Determination of LSD in blood by capillary electrophoresis with laser-induced fluorescence detection

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Abstract

Capillary electrophoresis (CE) with HeCd laser-induced fluorescence (LIF) detection and its application in forensic toxicology is demonstrated by the determination of D-lysergic acid diethylamide (LSD) in blood. Following precipitation of proteins, washing of the evaporated supernatant and extraction, the residue was reconstituted in methanol and injected electrokinetically (10 s, 10 kV). The total analysis time for quantification of LSD was 8 min using a citrate–methanol buffer, pH 4.0. With this buffer system it is possible to separate LSD, nor-LSD, iso-LSD and iso-nor-LSD. Using a specific sample preparation, electrokinetic injection, extended light path (bubble cell) capillaries and especially LIF detection (λ_{ex} 325 nm, λ_{em} 435 nm), a limit of detection of 0.1–0.2 ng LSD per ml blood could be obtained. The limit of quantitation was about 0.4–0.5 ng/ml. The quantitative evaluation for LSD was carried out using methylergometrine as internal standard. The precision expressed as coefficient of variation (C.V.) and accuracy of the method were <20% and 86–110%, respectively. The application of the method to human blood samples from two forensic cases and a comparison with radioimmunoassay demonstrated that the results were consistent.

Keywords: LSD; D-Lysergic acid diethylamide

1. Introduction

Recently, an increasing consumption of the hallucinogenic substance D-lysergic acid diethylamide (LSD) has been noticed in Germany. Among the illicit drugs of first-time consumers, LSD has shown the greatest increase (140.5%) [1]. Because of the extremely low doses and its extensive metabolism [2–4], the determination of LSD in body fluids is still a challenging analytical problem for forensic laboratories.

Several different approaches for detection have been described including thin-layer chromatography (TLC) [5–7], radioimmunoassay (RIA) [8,9], high-performance liquid chromatography (HPLC) with fluorescence detection [5,8,10–12], gas chromatography coupled with mass spectrometry and tandem mass spectrometry (GC–MS, GC–MS–MS) [4,7,11,13–15] and high-performance liquid chromatography combined with mass spectrometry and tandem mass spectrometry (LC–MS, LC–MS–MS) [16,17].

Capillary electrophoresis (CE) is a very useful technique for the determination of drugs in body fluids because of its high resolution and speed [18]. The main application in forensic toxicology is for the

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analysis of seized illicit drugs [19–25], but determinations of drugs in biological specimens such as urine and hair have also been reported [25–32]. LSD and its metabolites could be separated with CE and determined in human liver microsomal incubation extracts by mass spectrometry (CE–MS) [17].

Using CE with UV detection, the limit of detection is frequently insufficient for drugs in biological fluids due to the low concentration sensitivity of this technique. Using laser-induced fluorescence detection (LIF) it is possible to improve the limit of quantification for analytes with native fluorescence and allows the analysis of biological matrices with enhanced selectivity [33–35].

To improve especially the sensitivity we have introduced several modifications and after validation applied this methodology in two forensic cases.

2. Experimental

2.1. Chemicals

LSD was obtained from Sigma (Deisenhofen, Germany). N-Demethyl- and iso-N-demethyl-LSD (nor-LSD and iso-nor LSD) were purchased from Radian (Austin, TX, USA). The internal standard methylergometrine tartrate was kindly supplied by Sandoz (Basel, Switzerland). Iso-LSD was obtained from illicit LSD blotter by thin-layer chromatography and isolated from the spots by methanolic extraction. The fraction at the R_F value for iso-LSD described previously [36] was collected and identified by UV spectrum and GC–MS analysis. Only one peak was obtained with the typical spectrum. The stock solutions were prepared in methanol (HPLC grade) and stored at −18°C. All other chemicals were of analytical grade and commercially available.

2.2. Capillary electrophoresis and operating conditions

All separations were carried out on a P/ACE 5510 capillary electrophoresis system (Beckman, Palo Alto, CA, USA). Detection was performed using a Beckman LIF-detector and a HeCd-laser, Model 3074-30M (Omnichrome, Chino, CA, USA) with an

excitation wavelength of 325 nm (30 mW). Emission was measured at 435 nm using an interference filter. For UV detection a Beckman diode array detector (DAD) was employed. The capillary zone electrophoresis was performed in untreated fused-silica extended light path (bubble cell) capillaries (Hewlett-Packard, Waldbronn, Germany) 37 cm (30 cm effective length to the detector)×50 μm I.D., respectively ca. 150 μm at the detection window. Capillaries were treated before use by rinsing for 20 min with 0.1 M NaOH and after each sample with 0.1 M NaOH and running buffer for 1 min. Separations were carried out using a running buffer containing a solution of 250 mM citric acid (adjusted to pH 4.0 with 250 mM sodium acetate)–methanol (30:70, v/v). The applied voltage was 25 kV and the temperature was maintained at 20°C. Sample introduction was performed by electrokinetic injection for 10 s with 10 kV. The corrected peak areas were calculated using the Beckman System Gold software 8.10.

