ion mode. Discharge current and discharge voltage were set to 5 μA and 4.2 kV, respectively. The vaporizer temperature was optimized to 452 °C whereas sheath and auxiliary gas provided best results with flow rates of 40 and 20 arbitrary units (AU). The capillary temperature was set to 275 °C.

LSD and O-H-LSD were quantified using single reaction monitoring (SRM) of the corresponding mass transitions (LSD *m*/*z* 324.6→223.23, O-H-LSD *m*/*z* 356.33→338.33, LSD-d_3 *m*/*z* 327.21→226.2). The system was tuned and optimized for the detection of LSD.

Standard solutions

LSD and LSD-d_3 were bought as 1 mg/ml reference standards in acetonitrile, while O-H-LSD as 0.1 mg/ml reference standard in acetonitrile. Stock solutions in acetonitrile containing 100,000 ng/ml LSD, LSD-d_3, or 10,000 ng/ml O-H-LSD, respectively, were prepared in duplicate and stored at −20 °C in order to have different sets for quality control (QC) and calibration samples, respectively. Working solutions of each analyte at 1000, 100, 10, and 1 ng/ml in water were used for the preparation of QC and calibration samples as well as for matrix and selectivity experiments.

Table 1 Detailed extraction and analytical separation steps of the liquid chromatography method

Time (min)	Extraction				Analytical separation			
	Flow (μl/min)	%A	%B	%C	Flow (μl/min)	%A	%B	%C
0.00	2	–	100	–	0.30	99	1	–
0.83	0.50	–	100	–	0.30	99	1	–
0.92	0.50	–	–	100	0.30	80	20	–
1.58	0.05	–	–	100	0.30	55	45	–
2.03	0.50	–	–	100	0.30	40	60	–
4.03	0.03	–	–	100	0.30	2	98	–
9.03	0.01	–	–	100	0.50	2	98	–
11.03	2.	–	–	100	0.50	2	98	–
11.37	2	–	–	100	0.50	–	–	100
11.70	2	–	100	–	0.50	–	100	–
12.20	2	–	100	–	0.50	99	1	–
12.53	2	–	100	–	0.30	99	1	–

Sample preparation

To 100 μl of serum, 100 μl acetonitrile for protein precipitation and 10 μl of a LSD-d_3 internal standard solution (100 ng/ml) were added. An identical volume of urine was diluted with 50 μl of an ammonium acetate buffer (50 mM, pH 4) and 10 μl of the internal standard solution. The samples were then vigorously vortexed, centrifuged for 10 min at 13,200*g* and the supernatant afterwards transferred into autosampler vials.

Calibration

Calibration curve in serum was realized by spiking serum samples with LSD and O-H-LSD to concentrations of 0.10, 0.25, 0.50, 0.75, 1, 2.50, 5, 7.50, and 10 ng/ml plus a blank (matrix only) and zero sample (matrix plus internal standard). The highest calibration point in serum was adopted from the maximum plasma concentration out of available i.v. kinetic data [17].

The calibration curve in urine was realized by spiking urine samples with O-H-LSD to concentrations of 1.50, 5, 10, 25, 50, 100, 125, 250, and 500 ng/ml. LSD concentrations were 0.10, 0.50, 1, 2, 5, 10, 12, 25, and 50 ng/ml, respectively. The highest calibrator in urine was adopted from published data containing various analyzed urine samples [4].

Both calibration curves were fitted linearly using a weighting factor ($1/x^2$).

In order to demonstrate accuracy and precision of the method, five QC's in urine and six QC's in serum were used with every run. The concentrations of the QC samples can be seen in Tables 2 and 3.

Selectivity

Following the FDA validation guidelines [18], six urine and six serum samples from different patients and healthy volunteers were collected and analyzed to establish selectivity and check for unwanted interferences within both matrices.

Matrix effects and recovery

Matrix effects, recovery, and process efficiency were measured and calculated according to Matuszewski et al. [19]. Matrix effects in urine and serum were calculated as ratio of the peak area before extraction and divided by the peak area after extraction. In contrast to Matuszewski et al., the extraction step consisted of simple protein precipitation as bypassing the extraction step on our ion-trap system was not possible. Six serum and six urine samples were spiked once with LSD and O-H-LSD before and after extraction. The peak areas of the spiked samples were then compared with the area of the spiked mobile phase. Urine samples were spiked to 25 ng/ml LSD resp. 250 ng/ml O-H-LSD, serum samples to 10 ng/ml each. Recovery values were calculated as areas of standards spiked before extraction divided by the areas of standards spiked after extraction. The process efficiency was also adopted from Matuszewsky et al. and calculated as ratio between the area of the standard spiked before extraction, and the areas of the standard in neat solution.

