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Determination of lysergic acid diethylamide (LSD) by application of online 77 K fluorescence spectroscopy and a sweeping technique in micellar electrokinetic chromatography

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Abstract

The principal advantage of the use of Shopl'skii effect (low temperature spectrum) is that spectral sharpening occurs both in absorption and emission. However, thus far using the technique of capillary electrophoresis/low temperature fluorescence spectroscopy (CE/LTFS) either at 77 or 4.2 K remains difficult to obtain an on-line spectrum, if the analyte is present at low concentration. This paper examines the feasibility of combining the techniques of online concentration and CE/LTFS to identify LSD and related compounds in urine at 77 K. To improve sensitivity, sweeping-micellar electrokinetic chromatography (sweeping-MEKC) and cation-selective exhaustive injection-sweep-micellar electrokinetic chromatography (CSEI-sweep-MEKC) were used for on-line concentration which resulted in detection limits of ~20 ~60 ppt, respectively.

Keywords: Lysergic acid diethylamide (LSD); Iso-lysergic acid diethylamide (iso-LSD); Lysergic acid N,N-methylpropylamide (LAMPA); Capillary electrophoresis; Low temperature fluorescence spectroscopy; Sweeping; Cation-selective exhaustive injection-sweep-micellar electrokinetic chromatography (CSEI-sweep-MEKC); Urine

1. Introduction

Lysergic acid diethylamide (LSD) is a potent psychoactive and hallucinogenic drug that acts on the central nervous system. The detection of LSD (Fig. 1) and its metabolites in body fluids continues to represent a challenge because of the small dosage (usually approximately 1 μm kg^{-1}) which is typically taken [1,2]. A number of analytical methods have been developed for its identification and include techniques such as radioimmunoassay (RIA) [3], thin layer chromatographic analysis (TLC) [4], high performance liquid chromatography (HPLC)/fluorescence detection [5–7] alone, or combined with tandem mass spectrometry [8]. Needless to say, gas-chromatography/mass spectrometry (GC-MS) constitutes the most popular and powerful techniques for this analysis, not only in the area of forensic research, but also for clinical analysis [9–12]. However, each of the above

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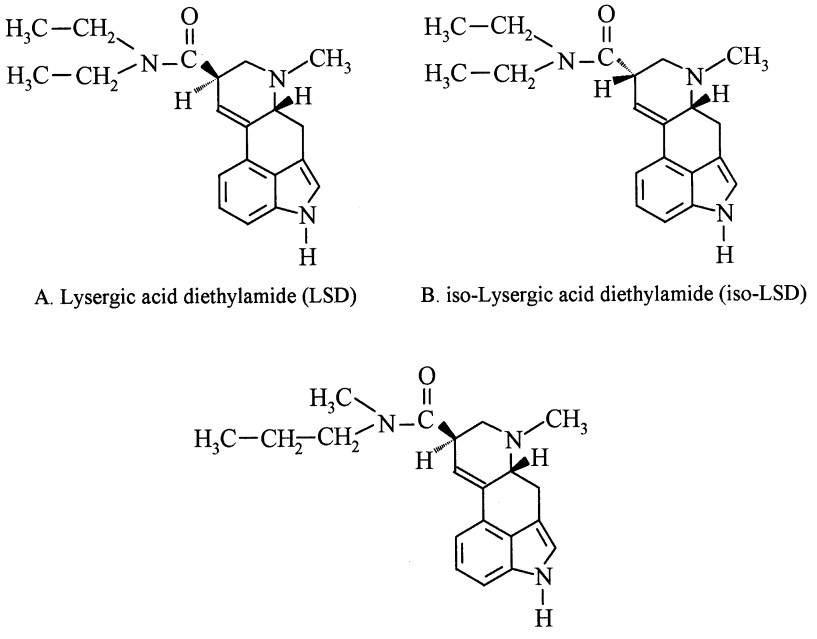

Fig. 1. Molecular structures of (A) LSD, (B) iso-LSD and (C) LAMPA.

methods have unique advantages and disadvantages with respect to sensitivity, precision and simplicity of use.