2.3. Fluorescence spectra

Fluorescence spectra were made with a Shimadzu RF 540 fluorescence spectrometer.

2.4. Extraction from whole blood samples

The method of Nelson and Foltz [15] was modified by successively adding 50 μl of the internal standard stock solution (200 ng methylergometrine/ml methanol) and 4 ml of acetonitrile to 2 ml of whole blood in a screw-cap glass tube. The blood–acetonitrile mixture was treated for 10 min in an ultrasonic bath. After vortexing for several minutes the suspension was centrifuged for 10 min at 3000 g. The supernatant was transferred to a clean tube. This initial step permits LSD extraction from either whole blood or hemolysed blood. The acetonitrile extract was evaporated to near dryness under a stream of nitrogen at ca. 60°C and reconstituted in 2 ml acetate buffer (250 mM, pH 4.0). The aqueous solution was washed with 1 ml n-hexane. After addition of 250 μl concentrated ammonia (pH was adjusted to 9–10) LSD was extracted from the basic aqueous solution with 4 ml dichloromethane. After centrifugation the organic layer was transferred to a small vial and completely dried under a gentle stream of nitrogen at

ca. 50°C. The residue was reconstituted in 50 μl of methanol. The extractions were carried out avoiding direct light.

2.5. Radioimmunoassay

The analysis was performed with a Coat-A-Count LSD RIA (Diagnostic Products, Los Angeles, CA, USA). This RIA normally is applicable to urine, but as described previously [37] it is possible to investigate LSD in serum, hemolysed blood and stomach contents with a satisfactory precision. Preliminary extraction of LSD from the samples is not compulsory. For determination of LSD 2 ml distilled water and 10 ml methanol were added to 2 ml blood, treated for 5 min in an ultrasonic bath and vortexed thoroughly. After centrifugation (10 min, 3000 g) the methanolic supernatant was added to 50 ml isopropanol and evaporated on a rotatory evaporator. The RIA was performed after reconstitution of the extract in 1 ml of phosphate buffer (0.15 M, pH 6.0).

3. Results and discussion

3.1. Development of the assay

3.1.1. Bubble cell capillary

Commercially available extended light path capillaries (bubble cell capillaries, Hewlett-Packard) were used to increase sensitivity. Using a bubble cell capillary the sensitivity was ca. 2–3-fold greater than compared to standard capillaries. By increasing the internal diameter a decrease in resolution occurs. So we renounced to use self-made bubble cell capillaries [38] with an enlarged bubble which improves sensitivity up to 4–5-fold.

3.1.2. Fluorescence intensity and separation

The buffer system has an influence both on separation and on fluorescence intensity. Measurements of solutions of LSD in phosphate buffer yielded in a lower fluorescence intensity than in acetate or citrate buffers at the excitation wave length maximum of 325 nm (Fig. 1). Therefore a citrate buffer system was used for further experiments. Similarly, an increase of organic solvents such as methanol or acetonitrile was associated with an increase of fluorescence intensity. Also, an elevation of the methanol fraction of the buffer up to 70% (v/v) led to a separation of LSD and nor-LSD using a 37 cm capillary. That was a prerequisite with regard to the selectivity of this method.

