



TECHNICAL NOTE

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LSD and 9,10-dihydro-LSD Analyses in Street Drug Blotter Samples via Easy Ambient Sonic-Spray Ionization Mass Spectrometry (EASI-MS)^{*}

ABSTRACT: Normally, the identification of the LSD drug is performed by forensic laboratories, using the Ehrlich spot test. However, this is a nonspecific analysis. Additionally, the Brazilian Federal Police has identified the presence of a new compound in seized blotters: 9,10-dihydro-LSD, an uncontrolled substance. In this work, easy ambient sonic-spray ionization mass spectrometry in the positive ion mode, EASI(+)-MS, was used to characterize LSD and 9,10-dihydro-LSD compositions directly from the surface of blotters. The presence of LSD in the seized blotter samples were also confirmed via high-performance liquid chromatography with ultraviolet detector. In a set of 41 blotters analyzed by EASI(+)-MS, 28 showed positive results for LSD, seven for 9,10-dihydro-LSD, and another six samples showed negative results for both LSD and 9,10-dihydro-LSD. The combination of thin layer chromatography with EASI-MS also demonstrated to be a relatively simple and powerful screening tool for forensic analysis of street drugs.

KEYWORDS: forensic science, criminalistics, lysergic acid diethylamide, LSD, 9,10-dihydro-LSD, illicit drug, ambient mass spectrometry, EASI-MS

LSD is a generic name for the hallucinogen lysergic acid diethylamide. Discovered by Dr. Albert Hofmann in 1938, LSD is one of the most potent mind-altering chemicals. LSD generally comes in "blotter" form, small squares of paper that have been soaked in solutions of LSD. The use of the hallucinogen LSD is usually associated with people in their teens and 20s (1,2). Although the main hallucinogen found in seized blotters is LSD, other substances have already been identified in these matrices. They are 4-bromo-2,5-dimethoxyamphetamine (DOB) (3) and bromobenzodifuranylisopropylamine (bromo-Dragon-FLY, ABDF) (4). ABDF, for instance, is also an extremely potent hallucinogen with longer duration than LSD.

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In 2009, the Brazilian Federal Police identified the presence of a new compound in seized blotters: 9,10-dihydro-LSD, an uncontrolled substance (5). 9,10-dihydro-LSD (with a nominal mass of 325 Da) displays a single difference in relation to the LSD structure (nominal mass: 323 Da) as the 9,10 double bond of the LSD molecule has undergone a hydrogenation reaction, producing 9,10-dihydro-LSD, as shown in Fig. 1. Clare (6) studied the dependence of activity of a series of hallucinogenic tryptamines as a function of parameters such as lipophilicity, amine nitrogen substituents, and orientation of nodes of occupied π -like orbitals. The author reports that 9,10-dihydro-LSD is an inactive substance, different from LSD, which is a potent hallucinogen. The results



FIG. 1-The structures of LSD and 9,10-dihydro-LSD.

obtained for 9,10-dihydro-LSD are consistent with other tryptamines studied.

Recently, a new class of ionization techniques for ambient mass spectrometry (MS) (7-18) has been developed. These techniques allow desorption, ionization, and MS characterization of analytes directly from their natural surfaces and matrices (19) in an open atmosphere with no or little sample workup, becoming therefore an attractive tool for direct characterization of street LSD blotter samples. Among these techniques, easy ambient sonic-spray ionization (EASI) is one of the simplest, gentlest, and most easily implemented (20). An EASI source can be constructed and installed in a few minutes from simple parts encountered in MS laboratories (Fig. 2) and can also be operated with self-pumping provided by the Venturi effect (21). EASI relies on the force of a high-velocity stream of N₂ (or even air) to accomplish analyte desorption and ionization by sonic-spray ionization (22). EASI has already been successfully tested with different analytes in different matrices and in various forensic applications such as the aging of ink writings on paper surfaces (23), authenticity of perfumes (24), identification of fake banknote (25) and vehicle documents (26), and analyses of m-CPP and ecstasy tablets (27). EASI has also been coupled to thin layer chromatography (TLC) (28) to study the presence of 3,4methylenedioxymethamphetamine (MDMA), methamphetamine, 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), amphetamine, caffeine, and lidocaine in street ecstasy tablets.

