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On-line identification of lysergic acid diethylamide (LSD) in tablets using a combination of a sweeping technique and micellar electrokinetic chromatography/77 K fluorescence spectroscopy

This work describes a novel method for the accurate determination of lysergic acid diethylamide (LSD) in tablets. A technique involving sweeping-micellar electrokinetic chromatography (MEKC) was used for the initial on-line concentration and separation, after which a cryogenic molecular fluorescence experiment was performed at 77 K. Using this approach, not only the separation of LSD from the tablet extract was achieved, but on-line spectra were readily distinguishable and could be unambiguously assigned. The results are in agreement with analyses by gas chromatography-mass spectrometry (GC-MS). Thus, this method, which was found to be accurate, sensitive and rapid, has the potential for use as a reliable complementary method to GC-MS in such analyses.

Keywords: Gas chromatography-mass spectrometry / Low-temperature fluorescence spectroscopy / Lysergic acid diethylamide (LSD) / Micellar electrokinetic chromatography / Sweeping
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1 Introduction

Lysergic acid diethylamide (LSD) is a powerful psychedelic drug that produces temporary hallucinations and a schizophrenic psychotic state [1–3], and is a social problem in many countries. Because of the rapid growing abuse of this compound, as well as of similar substances, a simple, economic, rapid and consistent method for their determination is necessary, not only for clinical research, but for forensic analysis as well. A number of analytical methods have been developed for the identification of LSD, and each has unique advantages and disadvantages with respect to sensitivity, precision and simplicity of use [4–14]. GC-MS currently remains the official prescribed method [15–20]. However, in many cases GC-MS requires a derivatization step and additional sample handling, all of which are time-consuming. This represents a major drawback when it is necessary to analyze hundreds of samples in a short period. Furthermore, in some cases, diet pills, such as phentermine, give mass spectral fragmentation ions that are similar to amphetamines. Thus, the exclusive use of GC-MS can lead to errors in identification and misjudgments. A rapid and complementary method would be highly desirable.

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Abbreviations: FS, fluorescence spectroscopy; **LSD**, lysergic acid diethylamide

Capillary electrophoresis (CE) represents a rapidly growing separation technique that might well meet this need, since it is now well-established and represents a widely used analytical method in many fields, such as bioscience, pharmaceutical and environmental studying, food science, and forensic research. However, in CE separation, migration time and spiking methods are normally used to identify sample constituents. It should be noted that a dependence on migration time and spiking methods could cause problems because of time scale shifts and unpredictable overlapping with extraneous compounds. To solve such problems, we previously reported on an on-line identification method using micellar electrokinetic chromatography-fluorescence spectroscopy (MEKC-FS) at 77 K [21–24].

In this study, we now report, for the first time, on a technique involving on-line sample concentration, which was successfully combined with MEKC/77 K FS for on-line identification. The analysis of LSD from two forensic cases (two suspect tablets) were selected as the samples. Optimum conditions for the identification of LSD were investigated.

2 Materials and methods

2.1 Chemicals

LSD and *iso*-LSD were acquired from Radian International (Austin, TX, USA). Acetonitrile and methanol (99.8%) were obtained from Fisher Scientific (Fair Lawn, NJ, USA), sodium dodecyl sulfate (SDS) and ammonium

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carbonate from Acros (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.

2.2 MEKC apparatus and method

The schematic diagram of the in-house fabricated MEKC setup is shown in Fig. 1, and is similar to that described previously [21–24]. Briefly, a high-voltage (HV) power supply was used to drive the electrophoresis and a 50 μm ID fused-silica capillary (110 cm in length/70 cm to the detector) was used for the separation. The excitation source was selected by a monochromator connected to a Xe lamp. 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, followed by detection using a photomultiplier tube. Electropherograms were collected with a data acquisition system, connected to a personal computer. A locally designed capillary-Dewar (CD) was custom-made and consisted of a double-walled quartz flask for introducing liquid nitrogen. The capillary was bent into a hoop, secured to a glass rod and positioned in the central region of the CD. The MEKC detection window, formed by removing the coating of the capillary was ~3 cm. The progress of the separation was observed on a computer monitor. When MEKC-separated analytes appeared on the screen, the HV power supply was immediately turned off and liquid nitrogen poured directly into the CD. Once frozen, arbitrary detection times can be used to completely characterize the separated analytes by 77 K FS. The capillary inside the CD can be moved up and down by a translator or manually, in order to locate the next/former MEKC-separated analytes.

