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Review

Chromatographic and mass spectrometric methods for determination of lysergic acid diethylamide (LSD) and metabolites in body fluids

Chad C. Nelson and Rodger L. Foltz

Northwest Toxicology, Inc., Salt Lake City, UT 84124-9906 (USA)

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ABSTRACT

Continued illicit use of the potent psychedelic drug lysergic acid diethylamide (LSD) has stimulated efforts to develop effective analytical methods for detection of the drug and its metabolites in body fluids from suspected LSD users. Recently reported methods based on gas and liquid chromatography, combined with single- and multiple-stage mass spectral analysis, now permit accurate detection and quantitation of LSD at sub-nanogram/milliliter concentrations.

CONTENTS

LIST OF ABBREVIATIONS

BSTFA	Bis(trimethylsilyl)trifluoroacetamide
CI	Chemical ionization
CID	Collision-induced dissociation
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
GC–MS–MS	Gas chromatography–tandem mass spectrometry
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography

Correspondence to: Dr. R. L. Foltz, Northwest Toxicology, Inc., Salt Lake City, UT 84124-9906, USA.

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LAMPA	Lysergic acid methylpropylamide
LC	Liquid chromatography
LC–MS	Liquid chromatography–mass spectrometry
LC–MS–MS	Liquid chromatography–tandem mass spectrometry
LSD	Lysergic acid diethylamide
MS	Mass spectrometry
MS–MS	Tandem mass spectrometry
NICI	Negative-ion chemical ionization
RIA	Radioimmunoassay
TFA	Trifluoroacetyl
TLC	Thin-layer chromatography
TMS	Trimethylsilyl

1. INTRODUCTION

Contrary to a widespread public perception that use of lysergic acid diethylamide (LSD) is no longer a societal problem, there is considerable evidence that this illicit drug continues to be used, and in some segments of the population its use is increasing [1]. According to the Home Office Forensic Science Service in Aldermaston, England, seizures of LSD have steadily increased since mid-1988 [2]; in the United States, seizures of LSD by the Drug Enforcement Agency doubled in 1990 over the previous year. Further cause for concern are reports that LSD is particularly popular among adolescents; in some areas it exceeds cocaine in popularity [3]. Factors that have contributed to its continued use are wide availability, low cost, and the difficulty of detecting LSD use by analysis of body fluids.

LSD is not considered highly toxic, although at least two cases where death was apparently a result of LSD toxicity have been reported [4,5]. However, the major reason many consider LSD to be highly dangerous is that it can have serious psychological and psychotic effects which sometimes cause users to commit irrational acts resulting in injury or death.

Conclusive identification of LSD in body fluids is a challenging analytical task. The usual oral dose of LSD is only 20–80 μg [3], and the drug is extensively metabolized, primarily to as yet unidentified metabolites. Furthermore, LSD is sensitive to ultraviolet light and elevated temperatures, and it is relatively unstable in solution at pH values below 4 [6]. However, the instability of LSD is not a major analytical problem if reasonable precautions are taken in the handling and storage of standards and specimens containing the drug. A far greater problem for analytical laboratories is the strong tendency for LSD and derivatized LSD to undergo adsorptive losses when subjected to gas chromatography (GC); this behavior often prevents detection of the drug at the sub-nanogram/milliliter concentrations normally encountered in body fluids from LSD users.

Illicit LSD is most frequently distributed as impregnated paper squares or microdot tablets [7]. LSD does not occur in nature but is easily prepared from ergotamine and related ergot alkaloids [8]. Iso-LSD, a non-psychoactive diastereoisomer of LSD, is often present in illicit preparations. Interconversion of the two isomers, which differ in their configuration at the carbon-8 position (Fig. 1), can occur under alkaline conditions. Assays for detection of LSD use by analysis of body fluids should be capable of detecting both isomers.

The primary purpose of this review is to describe the advances in chromatographic methods for analysis of LSD and its known metabolites that have occurred since the last review of the subject [9]. Current knowledge of the metabolism and pharmacokinetics of LSD is also summarized because an understanding of these processes is needed for interpreting the significance of measured concentrations of the drug.

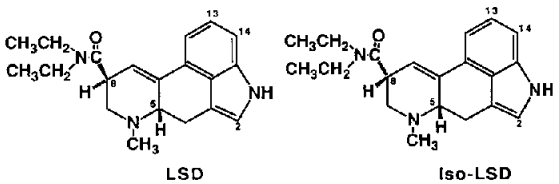

Fig. 1. Structures of LSD and iso-LSD.