LSD, its metabolite nor-LSD, the isomers iso- and iso-nor-LSD and methylergometrine were readily separated within 8 min (Fig. 2) with the CE conditions described in Section 2.2. Methylergometrine, also a lysergic acid derivative, was chosen as the internal standard because of its similar structure and fluorescence intensity at the same wavelength. The
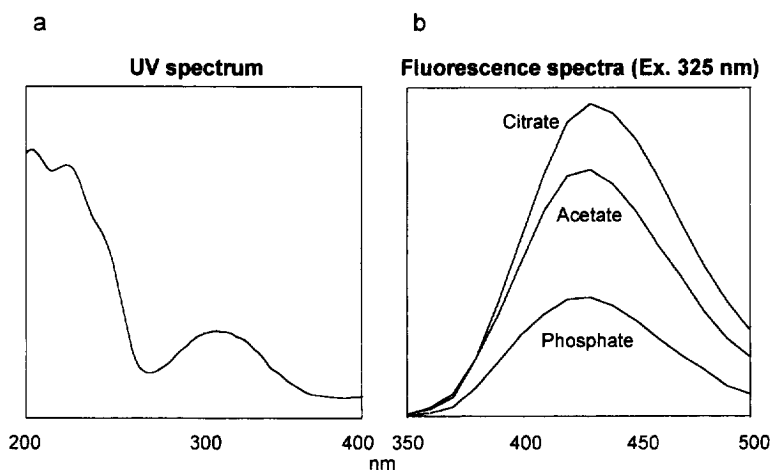

Fig. 1. UV spectrum (a) and fluorescence spectra of LSD (b). Conditions (b): citrate buffer pH 4.0, 100 mM, acetate buffer pH 4.0, 100 mM, phosphate buffer pH 4.0, 100 mM.

nor-LSD

LSD
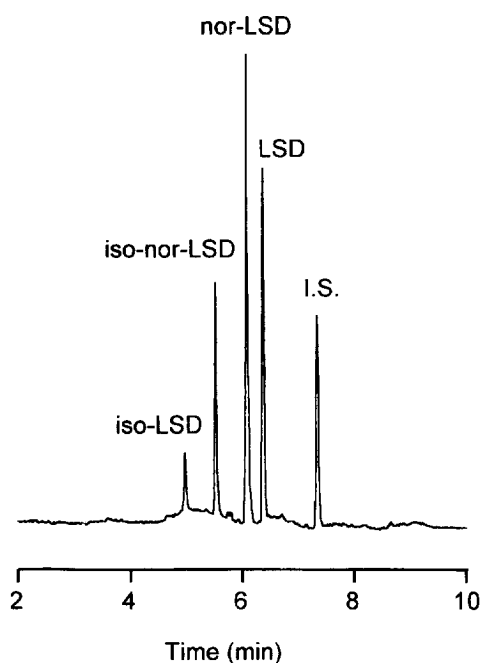
iso-nor-LSD

I.S.

iso-LSD

Fig. 2. Electropherogram of a standard mixture.

electropherogram of a blank blood and a blood sample spiked with LSD and nor-LSD (each 1 ng/ ml) showed no interfering peaks from blood at the corresponding migration time of LSD (Fig. 3).
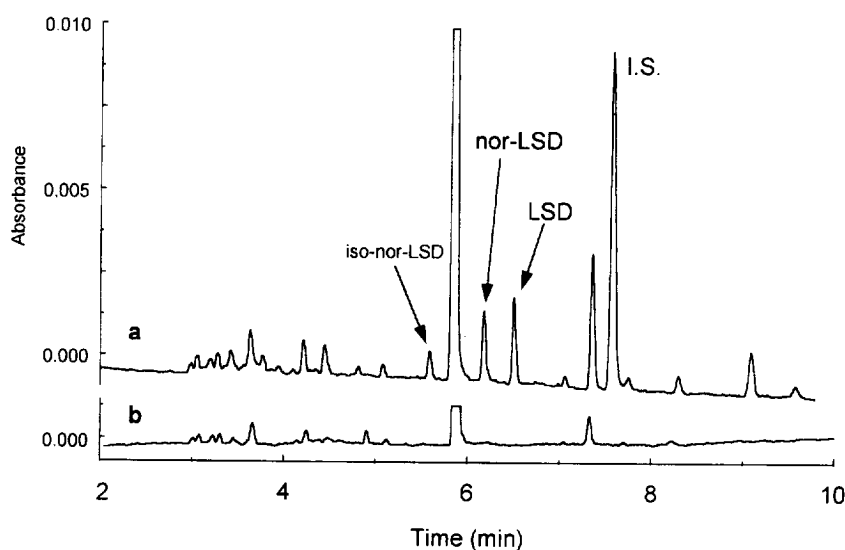
3.1.3. Electrokinetic injection

By preparing samples in a low-conductivity solution, e.g., water or methanol, and injecting the sample solution electrokinetically into the capillary, the stacking effect causes a sample concentration up to 100-fold [39,40]. To profit from this possibility the blood samples were prepared with the method described previously and reconstituted in methanol. The solution obtained was nearly free from matrix components. By using electrokinetic injection from this solution the gain in sensitivity in comparison to hydrodynamic injection was about 5–20-fold. A decrease in reproducibility of the injected analyte is balanced by the use of an internal standard.