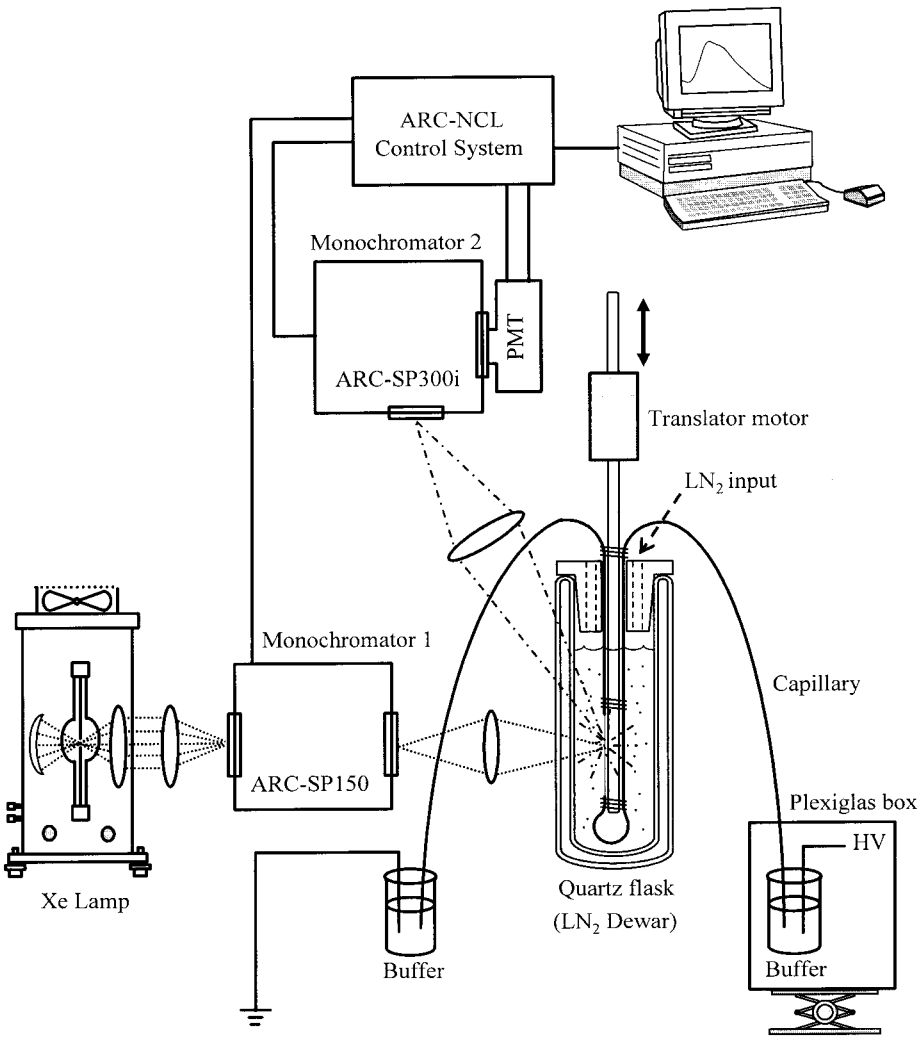

Figure 1. A schematic apparatus for the MEKC-FS system used for the low-temperature spectral fingerprint identification of MEKC-separated analytes.

2.3 GC-MS apparatus and methods

The GC-MS system (Hewlett-Packard, Palo Alto, CA, USA) consisted of a Model 6890 Series gas chromatograph, a Model 5973 mass spectrometer, and a Chemstation computer system. The column used was an HP-5 MS fused-silica capillary column (30 m × 0.32 mm ID) with a 0.25 μm film thickness of 5% phenylmethylsilicone. Injections were carried out in the splitless mode (injection volume, 2 μL). The carrier gas was high-purity helium at a flow rate of 2.0 mL/min. The mass spectrometer was used in the electron ionization (EI) mode at an electron energy of 70 eV. The temperatures of the source and the quadruple were 200°C and 100°C, respectively. Mass spectra were obtained from 40 to 500 amu at 1.64 scans per second. The injector and interface temperatures were maintained at 250°C and 220°C, respectively. The initial oven temperature was 180°C and was held for 1 min followed by a linear ramp to 270°C at 15°C /min. This was an additional held for 20 min. The total analysis time was 27 min.