2. METABOLISM AND PHARMACOKINETICS OF LSD

Because of the legal and ethical restrictions on administration of LSD to humans, current knowledge of how the drug is distributed, metabolized, and excreted in man is quite limited [9]. Plasma half-lives from 2.9 h [10] to 5.1 h [11] have been reported. N-Demethyl-LSD is normally present in the urine from LSD users, and preliminary evidence for urinary excretion of 13- and 14-hydroxy-LSD as conjugates has been presented [9,12]. Evidence for the remaining LSD metabolites shown in Fig. 2 comes from studies involving laboratory animals.

Two research groups have reported investigations of the metabolism of LSD by the rhesus monkey. Siddik *et al.* [13] found that 39% of an intramuscularly administered dose (150 μg/kg) of radiolabelled LSD was excreted in the urine within 48 h; 15–19% was excreted in the feces. Metabolites identified in the urine included: desethyl-LSD (1% of the dose) and the glucuronide conjugates of 13-hydroxy-LSD (2%) and 14-hydroxy-LSD (3%). Unchanged LSD accounted for 1–1.6% of the dose. At least five other radioactive spots were detected by thin-layer chromatography (TLC) but were not identified. The spot showing the greatest radioactivity (11% of the dose) gave a color reaction that was characteristic of a compound possessing a primary aromatic amino group.

Sullivan *et al.* [14] performed a similar study, but obtained significantly different results. After oral administration of radiolabelled LSD to male rhesus monkeys, 24% of the radioactivity appeared in the urine during the first 48 h, and none was detected in the feces. Analysis of the monkeys' urine by high-performance liquid chromatography (HPLC) with radio-detection showed seven chromatographic peaks, five of which also exhibited cross-reactivity in a radioimmunoassay (RIA) for LSD. However, the metabolic products were not further identified.

In all species investigated, including man, LSD is extensively metabolized so that a very small percentage of the dose is excreted in the urine unchanged. Following oral administration of a 1 μg/kg dose to a human volunteer, the unchanged

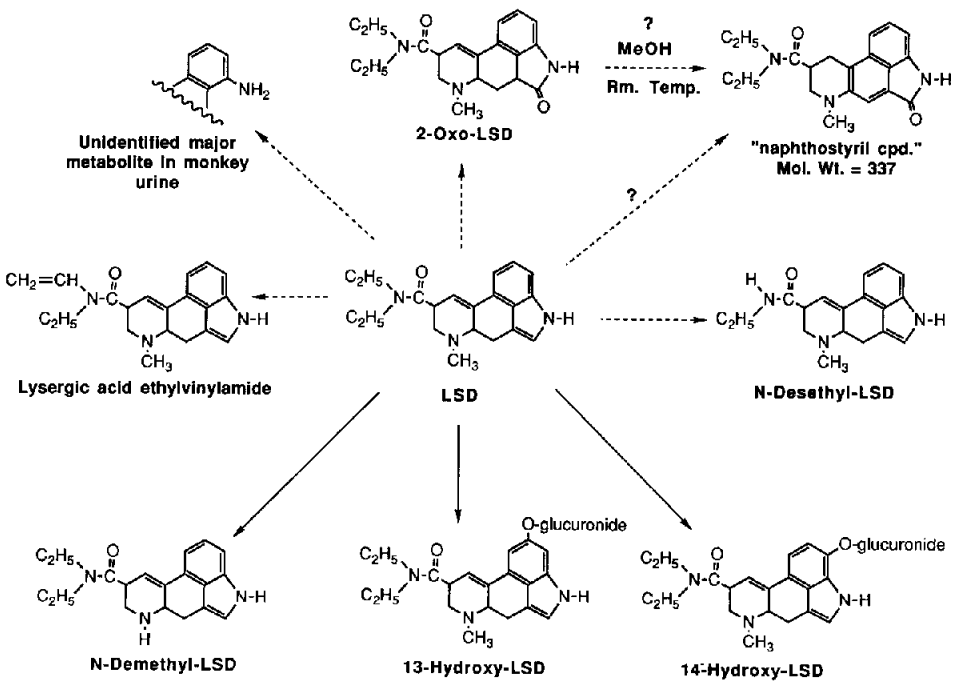

Fig. 2. Metabolites of LSD. Solid arrows indicate metabolic pathways identified in man.

LSD excreted in the urine amounted to 0.9% of the dose [12]. The amount of N-demethyl-LSD excreted in the urine was slightly higher (1.2% of the dose). The concentrations of LSD in plasma and in urine from this subject are plotted against time in Figs. 3 and 4, respectively. Other studies involving administration of known doses of LSD to human subjects have reported higher peak concentrations of LSD in plasma and urine [10,15]. Nevertheless, in most instances of LSD ingestion, the concentrations of the drug in plasma and in urine are likely to fall below 1 ng/ml within a few hours. Thus, attempts to detect use of the drug by analysis of body fluids must employ very sensitive and specific assays.