Capillary electrophoresis (CE) is a very useful method for the determination of drugs in body fluids because of its advantages in terms of speed, higher resolution, and a smaller injection volume than HPLC or GC. In particular, CE combined with laser-induced fluorescence (CE-LIF) detection provides high sensitivity and its use in the analysis of LSD has been reported [13,14]. Djordjevic et al. reported the D-LSD derivatives analysis using MEKC by adjusting column temperature and applied electric field for a fast separation [15]. However, with either fluorescence detection or UV detection, in CE separation, the migration time and spiking methods are normally used to identify sample constituents. It should be noted that a dependence on migration time could cause problems because of time scale shifts. To solve such problems, we previously reported on an online identification method by capillary electrophoresis/fluorescence spectroscopy (CE/FS) at 77 K [16–18]. However, thus far using the technique of CE/FS either at 77 or 4.2 K is difficult to obtain an online spectrum if these analytes are extremely slight. In this study, we report, for the first time, the online 77 K fluorescence spectral identification of LSD and iso-LSD using the same technique. To improve sensitivity, two techniques involving sweeping-micellar electrokinetic chromatography (sweeping-MEKC) and, for online sample concentration, cation-selective exhaustive injection-sweep-micellar electrokinetic chromatography (CSEI-sweep-MEKC) were introduced.

2. Materials and methods

2.1. Chemicals

LSD, iso-lysergic acid diethylamide (iso-LSD) and lysergic acid N,N-methylpropylamide (LAMPA) were acquired from Radian International (Austin, TX). Acetonitrile (ACN) and methanol (99.8%) were obtained from Fisher Scientific (Fair Lawn, NJ). Sodium dodecyl sulfate (SDS) and ammonium carbonate were obtained

from Acros (Belgium) and Sigma (St. Louis, MO), respectively. Brij-30 was acquired from Aldrich (Milwaukee, WI) All other chemicals were of analytical grade and are commercially available.

2.2. CE apparatus and operating conditions

The CE set-up was fabricated in-house and is identical to that described previously [16–18]. Briefly, a high-voltage power supply (Model RR30-2R, 0–30 KV, 0–2 mA, reversible, Gamma, FL) was used to drive the electrophoresis and a 50-μm I.D. fused silica capillary (J&W Scientific, CA) was used for separation (65 cm in length/60 cm to detector). The excitation source was selected by a monochromator (ARC, Acton Research Corporation; Model SP-150, 1200 grooves mm^{-1} grating) connected to a Xe lamp (Muller Elektronik Optik, SVX/LAX 1450, 500 W). The excitation wavelength was 320 ± 8 nm; emission was measured at 390 ± 3 nm. Fluorescence data were collected at a right angle to the light source and dispersed by a second monochromator (ARC Model SP-300i, 2400 grooves mm^{-1} grating), followed by detection using a photomultiplier tube (ARC Model P2-R928, for 190–900 nm). Electropherograms were collected at a speed of 200 ms $point^{-1}$ with a data acquisition system (ARC's Spectra-Sense NCL package), connected to a personal computer. The CE buffer basically was an acetonitrile–methanol–water solution (5:35:60, v/v), containing 100 mM SDS, 3 mM Brij-30 and 50 mM H_3PO_4. Details concerning CE buffer are described below. The LSD, LAMPA, iso-LSD standards were prepared in methanol (99.8%) for all experiments.

2.3. Methodology

2.3.1. Sweeping-MEKC

In the sweeping MEKC mode, 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), the pH of which was 2.06 (conductivity, 5.37 mS cm^{-1}). The samples were dissolved in the same solution (without SDS) resulting in a non-micelle buffer, and adjusted to the same conductivity as the 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^{-1}. When the injection was completed, −20 kV was applied to power the CE separation. This procedure permits the SDS-anionic surfactant micelles (in the inlet reservoir) to enter the sample zone. Thus, along the capillary axis the samples were being swept and concentrated near the junction between the sample solution and the background solution. As in the following step, the samples were separated by the MEKC mode.

