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Determination of lysergic acid diethylamide (LSD) in mouse blood by capillary electrophoresis/fluorescence spectroscopy with sweeping techniques in micellar electrokinetic chromatography

The separation and on-line concentration of lysergic acid diethylamide (LSD) in mouse blood was achieved by means of capillary electrophoresis/fluorescence spectroscopy using sodium dodecyl sulfate (SDS) as the surfactant. Techniques involving on-line sample concentration, including sweeping micellar electrokinetic chromatography (sweeping-MEKC) and cation-selective exhaustive injection-sweep-micellar electrokinetic chromatography (CSEI-sweep-MEKC) were applied; the optimum on-line concentration and separation conditions were determined. In the analysis of an actual sample, LSD was found in a blood sample from a test mouse (0.1 mg LSD fed to a 20 g mouse; ~1/10 to the value of LD_{50}). As a result, 120 and 30 ng/mL of LSD was detected at 20 and 60 min, respectively, after ingestion of the doses.

Keywords: Cation-selective exhaustive injection / Lysergic acid diethylamide (LSD) / Mouse blood sample / Sweeping micellar electrokinetic chromatography EL 5122

1 Introduction

Lysergic acid diethylamide (LSD) was first prepared in 1938 and synthesized by Stoll and Hoffman in 1943. It is a powerful psychedelic drug that produces temporary hallucinations and a schizophrenic psychotic state [1–3]. Because of the rapidly growing abuse of this drug [1], a simple, economic, fast and consistent method for its determination is needed. Thus far, a number of analytical methods have been reported to determine the distribution and metabolic profile of LSD. These methods include radioimmunoassay (RIA) [4], thin-layer chromatographic analysis (TLC) [5], gas chromatography/mass spectrometry (GC-MS) [6–11], CE combined with laser-induced fluorescence (CE-LIF) detection [12, 13], high-performance liquid chromatography (HPLC)/fluorescence detection alone [14–18], or combined with tandem mass spectrometry [19, 20]. Each method has unique advantages and disadvantages with respect to sensitivity, precision and simplicity of use. However, in a routine analysis, the detection of LSD in body fluids continues to present a challenge because of the extremely low doses that are typically ingested. CE has become a popular technique and is a very useful method for the determination of drugs in body fluids. Recently, a series of reports appeared by Terabe *et al.,* as well as other groups, concerning the so-called "sweeping" technique for on-line sample concentration [21–30]. In particular, an increase in sensitivity of a millionfold can be obtained by cation-selective exhaustive injection and sweeping (CSEI-sweep-MEKC) [23]; an anion selective exhaustive injection-sweep-MEKC (ASEI-sweep-MEKC) provides 1000–6000-fold increases in some aromatic carboxyl acids [24]. For neutral analytes, either sweeping or stacking techniques have also been discussed [25, 26]. In this study, we report on a simple and highly sensitive method by means of sweeping techniques for the detection of LSD in mouse blood at different times (20 and 60 min) after ingestion of the drug. Several electrophoretic parameters, such as the length of the sample injection (in sweeping-MEKC mode) and the time of electrokinetic injection (in CSEI-sweep-MEKC mode) were optimized.

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Abbreviations: BGS, background solution; **CSEI**, cation-selective exhaustive injection; **LAMPA**, lysergic acid *N*,*N*-methylpropamide; **LSD**, lysergic acid diethylamide

2 Materials and methods

2.1 Apparatus

The CE setup was fabricated in-house and is identical to that described previously [30–33]. Briefly, a high-voltage power supply (Model RR30-2R, 0–30 kV, 0–2 mA, reversible; Gamma, Ormond Beach, FL, USA) was used to drive the electrophoresis and a 50 μm ID fused-silica capillary

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column (J&W Scientific, Folsom, CA, USA) was used for the separation (total length, 65 cm; effective length, 60 cm). The excitation source was selected by a monochromator (Acton Research, Acton, MA; Model SP-150, 1200 grooves/mm grating) connected to a Xe lamp (Müller Elektronik Optik, Moosinning, Germany, SVX/LAX 1450). Fluorescence data were collected at a right angle to the light source and dispersed by a second monochromator (ARC Model SP-300i), followed by detection by means of a photomultiplier tube (ARC Model P2-R928, for 190–900 nm). Electropherograms were collected at a speed of 200 ms/point with a data acquisition system (ARC's Spectra-Sense NCL package), connected to a personal computer.