3. METHODS OF ANALYSIS FOR LSD

Commercial RIAs for LSD are available from several sources (Abuscreen, Roche Diagnostics Systems, Nutley, NJ, USA; Coat-a-Count, Diagnostic Products, Los Angeles, CA, USA) and they serve as a useful and relatively inexpensive method of screening for the presence of the drug. However, RIAs are not totally specific for LSD, so that a RIA-positive specimen should always be confirmed by a second and more specific assay if the results of the analysis can have punitive consequences. The manufacturers' recommended cut-off concentration for considering a sample positive for LSD is 0.5 ng/ml, although lower cut-offs have been used in investigations where legal consequences were not a concern [16,17]. The actual concentration of LSD in RIA-positive

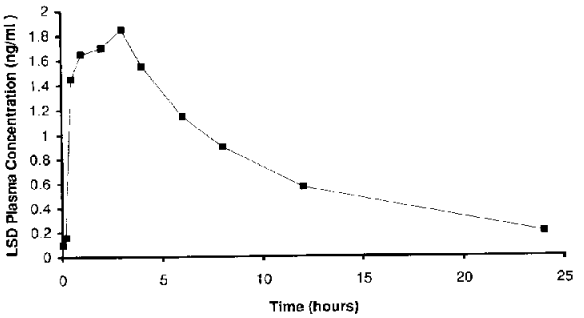

Fig. 3. Measured concentrations of LSD in plasma following oral administration of a 1 μg/kg dose to a male volunteer.

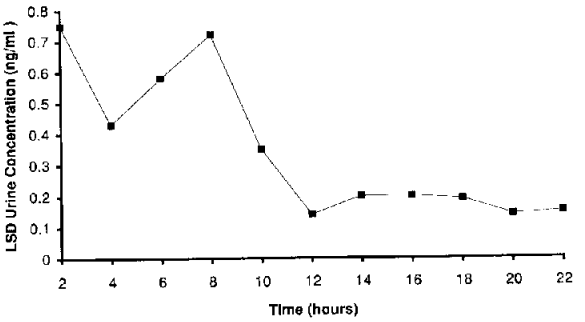

Fig. 4. Measured concentrations of LSD in urine following oral administration of a 1 μg/kg dose to a male volunteer.

urine specimens is generally lower than that indicated by the RIA, and often considerably lower [18,19]. Presumably the higher concentrations indicated by RIA are due to the cross-reactivity of LSD metabolites to the RIA antisera, but this conclusion cannot be substantiated until the major LSD metabolites in urine have been identified and their cross-reactivities determined.

To date, the only methods that have proven useful for the confirmation of LSD in body fluids have been based on a chromatographic separation and detection by either fluorescence emission or mass spectrometry (MS). To be an effective method of confirming the presence of LSD in urine, an assay should be capable of detecting and measuring LSD concentrations well below the RIA cut-off of 0.5 ng/ml. Support for this statement comes from the experience of the US Department of Defense in testing for LSD in urine from military personnel. Their current procedures include an initial RIA with a 0.5 ng/ml cut-off, followed by a GC–MS analysis of all RIA-positive specimens. To be confirmed positive for LSD a urine specimen must be shown by GC–MS to contain an LSD concentration greater than 0.4 ng/ml. However, with the current confirmation cut-off a majority of the RIA-positive urine specimens cannot be confirmed.

In addition to high sensitivity, a reliable LSD confirmation method must be extremely specific. This is because it is often very difficult to totally separate an analyte, at the sub-nanogram/milliliter concentration in a biological matrix, from interfering compounds by conventional chromatographic techniques.

3.1. Methods based on fluorescence detection

LSD's native fluorescence has been effectively exploited in numerous published LSD assays employing either TLC [18,20–25] or HPLC [7,8,16,19,20,22,26–32]. An instrumental high-performance thin-layer chromatographic (HPTLC) method for detection and quantitation of LSD in urine was recently described [18]. After addition of methysergide as the internal standard, the urine samples were made basic and extracted into petroleum ether–dichloromethane–isoamyl alcohol (70:30:0.5). The organic extracts were washed with 0.1 *M* ammonium hydroxide, evaporated to dryness and reconstituted in dichloromethane–isopropyl alcohol (19:1). The extracts were then spotted onto a Whatman 10 cm × 10 cm LHP-K high-performance silica gel plate with a layer thickness of 200 μm. The plate was initially developed with ethyl acetate to remove interfering substances and then redeveloped with chloroform–methyl alcohol (90:10). Fluorimetric measurement of LSD and methysergide was performed with a scanning densitometer at an excitation wavelength of 313 nm and a 320–400 nm emission wavelength filter. Further characterization of the LSD spots was achieved by spraying the plates with a reagent consisting of 2.5 g of *p*-dimethylaminobenzaldehyde dissolved in a mixture of 225 ml of ethyl alcohol and 25 ml of concentrated hydrochloric acid. This procedure caused the LSD (R_F = 0.53) and methysergide (R_F = 0.51) spots to turn blue against a yellow background. In spite of the similar R_F values, the LSD and methysergide could be distinguished due to the resolving power of the HPTLC system. Replicate analysis of urine control samples containing 1 ng/ml LSD gave a coefficient of variation of 10%. The assay's limit of detection, based on analysis of blank urine samples, was 0.4 ng/ml.