2.3.2. CSEI-sweep-MEKC

In the CSEI-sweep-MEKC mode, the background buffer consisted of only 75 mM H_3PO_4 in a mixture of acetonitrile–methanol–water (5:35:60, 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), and lastly by the injection of a short water plug (~ 1.0 mm). By electrokinetic injection at +25 kV, the cationic analytes were injected for a period of 20 min. Meanwhile, the cationic analytes could be concentrated into the water zone. 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 background solutions 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.

2.4. Liquid–liquid extraction procedure and recovery

A 2-ml aliquot of urine in a 15-ml glass tube was made alkaline by the addition of 150 μm of saturated ammonium carbonate and 100 μl of 2 M sodium hydroxide and briefly stirred for 5 min. Two milliliters of toluene/methylene chloride (7:3, v/v) were added, and the sample and solvent gently mixed for 30 min. After mixing, the tube was

centrifuged. The organic layer was transferred to a clean tube, 2.5-ml of 0.1 M ammonium hydroxide was added and the solution then mixed and centrifuged again. The toluene/methylene chloride organic layer, containing LSD, was transferred to a clean glass tube. To determinate the extraction efficiency, eight control urines containing 1 ppm of LSD were divided into equal aliquots. At this point, the internal standard (LAMPA, 100 ppm) was added to one set of aliquots after extraction. As a result, due to the fact that the fluorescence intensity of LAMPA and LSD are similar, by comparing the ratio to the LAMPA standard in each pair of aliquots, the extraction efficiency for LSD was determined to be approximately 56%.

3. Results and discussion

3.1. Separation condition and online 77 K fluorescence spectra

Fig. 2 shows the fluorescence spectra of LSD (— solid line), iso-LSD (· · · · · dotted line) and LAMPA (– · – dashed line) in a methanol matrix ($\lambda_{ex} = 320$ nm, monochromator resolution = 0.3 nm). The fluorescence spectra are analogous to each other. McNally et al. has previously reported on the stability of LSD under various storage conditions and pH [19,20]. It should be noted that LSD indeed is converted to iso-LSD under various different conditions, such as pH, temperature and UV-light. This is a competing reason for why the use of LAMPA as an internal standard is recommended. Fig. 3 shows a typical fluorescence electropherogram of the three analytes. The sample concentration was 134, 66 and 100 ppm for LSD, iso-LSD and LAMPA, respectively. For online spectral identification, a special capillary-Dewar was used [16–18]. After the separation is complete, the CE-separated analytes can be rapidly frozen by directly pouring liquid nitrogen in the capillary-Dewar. CE-separated analytes can be detected at 77 K for online spectral identification. The inset shows the online 77 K fluorescence spectra of LAMPA, LSD and iso-LSD. There is little structure to the fluorescence spectra at room temperature as shown in Fig. 2; whereas sharper