3 Results and discussion

3.1 MEKC separation condition

Figure 2 shows typical electropherograms of the LSD standard (electropherogram a) and the methanol extract from suspect tablet I (electropherogram b). Herein, the excitation and fluorescence emission wavelength were 320 ± 8 nm and 390 ± 3 nm, respectively. The inset (upper) shows a typical fluorescence spectrum of the standard of LSD (100 ppm in methanol). For the separation, the MEKC buffer was an acetonitrile-methanol-water solution (5:35:60 v/v/v) containing 100 mM SDS, 3 mM Brij-30 and 50 mM H_3PO_4. Brij-30 was used to stabilize the sample solution. In electropherogram a, the LSD standard was detected at 19.6 min; whereas a number of peaks were detected from the extract of tablet I, as shown in electropherogram b. However, only peak 4 had the same migration time as the standard of LSD. For further examination, we spiked standard LSD (40 ppm) to this extract and repeated the separation. As a result, peak 4 was increased in intensity, as shown in the inset. Thus, peak 4 can be assigned to LSD. However, as mentioned earlier, the use of only the dependence on migration time and a spiking method can lead to an ambiguous assignment. It is necessary to acquire additional information for the unequivocal assignment of peak 4, such as UV absorbance, fluorescence or mass spectra. In this study, with fluorescence detection the electropherogram was considerably more simple than UV detection due to the UV-absorption of numerous organic compounds in the sample. Although a CCD detector can record the fluorescence spectra of these peaks immediately, nearly all of the detectors currently used in CE systems are designed to obtain data at room temperature. In most cases, at room temperature these regular fluorescence spectra only provide a broad fluorescence spectrum, leading to difficulties in fingerprint identification, especially for the structurally similar compounds. Furthermore, as shown in electropherogram b, peak 4 shows a poor S/N ratio. Without sample concentration, it is difficult to obtain an on-line fluorescence spectrum with an acceptable S/N ratio for fingerprint identification because the peaks pass through the detection windows only for a few seconds. For this reason, we introduced the technique of "sweeping" for on-line concentration and this is described as follows.

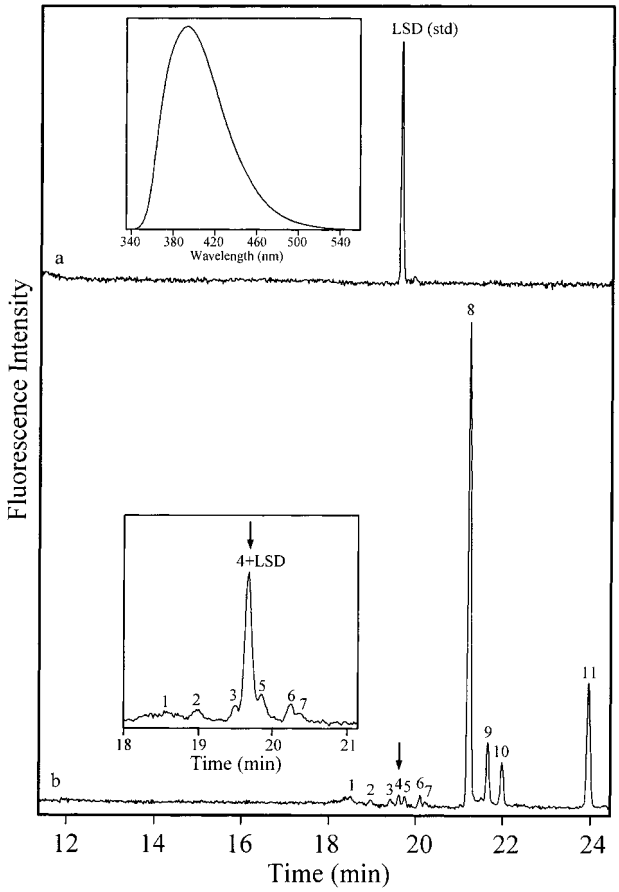

Figure 2. Electropherograms of (a) LSD and (b) a methanol extract of tablet I with fluorescence detection by CE. MEKC conditions: capillary, 110 cm (70 cm to the detector), 50 μm ID; buffer, 100 mM SDS, 3 mM Brij-30 and 5 mM H_3PO_4 in an acetonitrile-methanol-water solution (5:35:60, v/v/v); pH 2.1; applied voltage, − 25 kV, current ∼ −15 μA; λex = 320 nm, λem = 390 nm. Insets show the fluorescence spectrum of LSD (upper) and the electropherogram of extract from tablet I (lower), after spiking with a standard of 40 ppm of LSD (expanding peaks 1–7).