Concentrations of LSD in 48 clinical and forensic specimens were determined by the HPTLC method and compared to the concentrations determined by commercial RIAs. The quantitative agreement between the two methods was generally poor, but the concentration determined by RIA was nearly always greater than that determined by the HPTLC method.

A recent publication describes the application of HPLC with fluorescence detection for the confirmation of LSD in serum and urine samples from 31 emergency-room patients suspected of LSD intoxication [16]. The HPLC procedure was the same as that described by Francom *et al.* [19] except that methysergide was used as the internal standard rather than lysergol. The cited method employed an Ultrasphere ODS column (5 μm particle size) and elution with a solvent system prepared by adding 0.25% triethylamine to a mixture of 0.1 *M* ammonium acetate buffer (pH 8.0)–acetonitrile (70:30). The fluorescence emission was monitored at 420 nm with the excitation wavelength set at 330 nm. McCarron *et al.* [16] reported the method to be capable of detecting LSD concentrations as low as 0.2 ng/ml, but 0.5 ng/ml was suggested as a more reliable detection threshold due to the frequent occurrence of low-level interferences. Each of the samples was also analyzed by RIA using a detection threshold of 0.1 ng/ml. The HPLC method was successful in confirming nine of thirteen RIA-positive serum samples, and eleven of thirteen RIA-positive urine samples. Measured concentrations of LSD in serum ranged from 0.5 to 1.9 ng/ml and in urine from 0.2 to 7.7 ng/ml. However, since all of the cases involved emergency-room patients, it is likely that the quantities of drug ingested were higher than usual.

HPLC was used to confirm the presence of LSD in body fluids from forensic cases handled by the Metropolitan Police Forensic Science Laboratory in London [33]. The HPLC procedure was essentially the same as previously reported [4]; a 24 cm × 4.9 mm I.D. Spherisorb S5W column was eluted with 0.01 *M* ammonium perchlorate in methanol adjusted to pH 6.7 with 0.1 *M* sodium hydroxide in methanol. The fluorescence excitation wavelength was 308 nm and the emission was measured through a 370–700 nm filter. RIA was used for initial detection and quantitation of the LSD, but had a limit of detection for blood samples of only 2.8 ng/ml. The same report includes a summary of previously

published data on LSD concentrations in human body fluids. However, the authors caution that the methods used to obtain the early data were subject to gross quantitative inaccuracies.

3.2. Analysis for LSD in confiscated illicit drug samples

Although this review is primarily concerned with recently reported methods for determination of LSD in body fluids, several recent publications describe the application of HPLC, TLC, GC and GC–MS methods for identification of LSD in samples obtained in illicit drug seizures [7,8,34]. These reports contain chromatographic and MS data that are potentially useful in the determination of LSD in physiological specimens and are therefore summarized here. Because the concentrations of LSD in illicit drug samples are generally high relative to the concentrations of LSD in body fluids from users, significantly less sensitive methods can be used for analysis of the confiscated samples. However, it is important to be able to distinguish LSD from isomers and other ergot alkaloid-derived compounds.

LSD and fifteen compounds isomeric with LSD have been fully characterized as to their TLC R_F values, HPLC and GC relative retention times, and their electron ionization mass spectra [8]. TLC analyses were performed on commercial glass plates with 250-μm coatings of silica gel G. Developing solvents consisted of either acetone or chloroform–methanol (9:1). For conclusive identification of LSD the authors suggested that development be performed in both solvent systems, and that samples containing LSD be heated in alcoholic potassium hydroxide, which causes partial isomerization to iso-LSD and subsequent appearance of spots corresponding to both LSD and iso-LSD. For separation of LSD isomers by HPLC, a 25 cm × 4.6 mm I.D. column packed with 5-μm Supelcosil LC-18 was used with UV detection at 309 nm. The elution solvent consisted of 40 parts of methanol and 60 parts of a phosphate buffer prepared by addition of 10 ml of phosphoric acid to 1 l of water. Although complete resolution of all of the LSD isomers was not achieved, this system did permit differentiation of LSD from all but one isomer. For GC analysis of LSD isomers, a 12 m × 0.32 mm I.D. fused-silica capillary with a 0.52-μm-thick film of OV-1 was used. Samples were injected with a split ratio of 25:1, an injector temperature of 270°C, an oven temperature of 260°C, and analyzed by either electron ionization MS or by flame ionization detection. All of the LSD isomers gave similar mass spectra with prominent molecular ions at m/z 323. However, the various isomers could be distinguished by the differences in their relative retention times and in their fragment ion intensities.