2.2 Chemicals

LSD, *iso*-LSD and lysergic acid *N*,*N*-methylpropylamide (LAMPA) were acquired from Radian International (Austin, TX, USA). Acetonitrile and methanol (99.8%) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). SDS, ethyl acetate and ammonium carbonate were obtained from Acros (Geel, Belgium) and Sigma (St. Louis, MO, USA), respectively. Brij-30 was acquired from Aldrich (Milwaukee, WI, USA). All other chemicals were of analytical grade and are commercially available.

2.3 Animals and blood samples

Inbred male mice of the BALB/c and C57BL/6J strain (10–12 weeks) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Upon arrival, the mice were caged in a colony room where a 12-h light-dark cycle was maintained through artificial illumination. They received free access to both food and water throughout the experiment except as noted and a 2-week acclimation period prior to experimental manipulation. All animal manipulation was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996). For the blank experiments, the animal was placed on its back after ether anesthesia. Whole blood (~ 1 mL) was collected from the heart using a heart puncture through a 22-gauge needle into a syringe that contained 100 U/mL heparin. Blood was transferred to 1.5 mL microcentrifuge tubes and stored at −20°C until used for assay. The LD_{50} for mice, rats and rabbits has been reported to be 46, 16 and 0.3 mg/kg [1]. In this study, 0.1 mg of LSD (~1/10 of the LD_{50}-mouse value) was fed to a test mouse (C57BL/6J, 20 g) by gavage. 20 min after administration of the dose, the first test blood (~ 90 μL) was collected from the saphenous vein of the leg. After an additional 40 min (60 min after ingestion), another blood sample (~ 500 μL) was collected from the heart *via* heart puncture. The sample preparation was the same as that used for the blank blood preparation.

2.4 Extraction

An extraction method described by Nelson and Foltz [9] and modified by adding LAMPA as an internal standard was used. A 50 μL blood sample was added to 200 μL of ethyl acetate, followed by shaking for 10 min. The upper layer was transferred to a clean tube and the organic solvent removed in a vacuum chamber. The residue was acidified by the addition of 200 μL of 0.1 M HCl. After adding 200 μL of hexane and shaking for 10 min, the organic matrix was extracted into the organic phase. After removing the organic phase, the aqueous phase was made alkaline by the addition of 30 μL of saturated ammonium carbonate and 10 μL of 2 M sodium hydroxide, followed by stirring for 5 min. 250 μL of toluene/methylene chloride (7:3 v/v) was added, and the sample and solvent gently mixed for 30 min, followed by centrifugation. The organic phase (upper layer) was collected and evaporated to dryness. The residue was dissolved in 50 μL of sample matrix (50 mM H_3PO_4 and 3 mM Brij-30 in an acetonitrile-methanol-water solution; 5:35:60 v/v/v), and the conductivity of which was adjusted (5.37 mS/cm) by adding 100 mM of H_3PO_4 for the subsequent CE separation. To determine the extraction efficiency, blank blood samples containing 1 and 0.1 μg/mL of LSD were divided into equal aliquots. At this point, the internal standards (0.5 and 0.05 μg/mL LAMPA) were added to the aliquots after extraction.

3 Results and discussion

3.1 On-line sample concentration

3.1.1 Sweeping-MEKC

In this experiment, the background solution (BGS) consisted of 100 mM SDS, 3 mM Brij-30 and 50 mM H_3PO_4 in a mixed acetonitrile-methanol-water solution (5:35:60 v/v/v), the pH of which was 2.06 (conductivity, 5.37 mS/cm). The samples were dissolved in the same solution (without SDS) resulting in a nonmicellar buffer, and adjusted to the same conductivity of BGS by the addition of 100 mM H_3PO_4. Hydrodynamic injection was achieved by raising the sample reservoir to a height of 20 cm relative to the exit reservoir, thus generating a flow rate of 0.30 mm/s. By using this procedure, 4.5, 27, 32.7 and 36.4 cm column lengths (in times: 150, 900, 1080, 1200 s) of solution were injected into the capillary. When the injection was completed, −20 kV was applied to power the CE separation. As in the following step, the samples were separated by