3.2 On-line sample concentration

To improve the S/N ratio, a technique involving on-line concentration becomes increasingly important. Recently, a series of reports by Terabe *et al.* as well as other groups concerning the so-called "sweeping" technique has appeared [25–31]. In this study, sweeping-MEKC was introduced. In Fig. 3, the background solution (for sweeping) consists 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. The methanol extract of tablet I was dissolved in the same solution (without SDS) resulting in a nonmicelle buffer, and adjusted to the conductivity of the background solution (5.37 mS/cm) by the addition of 100 mM H_3PO_4, the pH of which was 1.92. Hydrodynamic injection was achieved by raising the sample reservoir 20 cm relative to the exit reservoir for a period of 15 min. This procedure permits the injection of a 30 cm length of solution into the capillary. When the injection was completed, −25 kV (current, ∼ −15 μA) was applied to run the MEKC separation. As a result, in comparison with a normal injection (10 cm relative to the exit reservoir for 3 s, ∼0.5 mm) and separation (in Fig. 2, electropherogram b), a ∼200-fold improvement (S/N = 3) in detection could be achieved.

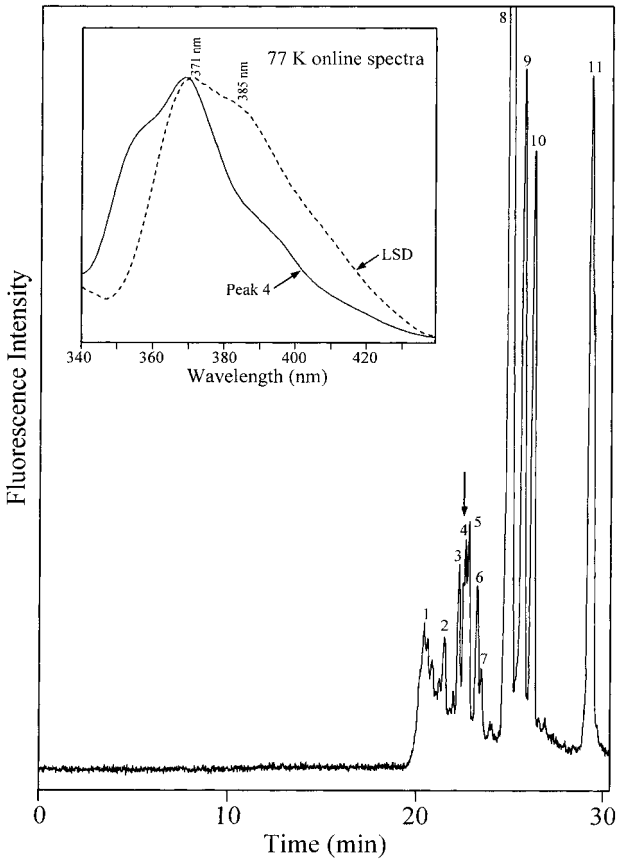

Figure 3. MEKC-separated chromatogram of the extract from tablet I obtained by the sweeping-MEKC technique. BGS, 100 mM SDS, 3 mM Brij-30, 50 mM H_3PO_4 in a mixed acetonitrile-methanol-water solution (5:35:60 v/v/v); S, the extract from tablet I in the same solvent (containing 3 mM Brij-30 and 50 mM H_3PO_4) adjusted to the conductivity of the BGS (5.37 mS/cm) by the addition of 100 mM H_3PO_4; injected length of S, 30 cm; applied voltage, − 25 kV; capillary, 110 cm to the detector (70 cm total). Inset: the on-line 77 K fluorescence spectra for the MEKC-separated peak 4 (solid line) and LSD standard (dash line).