Limit of quantification

Drug-free serum and urine samples were spiked with different concentrations of LSD and O-H-LSD for the determination of the lower limit of quantification (LLOQ). The parent substance and metabolite ratio was determined 1:1

Table 2 Intraday precision and accuracy data of LSD and 2-oxo-3-hydroxy LSD measured in serum and urine at different concentrations

	Weighed-in concentration [ng/ml]		Measured concentration [ng/ml]		Mean precision SD [%]		Mean accuracy±SD [%]	
	Serum	Urine	Serum	Urine	Serum (n=6)	Urine (n=6)	Serum (n=6)	Urine (n=6)
LSD	0.10	0.10	0.098±0.006	0.106±0.007	6.3	6.5	98.40±4.8	106±7.7
	0.40	0.25	0.38±0.03	0.28±0.03	6.6	12.3	96.20±6.5	112±13.1
	0.80	0.60	0.82±0.03	0.53±0.03	4.6	5.0	103±6.3	88.80±4.1
	4	3.30	3.92±0.22	3.32±0.20	5.7	6.1	97.80±4.7	101±7.0
	8	33	7.52±0.49	31.70±1.39	6.6	4.4	93.9±5.8	96.0±4.1
	10		9.53±0.53		5.5		95.3±5.7	
O-H-LSD	0.10	1.50	0.104±0.008	1.45±0.05	8.0	3.6	104±8.3	96.4±3.2
	0.40	2.50	0.44±0.02	2.20±0.16	3.8	7.2	110±4.2	88.20±6.5
	0.80	6	0.88±0.02	6.25±0.07	2.5	1.2	110±2.8	104±4.7
	4	33	4.04±0.38	33.90±2.5	9.5	7.3	101±9.6	103±8.2
	8	333	8.20±0.28	321±18	3.4	5.7	102±3.5	96.20±6.0
	10		11.29±0.11		0.9		113±1.1	

LSD lysergic acid diethylamide, *O-H-LSD* 2-oxo-3-hydroxy lysergic acid diethylamide

in serum and assumed 1:10 in urine samples [4]. The LLOQ concentrations had to give a response at least five times greater than the blank. Additionally, precision had to be <20 % and the accuracy between 80 and 120 % using at least five determinations per matrix and concentration.

Carryover

Carryover was determined by quantification of different blanks, running between patient samples, calibrations, and quality controls.

Reproducibility

According to the FDA guidelines, a minimum of five determinations per concentration are recommended for determination of precision and accuracy [18].

The reproducibility of quantification was determined by measuring serum (n=6) and urine (n=5) quality controls (QC) once on 1 day (intraday precision and accuracy) and on six different days (interday precision and accuracy). All values had to fulfill the criteria of a variation coefficient (CV) below 15 %, resp. below 20 % at the LLOQ and accuracy between 80 and 120 %. For serum, six quality controls from LLOQ to

Table 3 Interday precision and accuracy data of LSD and 2-oxo-3-hydroxy LSD measured in serum and urine at different concentrations

	Weighed-in concentration [ng/ml]		Measured concentration [ng/ml]		Mean precision SD [%]		Mean accuracy±SD [%]	
	Serum	Urine	Serum	Urine	Serum (n=6)	Urine (n=6)	Serum (n=6)	Urine (n=6)
LSD	0.10	0.10	0.11±0.01	0.10±0.02	4.60	15.00	110±5.10	104±14.60
	0.40	0.25	0.39±0.02	0.26±0.02	4.20	8.80	97.5±4.10	105±9.30
	0.80	0.60	0.82±0.07	0.55±0.02	8.50	4.00	103±8.70	91.1±3.60
	4	3.30	3.97±0.34	3.32±0.22	8.60	6.70	99.2±8.60	101±6.50
	8	33	7.41±0.59	32.8±2.3	7.90	6.90	92.7±7.30	99.3±6.30
	10		10.1±0.55		5.50		101±5.50	
O-H-LSD	0.10	1.50	0.10±0.08	1.58±0.19	8.10	12.50	105±8.43	105±13.10
	0.40	2.50	0.39±0.03	2.64±0.35	8.40	13.40	98.3±8.20	105±14.10
	0.80	6	0.79±0.08	5.56±0.16	9.80	3.00	98.5±9.70	92.6±2.70
	4	33	3.79±0.35	34.8±2.2	9.20	6.60	94.8±8.70	105±6.40
	8	333	8.14±0.58	327±16.8	7.20	5.10	102±7.30	98.3±5.00
	10	–	10.1±0.76		7.60	–	101±7.60	–