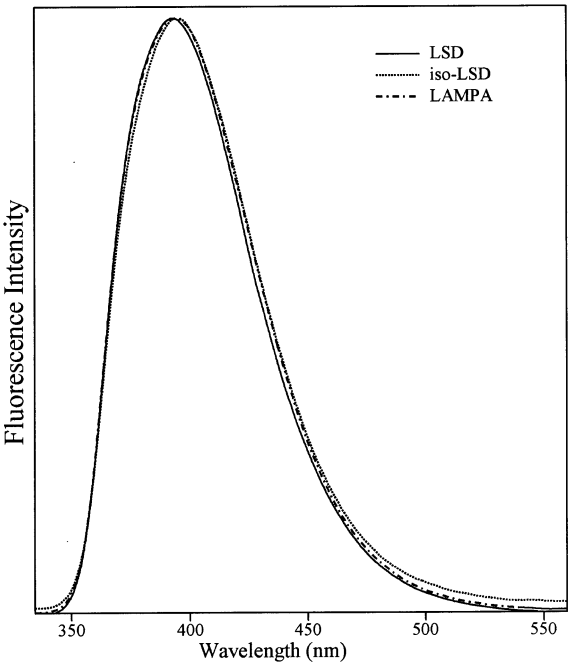

Fig. 2. Typical fluorescence spectra of LSD (— solid line), iso-LSD (--- dashed line) and LAMPA (· – · dashed line) in methanol ($\lambda_{ex} = 320$ nm, monochromator resolution = 0.3 nm); at room temperature.

spectral bands (~ 371 nm) appear when the temperature is reduced to 77 K. The on-line 77 K fluorescence spectra can be used as another evidence for the identification of LSD. However, to differentiate between LAMPA and LSD, a further low temperature, e.g. 4.2 K, is necessary because at such a low temperature, the (0, 0) origin band would be split into mutliplet structures when the sample is excited with lasers, i.e. the so called fluorescence line-narrowing spectroscopy (FLNS) [21–24].

3.2. On-line sample concentration

As mentioned above, the detection of LSD in body fluids continues to be difficult because of the small dosage used. Therefore, a technique involving online concentration becomes increasingly important. Recently, a series of reports appeared by Terabe et al., as well as other groups concerning the so called ‘sweeping’ technique [25–32]. In the

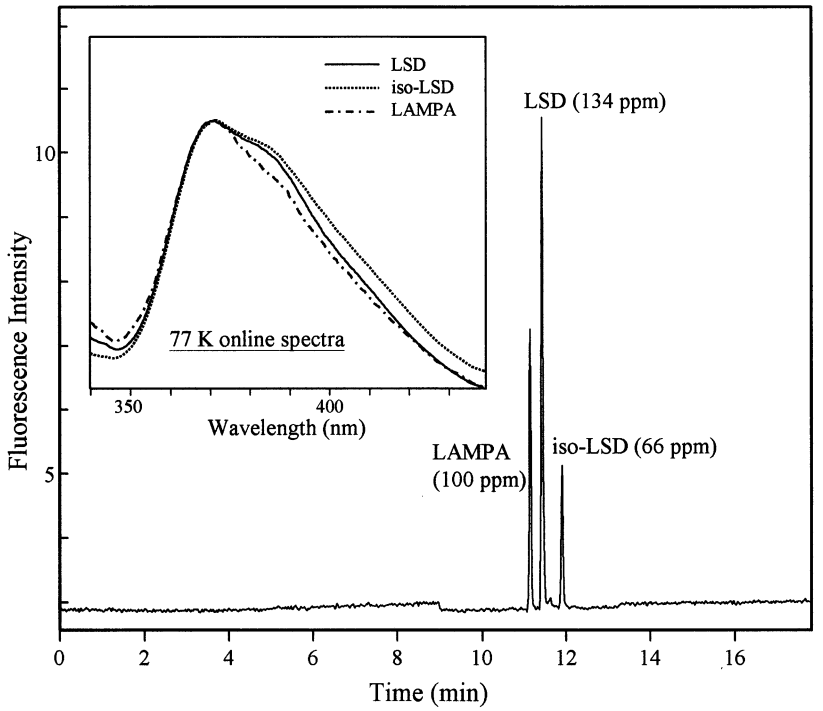

Fig. 3. A typical CE fluorescence electropherogram of LSD, iso-LSD and LAMPA. CE conditions: capillary, 65 cm (60 cm to detector), 50 μm I.D.; buffer, 100 mM SDS, 3 mM Brij-30 and 5 mM H_3PO_4 in an acetonitrile–methanol–water solution (5:35:60, v/v); pH 2.1; applied voltage, −20 kV, current ∼ −30 μA; $\lambda_{ex} = 320$ nm, $\lambda_{em} = 390$ nm. Sample concentration: 134, 66 and 100 ppm for LSD, iso-LSD and LAMPA, respectively. The inset shows the online 77 K fluorescence spectra of LSD and iso-LSD, where sharper spectral bands (371, 385 nm) emerged when the temperature was reduced to 77 K.

following discussions, the applications of sweeping-MEKC and the CSEI-sweep-MEKC model are described and compared.