3.2. Validation of the assay

3.2.1. Recovery

For recovery experiments blood was spiked with three different concentrations of LSD (0.5, 2.5 and 7.5 ng/ml). The internal standard was added after extraction at a final concentration of 10 ng/50 μl methanol. Five blood samples each with the various concentrations were extracted and analysed. The recovery was determined by comparison of the corrected peak area ratios (LSD/I.S.) with the corrected peak area ratio of standard solutions. For LSD the recovery was calculated to be 59.3±12.6% for

Fig. 3. Electropherogram of (a) human blood spiked with LSD and nor-LSD (1 ng/ml) and internal standard (I.S.), (b) blank blood.

0.5 ng/ml, 60.8±10.3% for 2.5 ng/ml and 63.8±7.6% for 7.5 ng/ml.

3.2.2. Linearity

Calibration samples were obtained by spiking blood with seven different concentrations of LSD (from 0.1 to 7.5 ng/ml) and the internal standard. All samples were processed further as described in Section 2.4. Calibration curves according to the internal standard were obtained by plotting concentration vs. corrected peak area ratios. As an example the calibration curve for LSD is described with the function $y = 0.3017x - 0.0329$. The correlation coefficient (r) of LSD from linear regression analysis by the least-squares method is 0.9988.

3.2.3. Precision and accuracy

Five blood samples spiked with concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 7.5 ng LSD/ml and internal standard were extracted and analysed in one series to assess intra-series variability as "within factor". The concentrations were calculated from the calibration curves. In our experiments the intra-series precision expressed as the coefficient of variation (C.V.) was between 2–20%. The accuracy was also satisfactory within the series with 86–110% (Table 1). The procedure was repeated three times to

estimate inter-series results as "grouping factor". Good results were obtained with C.V.s between 1 and 12%. The absence of interference from the matrix was confirmed by analysis of blank whole blood samples. The limit of detection for LSD (signal-to-noise ratio of 3) was approx. 0.1–0.2 ng/ml (Fig. 4) and the limit of quantitation about 0.4–0.5 ng/ml blood (signal-to-noise ratio of 10).

3.3. Application

The method for determination of LSD in blood was used in forensic cases where there was a suspicion of LSD consumption (Fig. 5). The electropherogram of a blood sample from a person who unknowingly took LSD is very similar to experimental separations (Fig. 5a). The person was worried by the disturbance of consciousness and visited a doctor during the intoxication phase and a blood sample was taken. Using the described method 0.51 ng LSD/ml blood was detected. Fig. 5b shows the electropherogram of a man who walked onto the highway under the influence of LSD and was run over. LSD could be detected in the blood sample at 0.2 ng/ml. However this is at the limit of detection of this method. With the CE method described the radioimmunological investigations from four cases

Table 1
Precision and accuracy of the determination of LSD in human blood with CE–LIF