LSD lysergic acid diethylamide, *O-H-LSD* 2-oxo-3-hydroxy lysergic acid diethylamide

the highest calibrator (0.10, 0.40, 0.80, 4, 8, 10 ng/ml) were measured once a day. For validation in urine, five QCs from 1.5 to 333 ng/ml were used.

Results

Selectivity

None of the blank urine or serum samples showed any interference within the measured mass range and time frame.

Matrix effects and recovery

The matrix effects in urine were 138 % for LSD and 122 % for O-H-LSD. Recovery in urine was calculated to be 90.00 and 87.80 %, respectively. Process efficiency in urine was 124 % for LSD and 107 % for O-H-LSD. Serum showed higher matrix effects with 128 % for LSD and 78.70 % for O-H-LSD. Recovery in serum was 64.00 % for LSD and 32.00 %, for O-H-LSD. The process efficiencies in serum were calculated to be 128 % for LSD and 79 % for O-H-LSD. No ion suppression was found for LSD or O-H-LSD in serum and urine, but as mentioned by Johansen and Jensen [10] LSD-d_3 would correct for any ion suppression. In various negative samples, small LSD concentrations below the LLOQ could be identified which derived from the deuterated internal standard. Following these findings, LSD-d_3 was measured ten times at different concentrations. The working solution of the standard (100 ng/ml) contained 0.12 % undeuterated LSD. This impurity in the peak area of LSD was subtracted from all calibrators, quality controls, and unknown samples.

Lower limits of quantification

The lowest accurate and precisely measurable concentration was 0.10 ng/ml and thereby determined as LLOQ for LSD and O-H-LSD in serum. In urine samples, the LLOQ was determined at 0.10 ng/ml for LSD and 1.50 ng/ml for O-H-LSD.

Carryover

No carryover was found for LSD and O-H-LSD in serum samples. In contrast, a slight carryover (0.10 %) was found for O-H-LSD in urine samples following the highest QC (333 ng/ml) and the highest calibration (500 ng/ml) in urine. As a consequence, a second consecutive blank was inserted between and the carryover was reduced to 0.01 %.

Reproducibility

Calibration curves in urine were linear for both substances, LSD and O-H-LSD with R^2 greater than 0.98. Mean intraday accuracy and precision in serum were 97.40 resp. 5.89 % for LSD and 107 resp. 4.70 % for O-H-LSD (see Table 2). Mean interday accuracy and precision for LSD and O-H-LSD were 101 resp. 6.56 % and 99.80 resp. 8.35 %, respectively (see Table 3).

Linearity

LSD and O-H-LSD calibration curves in serum were linear over the range from 0.10 to 10 ng/ml with a mean correlation coefficient (R^2) of 99.86 %. The calibration curves of the mean values are shown in Fig. 1. Error bars indicate the standard error of the mean.

Calibration curves of LSD and O-H-LSD in urine were linear over the concentration range from 1.50 ng/ml to 333 ng/ml. R^2 was found to be 99.93 %. The detailed calibration curve is shown in Fig. 2.

Toxicological cases

In the period from January to September 2014, five patients were admitted to the emergency department (ED) of the University Hospital Basel with suspected LSD intoxication. In all five cases, LSD consumption could be confirmed. Routinely, a LC-MS/MS method screening over 700 substances in serum was run to detect the intake of other medication and designer drugs.

As a summary, all in vivo measured concentrations in the matrices available from the emergency department can be found in Table 4.

Case 1

A 17-year-old girl was brought to the ED with acute confusion and loss of sense of time and orientation. She admitted consumption of two sugar cubes and one blot with LSD (estimated dose, 750 μg). A plasma sample for drug screening was taken approximately 3 h after ingestion. The patient was under chronic treatment with trazodone for depression. An additional LC-MS/MS screen in serum showed the presence of THC and trazodone. Quantification of LSD revealed a level of 14.70 ng/ml and a quantifiable O-H-LSD level of 0.99 ng/ml in serum. The only other published case where O-H-LSD could be detected in blood so far, was in a reanalyzed fatal case 10 years after collection [5]. Figure 3 shows the chromatogram of LSD, LSD-d_3 and O-H-LSD in the serum of this patient.