Investigators at the Home Office Forensic Science Laboratory in Aldermaston, England, analyzed illicit LSD preparations by capillary GC and by HPLC [7,34]. They extracted LSD from confiscated specimens by first cutting or crushing the specimen and vortex-mixing it with methanol–water (1:1). A small quantity of concentrated ammonia (about two drops) was then added along with 1 ml of methyl-*tert.*-butyl ether, and the mixture was subjected to ultrasonic agitation for 20 min. The ether layer was then removed and concentrated under a stream of nitrogen. Two HPLC systems were evaluated: a reversed-phase system consisting of a 16 cm × 5 mm I.D. column packed with 3-μm ODS-Hypersil and eluted with 60% methanol containing a phosphate buffer (pH 8.1), and a normal-phase system consisting of a 10 cm × 5 mm I.D. column packed with a 5-μm aminopropyl bonded phase (APS-Hypersil) and eluted with methyl-*tert.*-butyl ether–isopropanol (95:5). The normal-phase system did not resolve LSD from lysergic acid methylpropylamide (LAMPA), whereas nearly baseline separation could be achieved on the reversed-phase system, but only if the pH of the eluting solvent was raised to 11. The ergot compounds were detected either by UV absorption at 220 nm or by fluorescence emission at 400 nm with excitation at 312 nm. For GC analysis, underivatized extracts were injected split (20:1 ratio) at 275°C into a 25 m × 0.22 mm I.D. methylsilicone (BP1) fused-silica column (0.25 μm film thickness) operated isothermally at 275°C. GC

retention indices [7] and HPLC k' values [34] were determined for LSD and structurally related compounds.

3.3. Methods based on gas chromatography–mass spectrometry

For the analysis of LSD in body fluids it is necessary to employ a selective method of extraction and to derivatize the LSD before injection into a GC–MS system. Without derivatization LSD exhibits poor chromatographic characteristics unsuitable for detection and measurement at the sub-nanogram/milliliter concentrations most often encountered in body fluids from recent users of the drug. The first published GC–MS assay for LSD in urine consisted of a simple but selective *n*-butyl chloride extraction, conversion of LSD to its N-trimethylsilyl (TMS) derivative, and analysis by capillary GC and electron ionization MS [19]. The indole nitrogen is readily silylated by treatment with bis(trimethylsilyl)trifluoroacetamide (BSTFA). Although the TMS derivative is fairly stable in the presence of excess silylating agent, attempts to remove the BSTFA before injection into the GC–MS were unsuccessful. Fig. 5 shows the electron ionization mass spectrum of the TMS derivative of LSD. In this initial study, the derivatized extracts were injected splitless into a 12 m × 0.20 mm I.D. fused-silica column with a dimethylsilicone film thickness of 0.33 μm. The column was temperature programmed from 220 to 320°C at a rate of 20°C/min. The injection port and transfer lines were maintained at 250°C and helium served as the carrier gas at a linear velocity of 30 cm/s. Deuterium-labeled LSD was used as the internal standard. Selected-ion monitoring of the ion pairs at *m/z* 395 and 398, 293 and 296, and 268 and 271 permitted detection and quantitation of LSD in urine control samples at concentrations down to 0.5 ng/ml. Unfortunately, urine from actual LSD users often contained interfering compounds that prevented conclusive identification at concentrations below 2 ng/ml.

Paul *et al.* [6] at the Navy Drug Screening Laboratory in Norfolk, Virginia, were able to lower the limit of quantitation by modification of the extraction procedure and by substitution of LAMPA for deuterated LSD as the internal standard. After the initial *n*-butyl chloride extraction, either of two purification procedures was employed: (1) a back-extraction of the LSD into acid (phosphate buffer, pH 4.5), followed by basification with ammonium hydroxide and re-extraction into *n*-butyl chloride; or (2) solid-phase purification with cartridges containing a silica-based propylamine sorbent. The GC–MS analy-