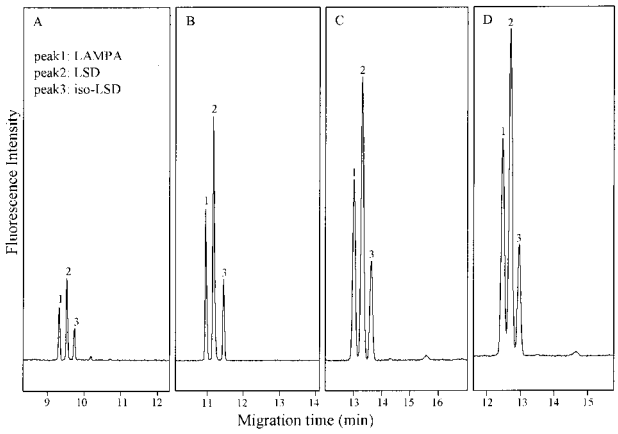

Figure 1. Effects of different injection lengths of sample solution in sweeping-MEKC separation. (A)–(D) 4.5, 27, 32.7 and 36.4 cm. CE conditions: capillary, 65 cm (60 cm to the detector), 50 μm ID; applied voltage, −20 kV; current ~ −26, −24, −22 and −23 mA; λex = 320 nm, λem = 390 nm. Sample concentrations: 1.67, 0.83 and 1.25 μg/mL for LSD, *iso*-LSD and LAMPA, respectively. Buffer: 100 mM SDS, 3 mM Brij-30 and 50 mM H_3PO_4 in an acetonitrile-methanol-water solution (5:35:60 v/v/v); pH 2.1.

the MEKC mode. The CE electropherograms are shown in Fig. 1A–D corresponding to different injection lengths. Herein, the sample concentrations of LSD, *iso*-LSD and LAMPA were 1.67, 0.83 and 1.25 μg/mL (peaks 1–3), respectively. As a result, 27 cm of sample injection (900 s in time) provides the best separation efficiency, as shown in Fig. 1B. Figure 2 demonstrates the relationship between the length of the sample injection and the corresponding fluorescence intensity in sweeping-MEKC. The

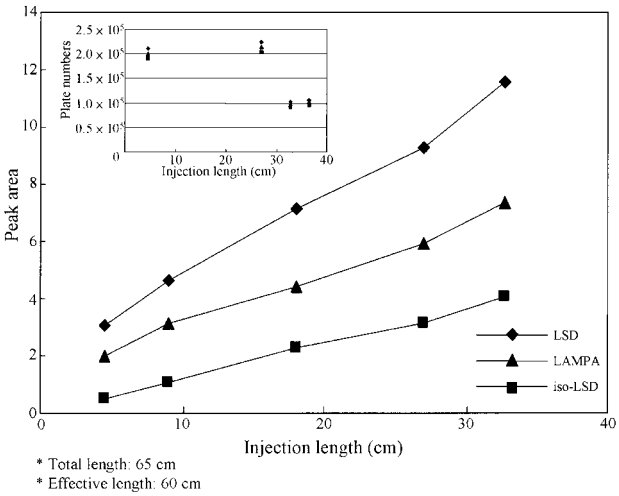

Figure 2. Relationship between sample injection length, related fluorescence intensity and plate numbers (inset) in sweeping-MEKC.

fluorescence intensity increases in a nearly linear manner with the length of the injection. The inset shows the relationship between the length of the sample injection and plate numbers. The plate numbers decreased when the injection length exceeded ~ 30 cm. Thus, ~ 30 cm is useful for sample concentration by sweeping, and the next ~ 30 cm is necessary for separation in this case.