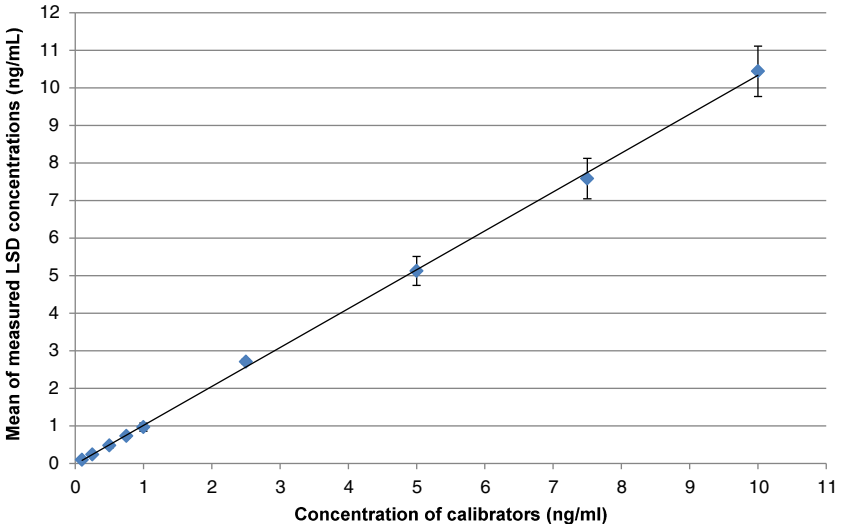

Fig. 1 Shows the mean calibration curve of LSD in serum from the validation measurements. The determination coefficient was 0.9995

Case 2

A 17-year-old male was brought by the ambulance to the ED with thoracic pressure, restlessness, and dyspnea. He admitted the intake of one sugar cube with LSD (estimated dose 250 μg) at 8 p.m. with concomitant consumption of cannabis. He reported onset of the symptoms at 10 p.m., 2 h post-consumption. In the emergency department, the patient was treated with lorazepam and acetaminophen. Serum analysis revealed a LSD concentration of 1.80 ng/ml in a blood sample taken at 11 p.m.

Case 3

A 21-year-old male was admitted to the ED by ambulance and the police because of aggressive and uncooperative behavior after consumption of an alleged LSD blot. No information about the time-point of the LSD ingestion was available from anamnesis. Serum analysis showed an LSD concentration of 6.10 ng/ml and an O-H-LSD concentration of 0.45 ng/ml. An additional LC-MS/MS screening revealed the presence of THC, cocaine, and amphetamine.

Case 4

A 45-year-old male presented himself to the ED with agitation, disorientation, and intense visual hallucinations. He was partying for 2 days and consumed alcohol, LSD, cocaine, and cannabis. The time-point of the LSD intake was not reported. The LC-MS/MS screening confirmed the intake of THC and cocaine. Quantification of the serum LSD level detected 4.10 ng/ml LSD, but no quantifiable O-H-LSD.

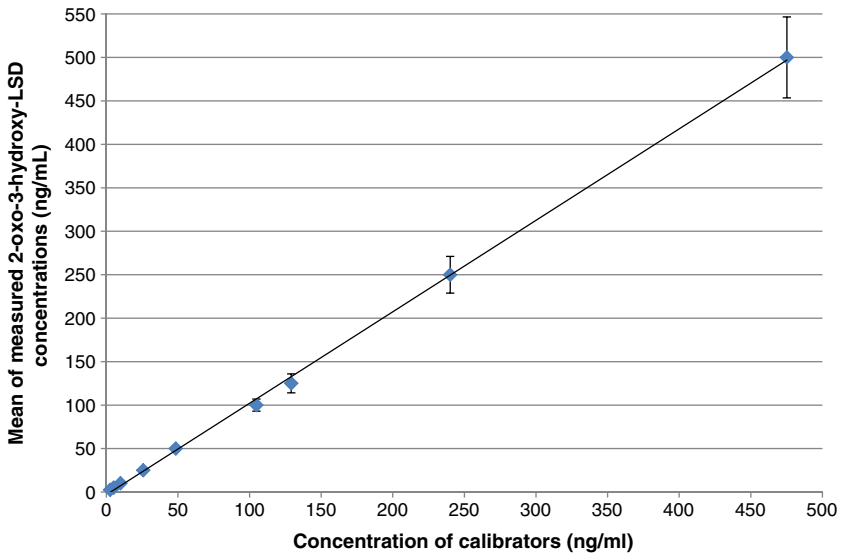

Fig. 2 Shows the mean calibration curve of 2-oxo-3-hydroxy LSD in urine from the validation measurements. The determination coefficient was 0.9993