3.3 On-line sample concentration combined with 77 K fluorescence spectral identification

In our laboratory, the MEKC method coupled with 77 K FS was developed and validated for the on-line identification of isomers [21–24]. The low-temperature spectrum was measured when the MEKC-separated analyte reached the MEKC-detection window and was then cooled to a temperature of 77 K by directly pouring liquid nitrogen

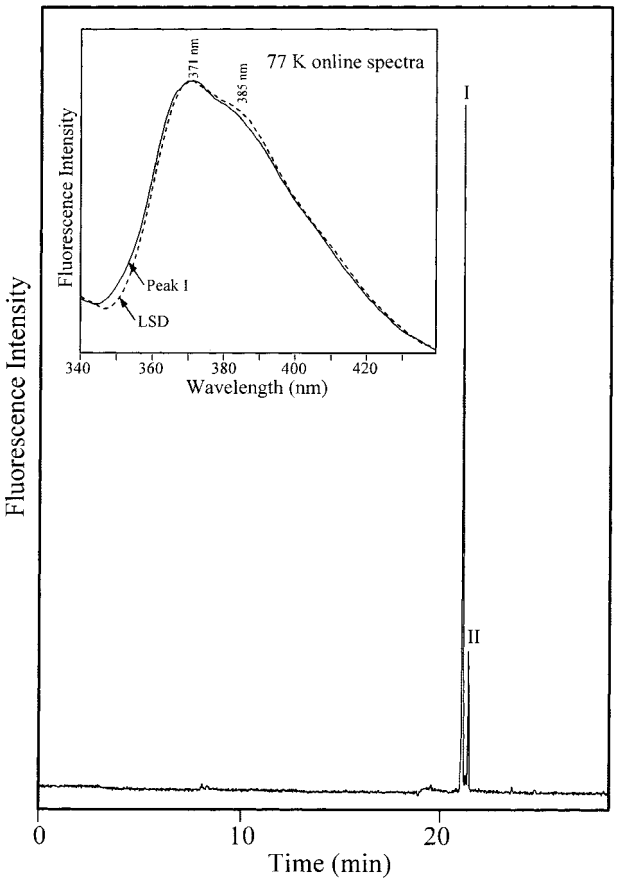

Figure 4. MEKC-separated chromatogram of the extract from tablet I. The MEKC conditions were the same as described in Fig. 2. Inset: the on-line 77 K fluorescence spectra for the MEKC-separated peak I (solid line) and LSD standard (dash line).

into the capillary-Dewar. In our previous studies, the 77 K spectra were much more characteristic for analyte identification than the ambient temperature spectra [21–24]. Using the same experimental methods, the extract from tablet I was separated and detected. With the help of on-line sample concentration, the measured 77 K on-line fluorescence spectra of standard LSD (dash line) and peak 4 (solid line) are shown in the inset of Fig. 3. For LSD, there is little structure to the fluorescence spectrum at room temperature (in Fig. 1); whereas sharper spectral bands (371, 385 nm) appear when the temperature is reduced to 77 K (monochromator resolution ~0.3 nm). By comparing the two spectra, it is clear that they represent not an identical compound. Thus, we conclude that tablet I did not contain LSD. Herein, we point out the possibility of misjudgment if the analysis was dependent only on migration time and a spiking method. This is especially important in forensic cases where a precise and valid analysis is critical. Although this is a negative example, the combination of the sweeping technique and CE/77 K FS could be also very useful for the analysis of LSD in urine or blood samples where the detection of LSD and its metabolites in body fluids continues to represent a challenge because of the small amounts present.

For the analysis of suspect tablet II, the electropherogram is shown in Fig. 4 using the same MEKC conditions as described above. Only two major peaks were detected within the wavelength range of 390 ± 3 nm. The fluorescence intensity was so strong that on-line concentration was not necessary. The measured 77 K on-line fluorescence spectra of standard LSD (dash line) and peak I (solid line) are shown in the inset of Fig. 4, respectively. As the result, the origin band was observed at 371 nm and the two spectra are clearly identical. Thus, we conclude that LSD was present in tablet II. Even though the accuracy of recovery of liquid-liquid extraction (by shaking with methanol in this experiment) was unknown, by comparing standard solutions with different concentrations, the content of LSD in this tablet (~1.5 mm in diameter, roughly a spherical black body) was greater than ~20 mg. We were curious about peak II, which could