Sweeping-MEKC is one of the most simple and convenient methods for online concentration. The concentration effect relies on the pseudostationary phase that enters the sample solution zones. As a result, in comparison with a normal injection (10 cm relative to the exit reservoir for 3 s) and separation (Fig. 3), ∼400-fold improvement (S/N = 3) in detection could be obtained, as shown in Fig. 4A. On the other hand, the CSEI-sweep-MEKC model was first reported by Terabe et al [26]. This method provides for a higher detection limit than sweeping-MEKC and is sufficiently flexible to offer the potential to extend the detection limit of positively chargeable analytes even in the parts per trillion (ppt) level. As shown in Fig. 4B, the sample concentrations of LSD, iso-LSD and LAMPA were 3.3, 1.6 and 2.5 ppb, respectively. Compared to the conditions shown in Fig. 3, a ∼100 000-fold improvement (S/N = 3) in detection sensitivity can be obtained. Using the conditions described in Fig. 4A and B, the limit of detection (LOD) values, linearity of peak area and migration times were calculated and are summarized in Table 1. LOD values (S/N = 3) for a normal injection were just 5.3, 8.0 and 7.3 ppm for LSD, iso-LSD and LAMPA, respectively; whereas the LOD was dramatically improved when the sweeping techniques were applied. Frost et al. reported 0.51 ppb of LSD in human blood by He–Cd laser and the detection limit was 0.1–0.2 ppb [13,14]. Although the Xe lamp source is still not superior to laser-induced fluorescence (LIF) detection, a combination of sweeping technique and LIF definitely can further improve the LOD values. This is considered to be important in the