In this work, EASI-MS was first used to characterize LSD and 9,10-dihydro-LSD compositions directly from the surface of seized blotter samples. Then, EASI-MS was coupled to TLC and tested. To verify the validity of the results from the EASI-MS and TLC-EASI-MS techniques, the presence of LSD in the seized blotter samples was also confirmed via high-performance liquid chromatography with ultraviolet detector (HPLC-UV).

Methods and Materials

Samples and Reagents

HPLC and PA grade methanol (CH₃OH), chloroform (CHCl₃), acetone (CH₃COCH₃), and formic acid (HCOOH) were



FIG. 2—Schematic representation of the EASI-MS system in operation on a solid surface. The sonic spray produces a bipolar stream of very minute charged droplets (spray) that bombards the solid surface causing desorption and ionization of the analyte molecules from the target spots (dots). Analytes are often ionized as $[M + H]^+$ or $[M - H]^-$, or both. EASI is assisted only by compressed nitrogen or air and causes no oxidation, electrical discharge, or heating interferences.

purchased from Merck (Darmstad, Germany) and Burdick and Jackson (Muskegon, MI). Seized blotter samples (n = 41) were provided by the Rio de Janeiro and Minas Gerais State Civil Policies as well as by the Brazilian Federal Police. LSD standard solution (1 mg/mL) was purchased from Radian (Austin, TX).

EASI-MS

The experiments were conducted on a single quadrupole (2010EV; Shimadzu Corporation, Kyoto, Japan) and on an ion trap (LCQ Fleet; Thermo Scientific, San Jose, CA) mass spectrometers. Both instruments operated in the positive ion mode and were equipped with a homemade EASI source. Acidified methanol (0.1% in volume of formic acid) at a flow rate of 20 μ L/min and compressed N₂ at a pressure of 100 psi were used to form the sonic spray. The entrance angle of the capillary to the sample surface was *c*. 45°. Each sample was directly analyzed by EASI-MS, without any preparation. Mass spectra were collected from each blotter surface for about 10 sec.

To confirm the LSD and 9,10-dihydro-LSD structures, data was also collected in a Fourier Transform-Ion Cyclotron Resonance Mass Spectrometer (FT-ICR MS; ThermoScientific, Bremen, Germany). The high mass resolving power $(m/\Delta m_{1/2} >$ 400000, in which $\Delta m_{1/2}$ is the peak width at the half-maximum height) and the mass accuracy (better than 1 ppm) of the FT-ICR MS instrument allow for the assignment of a unique elemental composition to each peak in the mass spectra. The FT-ICR MS data were acquired in the positive ion mode, whereas external frequency-to-m/z calibration were performed over the 190 < m/z < 2000 range using a solution provided by the manufacturer (all calibrations furnished errors lower than 0.5 ppm). All ionic species were found to be singly charged, as evidenced by the c. 1 Da spacing between the monoisotopic species and the corresponding nuclides containing one ¹³C atom. Mass spectra were accumulated over 100 microscans, centered and aligned using the Xcalibur 2.0 software (ThermoScientific, Germany).

TLC Coupled to EASI-MS

Precoated plates (silica gel 60 GF 254; Merck) were used. These plates were dried for 30 min at 80°C and then stored in a desiccator. The blotters were cut into pieces of c. 10 mg. Each blotter piece was extracted with 10 mL of methanol for 10 min under stirring. A volume of c. 3 µL of each final extract was carefully applied to the TLC plates, which were developed in a horizontal chamber (CAMAG HPTLC, Muttenz, Switzerland). The total developing distance was 8 cm. Two different solvent systems were tested as eluents: CHCl₃/CH₃OH (90/10 v/v%) and CHCl₃/CH₃COCH₃ (20/80 v/v%). After development, the plates were dried at 100°C for 15 min. Spots were detected under ultraviolet (UV) radiation at 254 and 365 nm. Each TLC spot was directly analyzed by EASI-MS, without any sample preparation. Mass spectra were collected on each spot for about 10 sec. The limit of detection (LOD) of LSD deposited on the TLC plates was set as the minimum concentration that could be visualized by UV with an acceptable level of precision (<15%) and accuracy (±15%) for 10 replicates.