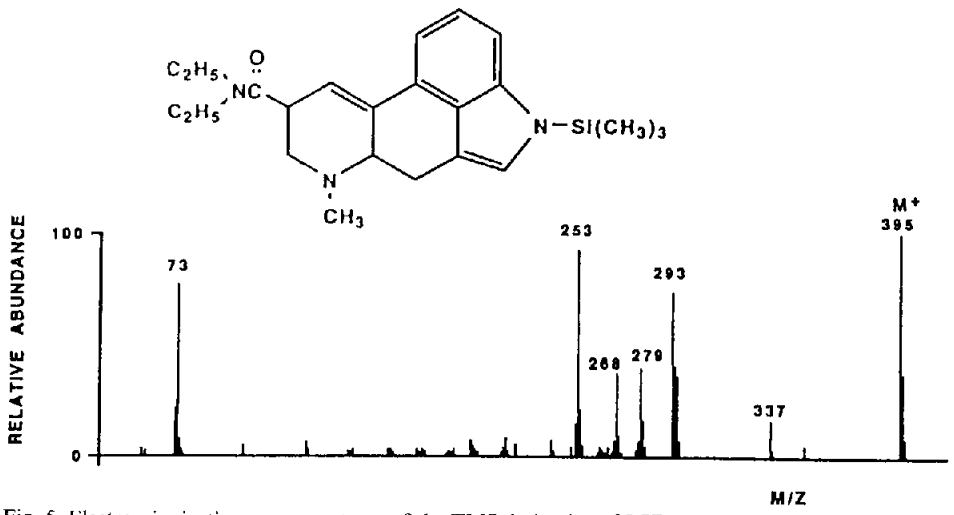

Fig. 5. Electron ionization mass spectrum of the TMS derivative of LSD.

ses were performed on a Hewlett-Packard mass-selective detector fitted with a 15 m × 0.25 mm I.D. DB-5 capillary column. The authors pointed out the importance of maintaining a well conditioned column, and recommended frequent conditioning by five successive injections of BSTFA or hexamethylene-disilazane, or, even better, four injections of a derivatized urine extract. LAMPA was preferred over deuterated LSD as the internal standard because it did not co-elute with LSD, and therefore permitted selected-ion monitoring of the three major ions (*m/z* 395, 293 and 253) for both compounds.

A further improvement in sensitivity was reported by researchers at the Center for Human Toxicology, University of Utah [12]. In their procedure, LSD and the N-demethyl metabolite are extracted with *n*-butyl chloride, derivatizated with trifluoroacetylimidazole in the presence of 1,4-dimethylpiperazine, and analyzed by GC and negative-ion chemical ionization (NICI). Under the derivatization conditions, LSD forms a mono-trifluoroacetyl (mono-TFA) derivative and N-demethyl-LSD forms a bis-TFA derivative, both of which are efficiently ionized by resonance capture of electrons within a chemical ionization (CI) source. The resulting mass spectrum for each compound consists of a single abundant ion corresponding to the molecular anion (M^-). This is ideal for optimum sensitivity, but does not provide the spectral detail generally considered necessary for conclusive identification.The GC–MS conditions consisted of splitless injection into a 12 m × 0.2 mm I.D. fused-silica column with a 0.3 μm film thickness, temperature programming from 160 to 300°C at 20°C/min, and NICI of the GC effluent. Hydrogen was used as the carrier gas at a linear velocity of 60 cm/s measured at 160°C. Methane was bled into the ion source as the reagent gas to give a total pressure of 90–107 mPa. The lowest concentrations of LSD and N-demethyl-LSD in urine that could be reliably measured were 50 and 30 pg/ml, respectively. This level of sensitivity permitted measurement of urinary concentrations of LSD for 24 h and N-demethyl-LSD for 60 h after oral administration of 70.5 μg of LSD to an experienced adult volunteer. Fig. 6 shows the selected-ion current profiles from the GC–NICI-MS analysis of an extract of enzyme-hydrolyzed urine collected 8–10 h after administration of the LSD. Both LAMPA and [$^2H_{10}$] LSD were added to the spec-

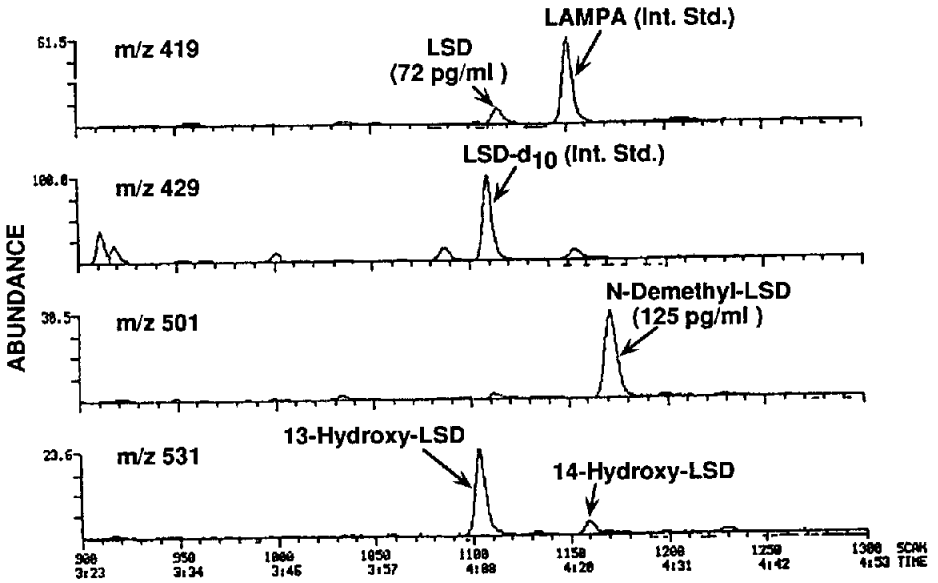

Fig. 6. Selected-ion current profiles from the GC–NICI-MS analysis of a hydrolyzed urine extract collected 8–10 h after oral administration of LSD.