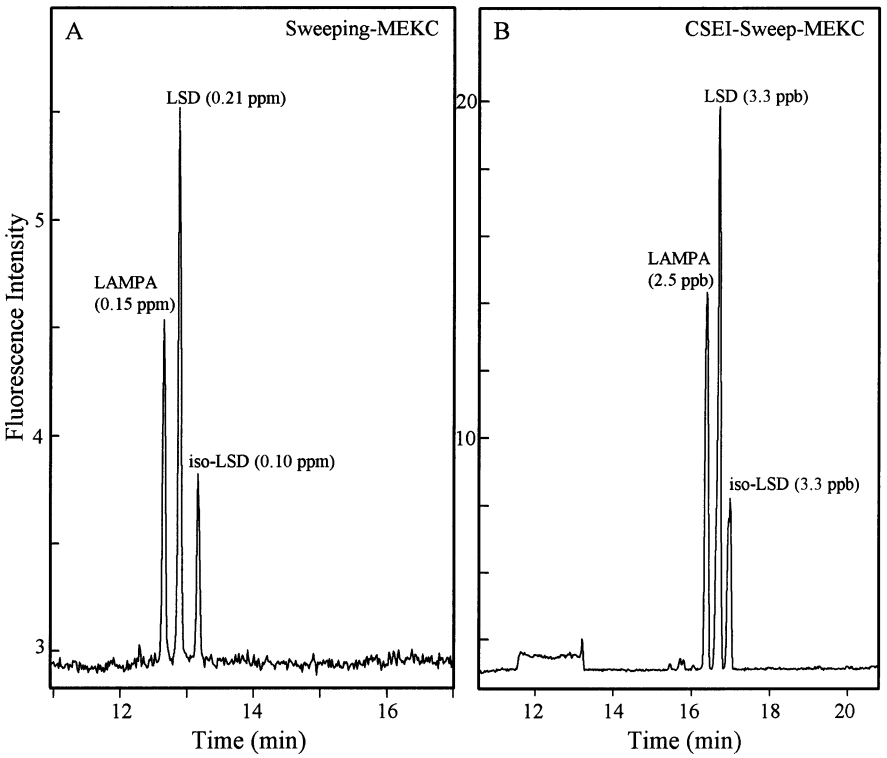

Fig. 4. (A) Sweeping-MEKC analysis of LSD, iso-LSD and LAMPA in the presence of a small amount of organic solvent in the sample matrix: BGS, 100 mM SDS, 3 mM Brij-30, 50 mM H_3PO_4 in a mixed acetonitrile–methanol–water solution (5:35:60, v/v); S, three analytes in the same solvent (containing 3 mM Brij-30 and 50 mM H_3PO_4) adjusted to the conductivity of the BGS (5.37 mS cm^{-1}) by the addition of 100 mM H_3PO_4; injected length of S, 30 cm; applied voltage, −20 kV; concentrations of LSD, iso-LSD and LAMPA were 0.21, 0.10 and 0.15 ppm; capillary, 60 cm to the detector (65 cm total). (B) CSEI-sweep-MEKC analysis of LSD, iso-LSD and LAMPA. Steps: 1, capillary was filled with the non-micelle buffer; 2, ten cm of capillary length of the 100 mM H_3PO_4 solution was injected; 3, a plug of water (~ 1.6 mm) was injected; 4, +25 kV was applied for 20 min; 5, shifting the voltage to −20 kV (~ −18 μA) for regular CE separation. The sample concentrations of LSD, iso-LSD and LAMPA were 3.3, 1.6 and 2.5 ppb, respectively.

field of forensics for the trace analysis of illicit drugs in actual cases.

3.3. Analysis of LSD in urine samples

In a real sample analysis, the application of sweeping techniques continue to be challenge because of the complicated matrix effects. In Fig. 5A, electropherograms a and b show typical electropherograms of a normal human urine extract and LSD (spiked 50 ppb using the same urine sample) after applying the technique of sweeping-MEKC. Only one major peak (marked as '*') at ~13 min was detected in the normal human urine sample. Thus, it is clear that in typical human urine samples, only a few native fluorescent compounds are present which fluoresce in the wavelength range of 390±3 nm. With this fluorescence detection, the electropherogram was much simple than UV detection due to the UV-absorption of numerous organic compounds in a urine sample. Using this approach, LSD can be efficiently extracted, concentrated and detected. This method is simple and reproducible. The inset shows the 77 K online spectrum of LSD (in CE buffer). This is very useful information in that a minor amount of LSD can be concentrated and detected as a characteristic spectrum. In contrast,

Table 1
Limit of detection values for LSD, iso-LSD and LAMPA with sweeping-MEKC (A), CSEI-sweep-MEKC (B) and normal MEKC (C)

	LSD	iso-LSD	LAMPA
A. *Sweeping-MEKC*			
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	$r^2 = 0.9934$	$r^2 = 0.9934$	$r^2 = 0.9914$
RSD			
(a) Migration time ($n = 6$)%			
Intra-day	1.0	1.01	1.04
Inter-day	2.36	2.30	2.69
(b) Peak area (n = 6)%			
Intra-day	3.36	4.37	4.54
Inter-day	5.03	7.80	7.36
Linear range	1.67–0.052 ppm	0.83–0.026 ppm	1.25–0.040 ppm
LOD (S/N = 3)	15.6 ppb	22.2 ppb	18.0 ppb
B. *CSEI-Sweep-MEKC*			
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	$r^2 = 0.9904$	$r^2 = 0.9963$	$r^2 = 0.9948$
RSD			
(a) Migration time ($n = 6$)%			
Intra-day	2.29	2.78	2.40
Inter-day	4.32	4.40	4.26
(b) Peak area ($n = 6$)%			
Intra-day	5.66	6.43	5.01
Inter-day	9.35	8.87	8.38
Linear range	6.68–0.83 ppb	3.32–0.42 ppb	5.0–0.625 ppb
LOD (S/N = 3)	58 ppt	68 ppt	80 ppt
C. *Normal MEKC*			
LOD (S/N = 3)	5.3 ppm	8.0 ppm	7.3 ppm