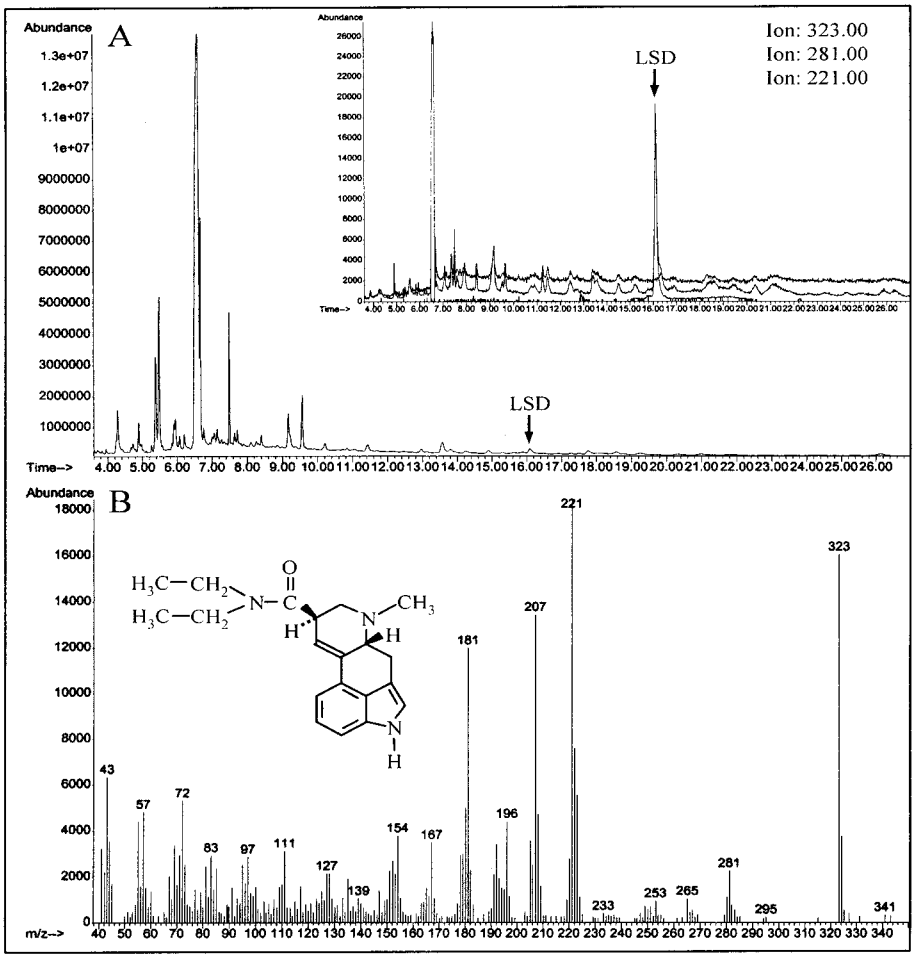

Figure 5. (A) Total ion chromatograms of the methanol extract from tablet II. Inset: SIM chromatograms (*m/z* of 323, 281 or 221). (B) Mass fragmentation spectrum of the peak at 16.1 min migration time.

represent *iso*-LSD because LSD is converted to *iso*-LSD under a variety of conditions, such as pH, temperature and UV-light [32, 33]. We spiked the extract of tablet II with *iso*-LSD and found that peak II was not *iso*-LSD.

3.4 Comparison with GC-MS

The results obtained by MEKC/FS were compared with GC-MS. Due to the high content of LSD in tablet II, the signal is easily detected even without a derivatization step. Figure 5A shows the total ion chromatogram profile for the extract of the tablet II. We found a tiny peak with a retention time of 16.1 min. With the SIM profile, this peak could be simplified. The inset shows the selected ion current (m/z = 323, 281 or 221) profile for the extract from tablet II. LSD was detected as a single peak at 16.1 min. Figure 5B shows the corresponding mass fragmentation spectrum. Using the same experimental conditions, LSD was not detected from the suspect tablet I. This is in agreement with the result obtained by MEKC-FS.

In conclusion, we demonstrated here that the combination of a sweeping technique and MEKC/FS can be successfully used for the analysis of LSD in suspect tablets or other solid samples. Sweeping-MEKC was initially used for on-line concentration and separation, and LSD was then detected and identified on-line, using a cryogenic molecular fluorescence technique at 77 K. This method resolves problems with weak intensity and broader fluorescence behavior of analytes at room temperature. The proposed method permits the excellent separation and on-line identification of LSD from suspect tablets, and represents a useful complementary method for GC/MS.

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