imen as internal standards. The two peaks in the *m/z* 531 profile were attributed to the TFA derivatives of 13- and 14-hydroxy-LSD, although authentic standards were not available to confirm this identification. The hydroxylated LSD compounds appear to be minor metabolites in man and are excreted in the urine as glucuronide conjugates [12].

The extraction procedure used for the NICI assay was subsequently modified to permit analysis of LSD in plasma samples [11]. The modification consisted of an initial hexane extraction to remove some of the lipophilic material in the plasma. Even with this additional clean-up, the amount of cholesterol in the final extract was sufficient to cause chromatographic problems when an HP Ultra-1 capillary column was used for the analysis. However, with a more polar column (DB-5), cholesterol no longer co-eluted with LSD and did not interfere with the analysis.

3.4. Methods employing tandem mass spectrometry

Over the past several years tandem MS (MS–MS) has come to be recognized as an important technique for identification, structural characterization, and quantitation of drugs, metabolites, and other biomolecules [35–37]. Although an increasing number of applications of MS–MS are being reported in toxicology and pharmacology journals, relatively few laboratories that deal with routine types of analyses, such as laboratories concerned with the analysis of drugs of abuse, are currently employing MS–MS assays. However, as laboratories continue to require more sensitive and specific means of analysis, MS–MS technology will certainly become more commonplace.

MS–MS, as the name implies, involves two stages of mass-to-charge analysis. Following ionization, the first mass analyzer selectively transmits a single parent ion mass, which is often the molecular ion or protonated molecule. Typically, the selected parent ion is then activated by energetic collision with a neutral gas, in a process called collision-induced dissociation (CID). CID imparts internal energy, causing the parent ion to dissociate into daughter ions. The daughter ions are then subjected to a second stage of mass analysis. The process of transmitting ions through two mass analyzers does result in lower absolute ion intensities, as compared with analysis using a single-stage mass spectrometer. However, because chemical noise is greatly reduced, a higher signal-to-background ratio is often achievable with MS–MS.

The increased selectivity and specificity gained with MS–MS can be particularly important when one is dealing with complex sample matrices such as urine, blood, and other biological fluids and tissues. In addition, various MS–MS scan functions can serve as valuable tools for determining dissociation pathways based on parent-to-daughter ion transitions. This information is useful for structural determinations and can be a powerful means of identifying compounds with similar structures, such as drug metabolites [37,38].

A drawback of MS–MS analysis is the cost for equipment. Several configurations of MS–MS instruments are commercially available, including ion traps, triple quadrupoles, and various sector-type tandem mass spectrometers. These instruments are relatively expensive, with increasing cost in the fore-mentioned order. Also, a relatively high level of expertise is required for methods development and for operation of a tandem mass spectrometer, since more operational parameters need to be understood, controlled, and optimized.

Recently, several methods based on MS–MS have been reported for the analysis of LSD, iso-LSD and N-demethyl-LSD. These methods include GC–MS–MS and liquid chromatography (LC)–MS–MS performed on triple-stage quadrupole mass spectrometers. Two GC–MS MS assays developed at Northwest Toxicology in Salt Lake City rely on GC and either positive- or negative-ion chemical ionization in combination with MS–MS analysis to identify LSD, iso-LSD, and N-demethyl-LSD in urine or blood extracts [39]. During development of these assays, TMS and TFA derivatives of LSD were compared for relative CI efficiencies and overall daughter-ion

sensitivities. Methane and ammonia CI reagent gases were evaluated in both positive- and negative-ion modes for the production of protonated (MH^+) or negatively charged (M^-) molecular species, respectively; ammonia was found to produce MH^+ or M^- at a factor of three to five times greater intensity than methane.