HPLC-UV

To confirm the results arising from EASI(+)-MS and TLC-EASI(+)-MS analysis, that is, the presence or absence of

LSD in the seized blotters, additional analysis were performed by using a liquid chromatograph coupled with a UV detector (LC-10AD; Shimadzu Corporation). LSD was extracted from the blotters using 2 mL of a mixture of methanol/water 50/50 (v/v%) during 5 min. An ultrasonic bath was used to improve the extraction efficiency. This procedure was repeated five times and the extracts were combined to yield a final volume of about 10 mL. Finally, a volume of 50 μ L of each final extract was injected into the liquid chromatograph at room temperature.

Results and Discussion

EASI(+)-MS Analysis: Chemical Profiles of the Seized Blotters

Initially, the chemical profiles of 41 seized blotters were obtained via EASI(+)-MS (by employing a monoquadrupole or an ion trap mass spectrometer—see further details in the previous section) directly from the surface of each sample. Among the samples analyzed, 28 showed positive results for LSD, whereas 9,10-dihydro-LSD was exclusively detected in seven (this compound has not been included in lists of controlled psychotropic substances). In six samples, a negative detection was verified for both compounds. All the findings, that is, the presence or absence of LSD in the seized blotters, were confirmed by the results arising from the HPLC-UV analysis.

In the EASI(+)-MS displayed in Fig. 3a, which typifies the 28 seized blotters containing LSD, the preponderant presence of the ions of *m/z* 304, 324, 399, 421, 540, and 556 is verified. LSD was identified as $[LSD + H]^+$ of m/z 324, whereas cocaine, found in only five of 28 samples, was detected as $\left[\operatorname{cocaine} + H\right]^+$ of m/z 304. To confirm further these assumptions, EASI(+)-MS were obtained for the 28 seized blotters using an ultrahigh resolution and mass accuracy mass spectrometer (see, i.e., Fig. 3b that shows an expanded vision of a typical EASI(+)-FT-ICR MS, ranging from 324.00 to 326.00). Hence, excellent agreements between the experimental and calculated m/z values for $[LSD + H]^+$ and $[cocaine + H]^+$, with relative errors less than 0.650 ppm, were achieved for both species in all samples. The other ions detected in Fig. 3a (of m/z 399, 421, 540, and 556) could not be associated with other ergot-type alkaloids that could also be present in the blotters such as ergovaline ([M+H]⁺: m/z 534), ergotamine ([M+H]⁺: m/z 582), ergocornine ([M+H]⁺: m/z 562), ergocryptine ([M+H]⁺: m/z 576), and ergocrystine ($[M+H]^+$: m/z 610) (29). These species are probably related to impurities or pigments usually employed in the blotter manufacturing process.

Figure 4*a* shows a typical EASI(+)-MS for the blotter samples (a total of seven) containing 9,10-dihydro-LSD, which was detected in its protonated form of m/z 326. Again, high resolution and accurate mass measurements recorded for these samples revealed an excellent agreement between the measured and calculated m/z values for [9,10-dihydro-LSD + H]⁺, with relative errors smaller than 0.768 ppm (see, i.e., Fig. 4*b* that shows an expanded region of a typical EASI(+)-FT-ICR MS, ranging from 326.10 to 327.30). In the latter EASI(+)-FT-ICR MS, besides [9,10-dihydro-LSD + H]⁺ (of m/z 326.2229), note the presence of another ion of m/z 326.1760 (ascribed to be the protonated form of a compound with the same nominal mass than 9,10-dihydro-LSD). In principle, the emergence of such an ion was thought to be due to the presence of ergonovine (an ergot-type alkaloid) in this seized blotter. This first



FIG. 3—Mass spectrum of seized LSD blotters obtained via: (a) EASI(+)-MS (low-resolution mass spectrometer over a 50 < m/z < 700 range); (b) EASI(+)-FT-ICR MS (ultrahigh resolution and mass accuracy FT-ICR mass spectrometer over a range from 324.00 to 326.00); and (c) product ion mass spectrum (MS/MS) of [LSD + H]⁺ (of m/z 324).

assumption, however, was not confirmed because of the high error (69 ppm) between the measured (326.1760) and theoretical (326.1988) m/z values for protonated ergonovine. Finally, note that an important result emerges when one has access to an FT-ICR MS: the detection of false-positive results can be dramatically reduced or even eliminated.