3.1.2 CSEI-sweep-MEKC

This method provides for a more sensitive detection than sweeping-MEKC and is sufficiently flexible to offer the potential of affording a detection limit for positively chargeable analytes even in the parts per trillion (ppt) levels [23]. In these experiments, the background buffer consisted of only 75 mM H_3PO_4 in a mixture of acetonitrile-methanol-water (5:35:60 v/v/v). The capillary was initially filled with this background buffer, followed by the injection of a high-conductivity buffer (~ 9 cm length of capillary with 100 mM H_3PO_4 solution) void of organic solvent, and, lastly by the injection of a short water plug (~ 1.0 mm). The samples were prepared in a solution of methanol-water (1:1 v/v). By electrokinetic injection at +25 kV, the cationic analytes were injected for a period of 20 min, and the current changed from ~ 10 to ~ 20 μA. Finally, the cationic analytes focus or stack at the interface between the water zone and the high-conductivity buffer. The injection was then stopped and the micellar BGSs were placed at both ends of the capillary. Following this, by quickly shifting the voltage to −20 kV, the negative polarity high voltage permitted the entry of micelles from the inlet vial into the capillary to sweep the stacked and to introduce analytes to the narrow brands. The separation was performed using MEKC within the next ~ 20 min. The electropherograms are shown in Figs. 3A–D. Herein, the sample concentrations of LSD, *iso*-LSD and LAMPA were 2.0, 1.0 and 1.5 ng/mL (peaks 1–3), respectively. The electrokinetic injection times were 300, 600, 1200 and 1800 s (Figs. 3A–D), respectively. The 1200 s (Fig. 3C) sample injection was found to provide the most satisfactory results both for separation efficiency (plate numbers ~ 3.5×10^5) and sensitivity. In comparison with a normal injection and separation, a ~ 100 000-fold increase (S/N = 3) in detection sensitivity was obtained. However, at longer injection times (1800 s, Fig. 3D), the separation became incomplete. Figure 4 shows the relationship between injection times and related fluorescence intensity in CSEI-sweep-MEKC. The inset shows the relationship between injection times and plate numbers. No changes were found when the injection time was increased. Using the conditions described in Figs. 1 and 4, the linearity, limit of detection (LOD) values, and plate numbers were examined and these data are summarized in Table 1.

Table 1. LODs, RSDs, and plate numbers for LSD, *iso*-LSD and LAMPA using sweeping-MEKC/CSEI-sweep-MEKC

A. Sweeping-MEKC	LSD	*iso*-LSD	LAMPA
Equation of the line	$y = 4.58 \times 10^5{}_\chi - 1.65 \times 10^4$	$y = 3.23 \times 10^5{}_\chi - 3.75 \times 10^3$	$y = 3.74 \times 10^5{}_\chi - 1.07 \times 10^4$
Coefficient of variation	$r^2 = 0.9934$	$r^2 = 0.9934$	$r^2 = 0.9914$
LOD (S/N = 3)	16 ng/mL (4.8×10^{-8} M)	22 ng/mL (6.9×10^{-8} M)	18 ng/mL (5.6×10^{-8} M)
Plate number	1.8×10^5	2.0×10^5	1.8×10^5
B. CSEI-Sweep-MEKC	**LSD**	***iso*-LSD**	**LAMPA**
Equation of the line	$y = 2.59 \times 10^5{}_\chi - 5.69 \times 10^4$	$y = 1.98 \times 10^5{}_\chi - 3.24 \times 10^4$	$y = 2.01 \times 10^5{}_\chi - 3.53 \times 10^4$
Coefficient of variation	$r^2 = 0.9904$	$r^2 = 0.9963$	$r^2 = 0.9948$
LOD (S/N = 3)	58 pg/mL (1.8×10^{-10} M)	68 pg/mL (2.1×10^{-10} M)	80 pg/mL (2.5×10^{-10} M)
Plate number	3.5×10^5	3.6×10^5	3.3×10^5
C. Normal MEKC	**LSD**	***iso*-LSD**	**LAMPA**
LOD (S/N = 3)	5.3 μg/mL (1.6×10^{-5} M)	8.0 μg/mL (2.5×10^{-5} M)	7.3 μg/mL (2.4×10^{-5} M)
Plate number	2.0×10^5	2.1×10^5	1.9×10^5