Table 4 Measured concentration of LSD and 2-oxo-3-hydroxy LSD in serum and/or urine in different patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Serum LSD	14.70 ng/ml	1.80 ng/ml	6.10 ng/ml	4.10 ng/ml	N/A
Serum O-H-LSD	0.99 ng/ml	<LLOQ	0.45 ng/ml	<LLOQ	N/A
Urine LSD	N/A	N/A	N/A	N/A	1.30 ng/ml
Urine O-H-LSD	N/A	N/A	N/A	N/A	9.70 ng/ml

N/A matrix was not available from the emergency department; *<LLOQ* value was below the lower limit of quantification

Case 5

A 36-year-old male presented himself to the ED with tactile and visual hallucinations after consumption of an alcoholic beverage in a club. He suspected someone to have mixed some drugs in his drink. A screening for LSD in urine revealed 1.30 ng/ml LSD and 9.70 ng/ml O-H-LSD, respectively. An additional LC-MS/MS screening in urine confirmed the presence of THC. No time-point of the drink consumption or start of the LSD effect was reported.

Discussion and conclusion

The development of a sensitive method for the measurement of LSD and its metabolite is an analytical challenge due to its low concentrations in serum and urine.

Purification procedures with solid-phase or liquid-liquid extraction can certainly lead to better sensitivity of the LC-MS/MS method, but form a time-consuming procedure [5]. The short run time of 12 min was mainly given by retention times of LSD, LSD-d_3, and O-H-LSD. The additional time following the LSD and LSD-d_3 peak was necessary to ensure clean peak separation and flushing the columns to minimize carryover.

Our purpose was to establish a fast and reliable method for application in emergency toxicological cases where time is crucial. With a short method run of 12 min and minimum sample preparation, results will be more quickly available so that a fast diagnosis is possible. The method was applied in five toxicology cases where consumption of LSD could be confirmed four times in serum and once in urine.

Due to the fast method and obviation of purification steps, a slight loss in sensitivity was accepted. LLOQ and LOD in serum were hence higher than in other comparable methods [5, 8–10, 13]. Some showed LOQ's as low as 0.02 ng/ml for LSD but needed sample preparation and a longer run time [5]. In contrast, our method was mainly developed to rapidly detect levels of LSD that occur during acute intoxication. The range of expected LSD concentrations in serum was difficult to determine because only few pharmacokinetic data is available. In fact, only one pharmacokinetic study with controlled administration of LSD exists. In this study, peak plasma concentrations of LSD were 4–6 ng/ml 1–2 h after administration of LSD (intravenously at 2 μg/kg) [17]. Therefore, we chose 10 ng/ml as highest calibrator to cover typically used oral doses of LSD (100–400 μg) [1]. However, one case