|	Concentration added (ng/ml)						
	0.1	0.25	0.5	1.0	2.5	5.0	7.5
Concentration found (ng/ml) arithm. mean value							
Series 1 ($n=5$)	0.09	0.27	0.48	0.96	2.59	5.12	7.28
Series 2	0.10	0.25	0.51	1.00	2.54	5.23	7.34
Series 3	0.11	0.27	0.48	0.95	2.60	5.11	7.62
Inter-series	0.10	0.26	0.49	0.97	2.58	5.15	7.41
Precision (C.V.%) arithm. mean value							
Series 1 ($n=5$)	15.4	12.8	9.8	8.9	7.2	6.8	5.0
Series 2	16.4	8.8	6.6	5.0	4.3	4.2	2.6
Series 3	10.1	6.6	9.6	7.5	6.8	3.4	3.2
Inter-series	11.3	4.6	3.7	3.2	1.4	1.2	2.4
Accuracy (%) arithm. mean value							
Series 1 ($n=5$)	86.7	106.7	95.9	95.9	103.6	102.3	97.1
Series 2	96.0	97.9	102.5	100.8	101.5	104.7	97.8
Series 3	109.5	105.9	96.7	95.3	104.2	102.2	101.6
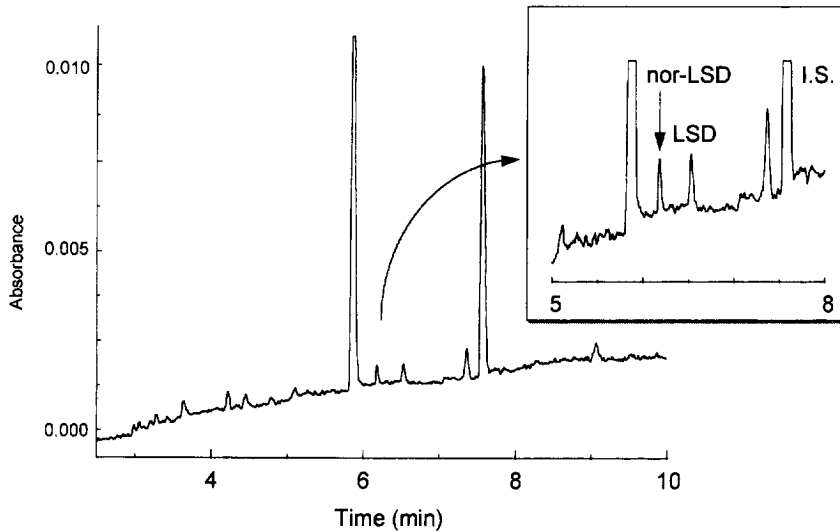
Fig. 4. Electropherogram of human blood spiked with LSD and nor-LSD (0.2 ng/ml) and internal standard (I.S.).
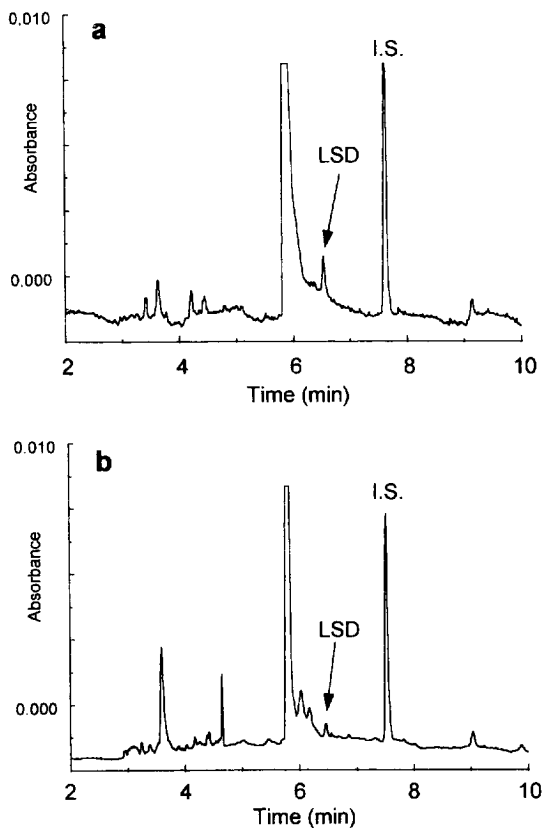

Fig. 5. Electropherograms of blood from two forensic cases. For further information see Section 3.3.

Table 2
Comparison of blood and urine concentrations of LSD obtained by RIA and CE

Subjects	RIA		CE	
	Blood	Urine	Blood	Urine
H	0.25	–	~0.2	–
W	~0.07	0.59	Non detectable	0.75
A	0.54	–	0.74	–
S	Not determined	0.67	0.51	0.45

were qualitatively and quantitatively confirmed (Table 2). Urine samples were also investigated with the method validated for blood after extraction (see Section 2.5).

4. Conclusion

The results of this investigation show that using capillary electrophoresis it is possible to detect very low doses of drugs in body fluids. The low limit of detection using UV detection can clearly be increased by the application of laser-induced fluorescence detection (LIF). A further increase in detection sensitivity could be achieved using extended light path (bubble cell) capillaries and electrokinetic injection. The detection of LSD was very satisfactory with regard to the precision and accuracy. The use of

capillary electrophoresis as an orthogonal technique for chromatography is an additional tool for confirmation of positive LSD results. A comparison with other techniques shows that the CE–LIF method presented has a similar limit of detection, e.g., HPLC fluorescence detection [10] about 0.5 ng/ml. Better results could be obtained with GC–MS–MS [15] (about 20 pg/ml), LC–MS–MS [17] (about 50 pg/ml) and RIA (about 20 pg/ml).

Acknowledgments

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