Light source: Xe lamp (total ~ 6 W);
λex = 320 ± 8 nm; λem = 390 ± 2 nm

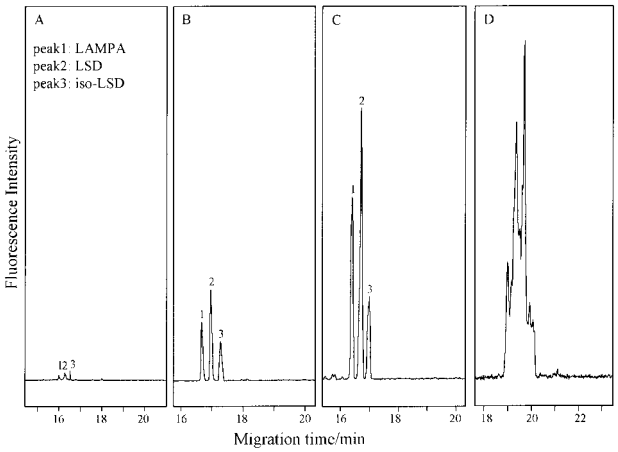

Figure 3. Effects of different injection times by electrokinetic injection at 300, 600, 1200 and 1800 s (A–D) in CSEI-sweep-MEKC. CE conditions as in Fig. 1, except current ~ −15, −13, −13 and −12 μA. Sample concentrations: 2.0, 1.0 and 1.5 ng/mL for LSD, *iso*-LSD and LAMPA, respectively. Buffer: 100 mM SDS, 3 mM Brij-30 and 50 mM H_3PO_4 in an acetonitrile-methanol-water solution (5:35:60 v/v/v); pH 2.1.

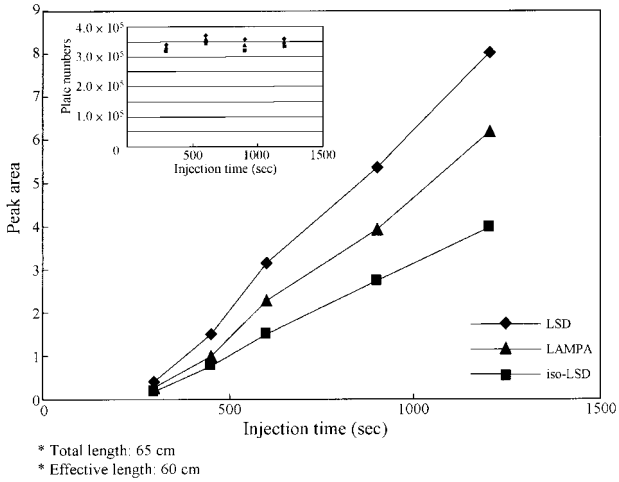

Figure 4. Relationship between sample injection length, related fluorescence intensity and plate numbers (inset) for CSEI-sweep-MEKC.

3.2 Analysis of LSD in blood samples

In terms of the efficiency of liquid-liquid extraction, LSD (1.0 μg/mL) was first added to a blank sample, and LAMPA (0.5 μg/mL) was then added after extraction. The CE electropherogram for the sweeping-MEKC mode is shown in Fig. 5. Peaks a–g are natural fluorescent compounds in the blood extract which fluoresce in the wavelength range of 390 ± 2 nm. The peak indicated as "*" was an impurity not related to *iso*-LSD. Due to the fact that the fluorescence intensity of LAMPA (in methanol) and LSD is 8–10, liquid-liquid extraction recovery of LSD at high (1.0 μg/mL) and low concentrations (0.1 μg/mL) were ~ 56 ± 4% and ~ 54 ± 1.8%, respectively. These

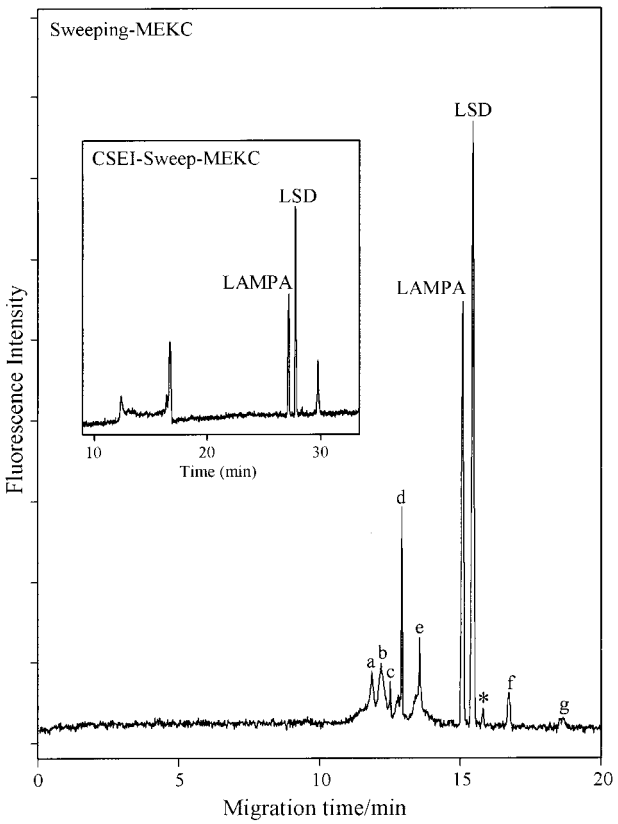

Figure 5. CE electropherogram of a blank blood sample from a normal subject, spiked with LSD and LAMPA at a level of 1.0 and 0.5 μg/mL, respectively, after applying the sweeping-MEKC technique. CE conditions are as stated in Fig. 1. The inset shows the CE electropherogram obtained by spiking with LSD and LAMPA at levels of 10 and 5.0 ng/mL, respectively, after applying the CSEI-sweep-MEKC technique. The CE conditions are as stated in Fig. 3.