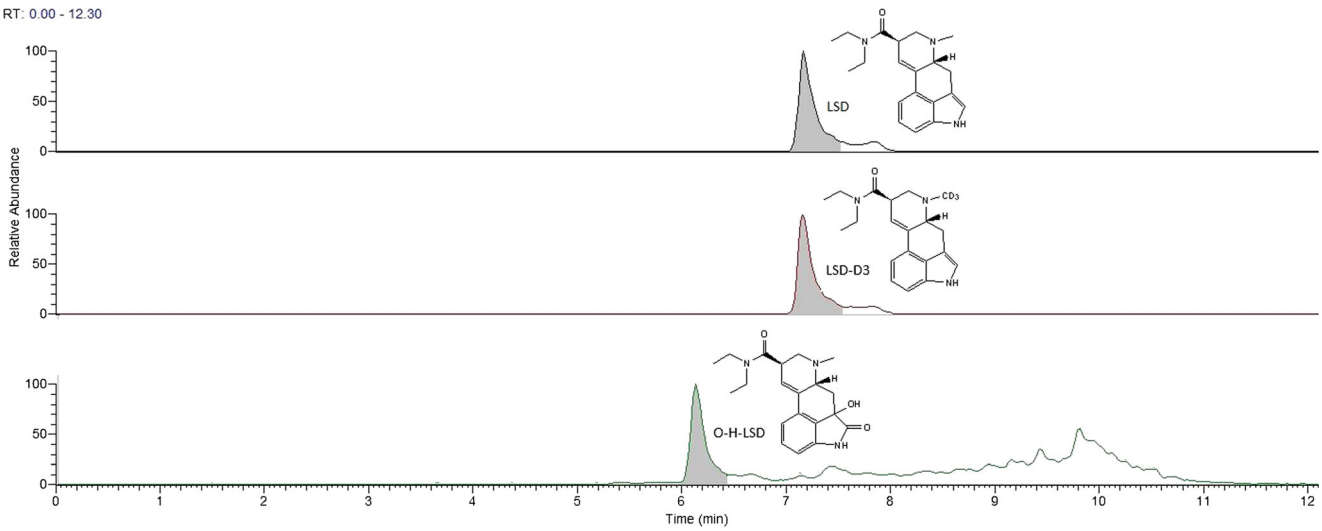

Fig. 3 Chromatogram and the respective structural formulas of LSD, LSD-d_3, and 2-oxo-3-hydroxy LSD in the serum sample of patient 1

was found with a LSD concentration of 14 ng/ml in plasma among the intoxication cases presented here.

This sample had to be diluted (1:1 with distilled water) in order to determine the correct result. Expected urine concentrations and the calibration range were established considering already published data [3, 4]. Our method fulfilled all criteria for measurement of emergency toxicological cases. All four cases showed concentrations of LSD in serum in the range of 1.80–14.70 ng/ml. Additionally, to our knowledge, for the first time, we describe the quantification of O-H-LSD in two patients in a concentration well above the LLOQ of our method.

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References

1. Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A (2008) The pharmacology of lysergic acid diethylamide: a review. CNS Neurosci Ther 14(4):295–314
2. Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, Brenneisen R (2014) Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. J Nerv Ment Dis 202(7):513–520
3. 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(3):170–179
4. 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 Biomed Sci Appl 724(1):23–33
5. 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(5):253–259
6. Klette KL, Horn CK, Stout PR, Anderson CJ (2002) 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. J Anal Toxicol 26(4):193–200
7. Li Z, McNally AJ, Wang H, Salamone SJ (1998) Stability study of LSD under various storage conditions. J Anal Toxicol 22(6):520–525
8. Berg T, Jorgenrud B, Strand DH (2013) Determination of buprenorphine, fentanyl and LSD in whole blood by UPLC-MS-MS. J Anal Toxicol 37(3):159–165
9. Canezin J, Cailleux A, Turcant A, Le Bouil A, Harry P, Allain P (2001) Determination of LSD and its metabolites in human biological fluids by high-performance liquid chromatography with electrospray tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 765(1):15–27
10. Johansen SS, Jensen JL (2005) Liquid chromatography-tandem mass spectrometry determination of LSD, ISO-LSD, and the main metabolite 2-oxo-3-hydroxy-LSD in forensic samples and application in a forensic case. J Chromatogr B Anal Technol Biomed Life Sci 825(1):21–28
11. Musshoff F, Daldrup T (1997) Gas chromatographic/mass spectrometric determination of lysergic acid diethylamide (LSD) in serum samples. Forensic Sci Int 88(2):133–140
12. Nelson CC, Foltz RL (1992) Determination of lysergic acid diethylamide (LSD), iso-LSD, and *N*-demethyl-LSD in body fluids by gas chromatography/tandem mass spectrometry. Anal Chem 64(14):1578–1585
13. 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(4):259–265
14. Caban M, Migowska N, Stepnowski P, Kwiatkowski M, Kumirska J (2012) Matrix effects and recovery calculations in analyses of pharmaceuticals based on the determination of β-blockers and β-agonists in environmental samples. J Chromatogr A 1258:117–127
15. Dams R, Huestis MA, Lambert WE, Murphy CM (2003) Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. J Am Soc Mass Spectrom 14(11):1290–1294
16. Mueller DM, Duretz B, Espourteille FA, Rentsch KM (2011) Development of a fully automated toxicological LC-MS(n) screening system in urine using online extraction with turbulent flow chromatography. Anal Bioanal Chem 400(1):89–100
17. Aghajanian GK, Bing OH (1964) Persistence of lysergic acid diethylamide in plasma of human subjects. Clin Pharmacol Ther 5:611–614
18. Service USDoHaH (2001) Guidance for the industry—bioanalytical method validation
19. Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 75(13):3019–3030