Figures 3*c* and 4*c* display the product ion mass spectra of $[LSD + H]^+$ (of *m/z* 324) and [9,10-dihydro-LSD + H]⁺ (of *m/z* 326), respectively. These results indicate that both ions fragment in a similar way. Hence, the fragment ions of *m/z* 251 (from $[LSD + H]^+$) and 253 (from [9,10-dihydro-LSD + H]⁺) are formed via the loss of a diethylamine molecule (C₄H₁₁N).



FIG. 4—Mass spectrum of seized blotters containing 9,10-dihydro-LSD obtained via: (a) EASI(+)-MS (low-resolution mass spectrometer over a 50 < m/z < 700 range); (b) EASI(+)-FT-ICR MS (ultrahigh resolution and mass accuracy FT-ICR mass spectrometer over a range from 326.10 to 327.30); and (c) product ion mass spectrum (MS/MS) of [9,10-dihydro-LSD + H]⁺ (of m/z 326).

Furthermore, both fragment ions dissociate further (via loss of CO) to yield the product ions of m/z 223 and 225, respectively. Note that in these fragmentation routes, the lysergic ring moieties of LSD and 9,10-dihydro-LSD remain intact. The hydrogenation of the C9–C10 double bond at the lysergic ring of 9,10-dihydro-LSD can thus be firmly established owing to the difference of 2 units between the main fragments arising from the dissociation of such protonated molecules.

For the six samples that showed negative results for both LSD and 9,10-dihydro-LSD, EASI(+)-MS generally detected main ions of m/z 291 and 435. As previously verified for the

previous blotters, the structure of these ions could not be determined.

TLC-EASI(+)-MS Analysis

TLC is a simple, low-cost, versatile, and popular technique used widely in forensic screening of illicit drugs, but may lead to false positives or erroneous attributions owing to limited resolution and lack of an undisputable and selective method for structural characterization, particularly for unexpected components. However, when combined with EASI-MS, such limitations can be easily overcome. Hence, TLC separation of the soluble constituents of eight common seized blotters samples containing LSD (blotters 2, 3, 4, 6, 7, and 8) and 9,10-dihydro-LSD (blotters 1 and 5) as well as standard LSD were evaluated using two different eluents: CHCl₃/CH₃OH (90:10) v/v% (Fig. 5a) and CHCl₃/CH₃COCH₃ (20:80) v/v% (Fig. 5b). The best resolution was achieved by using the latter eluent ($R_{\rm f} = 0.44$ for 9,10-dihydro-LSD and $R_{\rm f} = 0.69$ for LSD). For the first eluent, on the other hand, the R_f values for 9,10-dihydro-LSD and LSD showed to be undesirably close ($R_f = 0.78$ and 0.83, respectively).

Figure 6a-c show the "on-spot" EASI(+)-MS acquired directly from the surface of the TLC spots of blotters 1 and 2 and for the LSD standard. The unambiguous characterization of each drug is evident, mostly as a single ion (which facilitates spectra interpretation and analyte characterization) corresponding to their protonated molecules, $[M + H]^+$. It is also clear that even when two components elute with very close R_f values (as exemplified in Fig. 5*a*), which could hamper an unambiguous identification via single TLC, the application of EASI(+)-MS directly on each spot could promptly and reliably characterize



FIG. 5—TLC data for LSD standard solution and eight common seized blotters using as eluents: (a) $CHCl_3/CH_3OH$ (90:10) v/v% and (b) $CHCl_3/CH_3COCH_3$ (20:80) v/v%. Spots developed by UV are represented by gray ellipses.



FIG. 6—EASI(+)-MS collected directly on the surface of the TLC spots corresponding to (a) blotter 1; (b) blotter 2; and (c) LSD standard samples.

them. Finally, the LOD of LSD as evaluated for TLC and found to be of 0.1 μ g per blotter at 365 nm and 0.5 μ g per blotter, at 254 nm.

Conclusions

EASI-MS and TLC-EASI-MS showed to be viable complementary techniques to TLC for the screening of street drug samples of LSD, with sensitivity comparable to that of HPLC-UV. Both techniques provides a relatively simple, fast, and unequivocal screening tool to detect LSD and 9,10-dihydro-LSD in seized blotter samples. Additional confirmation can be obtained via MS/MS and FT-ICR MS measurements. The coupling of TLC with EASI-MS seems to provide therefore a most valuable tool in forensic investigations. TLC can first be used as a simple screening tool for specific target drugs and then, for the positive samples, on-spot EASI-MS analysis can be performed as an undisputable confirmation tool.

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