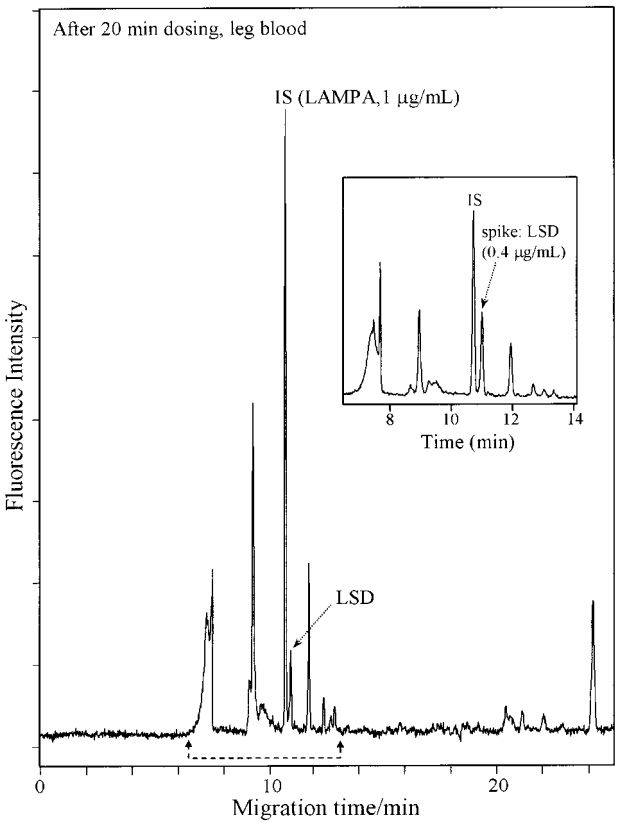

Figure 6. CE electropherogram of a blood sample extract (after ingestion of LSD 20 min, leg) from the LSD dosing mouse, spiked with 1.0 μg/mL of LAMPA as an internal standard. The inset shows this CE electropherogram after spiking with the LSD standard at a level of 0.4 μg/mL.

details are summarized in Table 2. The inset shows the CE electropherogram for the CSEI-sweep-MEKC mode. LSD (10 ng/mL) was first added to a blank sample, and LAMPA (5 ng/mL) was then added after extraction. Because of the numerous other conductive components in blood sample, the detection limit (~0.6 ng/mL) was poorer than that of a model mixture (model substances in pure methanol-water). This value is poorer than the model mixture because of other conductive components in which the detection limit was ~0.06 ppb. Thus, the application of CSEI-sweep-MEKC still remains problematic in the real world.

Table 2. Precision data for the method described

	LSD	LSD	LAMPA	LAMPA
Added (ng/mL)	1000	100	1000	100
Found (ng/mL)	550	54	540	51
RSD (%)	4.25	1.83	2.04	1.63
Recovery (%)	55.6	53.8	54.3	50.6

$n = 4$

Figure 6 shows a typical CE electropherogram of a blood sample extract from the LSD test mouse (20 min after ingestion of the LSD) by applying the sweeping-MEKC technique. This extract has been spiked with 1000 ng/mL LAMPA as an internal standard before extraction. LSD appears to the right of LAMPA (arrow). In order to examine this peak, we spiked 400 ng/mL of LSD standard and found that this peak indeed increased, as shown in the inset. Because the ratio of the recovery of LSD and LAMPA was 10:9.7, the concentration of LSD can be calculated. We assigned this peak as LSD and its concentration was determined to be 120 ng/mL. Using the same procedure, Fig. 7 shows the CE electropherogram for another blood sample extract from the same LSD test mouse (60 min after ingestion of the LSD). This extract was also spiked with 1000 ng/mL LAMPA as the internal standard and LSD can be detected (arrow mark) at a level