Light source: Xe lamp (total $\sim$6 W); $\lambda_{ex} = 320$ nm; $\lambda_{em} = 390$ nm.

using CSEI-sweep-MEKC, Fig. 5B (electropherograms c and d) shows typical electropherograms of a normal human urine extract, and LSD (spiked 3 ppb using the same urine sample). Because of the numerous unknown matrix effects, many unidentified peaks appear when the technique of CSEI-sweep-MEKC was applied. The application of CSEI-sweep-MEKC still remains problematic in the real world.

4. Conclusions

We demonstrated here that a capillary electrophoresis/fluorescence spectroscopy method can be successfully used for the separation and on-line spectral identification of LSD and iso-LSD in urine samples, via low-temperature fluorescence spectroscopy at 77 K. The proposed method permits the excellent separation of LSD and related compounds from urine samples. The CE method coupled with 77 K fluorescence spectroscopy was applied for the study of LSD. The low temperature spectrum was obtained when the CE-separated analyte reached the CE detection window and was then cooled to a temperature of 77 K. The various migration times and the (0, 0) origin band (371 nm) were easily observed and recognized.

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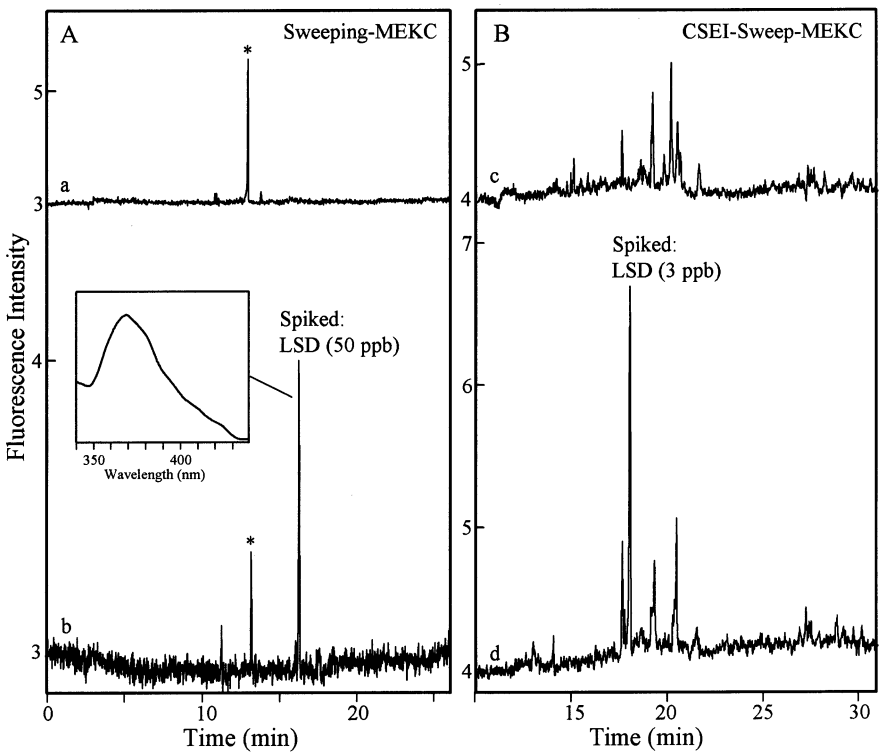

Fig. 5. (A) Electropherograms a and b, normal human urine extracts and LSD (spiked at a level of 50 ppb to the same urine sample) after applying the technique of sweeping-MEKC. Inset, online 77 K fluorescence spectrum of spiked LSD (50 ppb). (B) Electropherograms c and d, normal human urine extracts and LSD (spiked at a level of 3 ppb to the same urine sample) when CSEI-sweep-MEKC was applied.

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