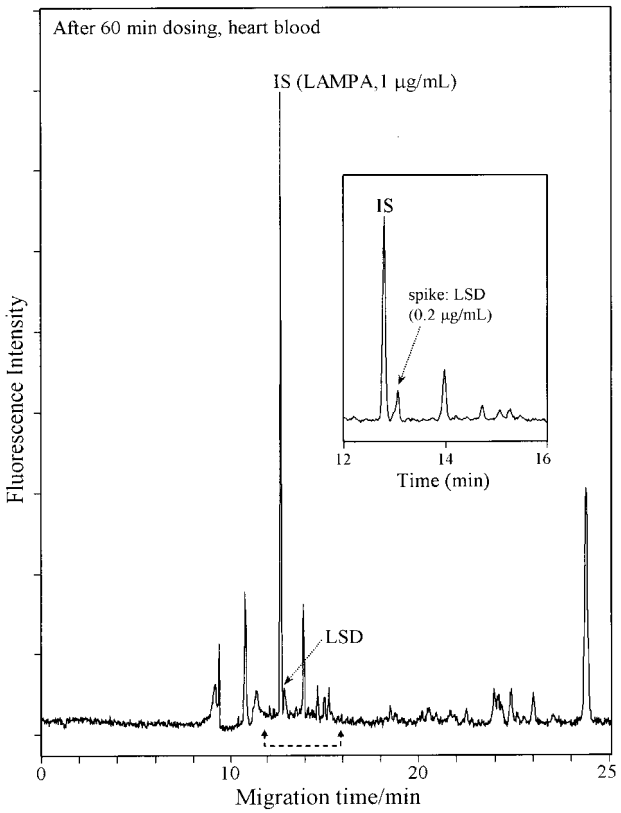

Figure 7. CE electropherogram of a blood sample extract (after ingestion of LSD 60 min, heart) from a mouse, spiked with 1.0 μg/mL of LAMPA as an internal standard. The inset shows this CE electropherogram after spiking with the LSD standard at a level of 0.2 μg/mL.

of 30 ng/mL. Finally, we spiked 200 ng/mL of LSD standard and found that this peak increased, as shown in the insert. Thus, we conclude that LSD can be detected in this test mouse blood at levels of 120 and 30 ng/mL, 20 and 60 min after ingestion of the LSD, respectively. Meanwhile, the LSD dosage was 5 μg/g. The concentration of LSD decreased quickly in body. This is consistent with the report, in which the blood half-life of LSD in mice, cats, monkeys was reported to be 7, 130, and 100 min, respectively [1]. We are the first group to have successfully detected LSD in blood after different dosing times. By applying this approach to humans, if the relationship between LSD concentration in the blood and various dosing times can be determined, it would be of use in determining the quantity of LSD ingested, after a period of days.

4 Concluding remarks

We demonstrate here that CE/fluorescence spectroscopy can be successfully used for the separation and on-line concentration of three similar analytes (LSD, *iso*-LSD and LAMPA) using the sweeping-MEKC and CSEI-sweep-MEKC techniques. The optimum CE conditions for the analysis of the three analytes were achieved using a mixture of acetonitrile-methanol-water solution (5:35:60 v/v/v) containing phosphate (50 mM), SDS (100 mM) and Brij-30 (3 mM). In the sweeping-MEKC mode, for a 65 cm capillary (effective length, 60 cm) the optimum injection length is ~27 cm, whereas in the CSEI-sweep-MEKC mode, the optimum electrokinetic injection time is ~1200 s. In comparison with the normal injection used in the CE separation by the MEKC mode, ~400 and ~100 000-fold improvement (S/N = 3) in detection sensitivity, respectively, can be obtained. Although the Xe lamp source is still not superior to laser-induced fluorescence (LIF) detection, a combination of the sweeping techniques and LIF, especially a He-Cd laser (325 nm), can clearly lead to further improvements in the analysis of LSD in blood. Furthermore, when the sweeping-MEKC technique was applied to a blood sample from an LSD test mouse, the analysis of LSD can be achieved in a short time, without the need for a derivatization step and additional sample handling, which are necessary when MS is used. LSD was detected in these blood samples at levels of 120 and 30 ng/mL, where the blood samples were obtained 20 and 60 min after ingestion of the LSD, respectively.

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