The research at Northwest Toxicology included an investigation of the fragmentation pathways resulting from CID of protonated LSD and the protonated TMS derivative of LSD [39]. The CID spectrum of protonated LSD-TMS (MH^+ 396), shown in Fig. 7, indicates several characteristic daughter ions of LSD. For instance, ions at *m/z* 353, 295 and 280 correspond to direct eliminations of CH_2NCH_3, diethylformamide, and diethylamine, respectively, from the protonated molecule. Similar daughter ions were generated as a result of CID of the protonated isomers of LSD, iso-LSD and LAMPA (used as internal standard); however, relative daughter-ion intensities differed for these isomers. Fragmentation paths analogous to the dissociation of LSD were also observed for CID of protonated N-demethyl-LSD-TMS, such as loss of diethylformamide from MH^- (*m/z* 382) to yield a daughter ion at *m/z* 281. Selected-reaction monitoring of these daughter ions permitted the analysis of LSD, iso-LSD, N-demethyl-LSD, and LAMPA in a single GC–MS MS experiment. Fig. 8, for example, shows the ion current profiles resulting from positive-ion CI GC MS MS analysis of a urine containing 400 pg/ml each of LSD, iso-LSD, and LAMPA. With this GC–MS–MS assay LSD, iso-LSD, and structurally related compounds could be identified, with no interfering peaks, from extracts of complex biological matrices. Detection limits for LSD-TMS were determined to be 10 pg/ml for *m/z* 295 and 25 pg/ml for *m/z* 353 and 280; iso-LSD-TMS detection limits were 10 pg/ml for *m/z* 353 and 280, and 25 pg/ml for *m/z* 295. Selected-reaction monitoring of the daughter ion at *m/z* 281 from CID of protonated N-demethyl-LSD-TMS was less sensitive with a detection limit of 100 pg/ml.

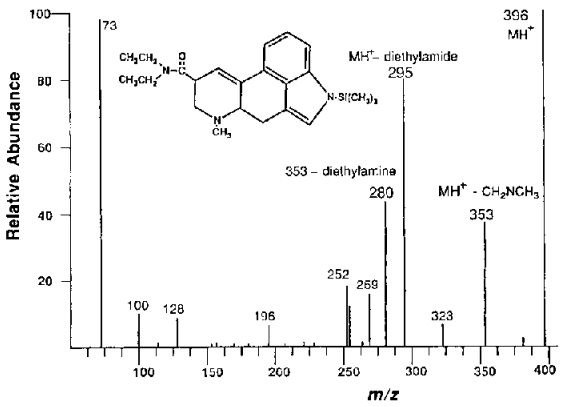

Fig. 7. Daugther ion spectrum resulting from CID of the protonated molecule of the TMS derivative of LSD.

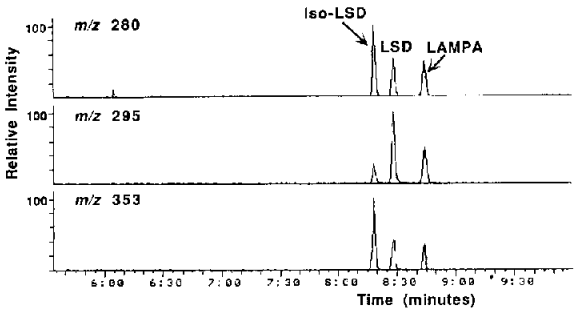

Fig. 8. Ion current profiles from the GC–MS–MS (positive-ion ammonia CI) of an extract of urine containing 400 pg/ml each of LSD, iso-LSD and LAMPA.

NICI of the TFA derivatives was significantly more sensitive (approximately ten times greater) for N-demethyl-LSD than for LSD or iso-LSD [39]. This difference was probably due to the substantially greater efficiency of NICI of the bis-TFA derivative of N-demethyl-LSD compared to the mono-TFA derivative of LSD. The CID spectra of M^- of LSD-TFA, iso-LSD-TFA, LAMPA-TFA, and N-demethyl-LSD-TFA showed elimination of TFA (97 mass units) as the principal CID product. GC–MS–MS analysis of N-demethyl-LSD-TFA allowed detection at a level of 10 pg/ml and quantitation down to 50 pg/ml in urine, whereas GC–MS–MS analysis for LSD-TFA was significantly less sensitive, and the limit of quantitation was only 500 pg/ml. Under the chromatographic conditions used in this study, the TFA derivatives of LSD and iso-LSD nearly co-eluted, making identification and quantitation inherently difficult in cases where iso-

LSD and LSD were both present in a sample. In contrast, the TMS derivatives of iso-LSD and LSD gave chromatographic peaks that were well separated (Fig. 8).

For both the positive- and negative-ion CI GC–MS–MS assays, urine specimens were prepared by extracting LSD into toluene–methylene chloride (7:3, v/v) from a basic solution of the sample. Extraction of plasma samples involved addition of acetonitrile, centrifugation and separation of the acetonitrile extract, evaporation and reconstitution of the extract residue into dilute hydrochloric acid, extraction into toluene methylene chloride (7:3, v/v), and finally washing the organic layer with 0.1 *M* ammonium hydroxide. Hemolyzed blood samples required an additional solid-phase extraction clean-up. TMS and TFA derivatives were prepared by reaction of the dried extract with BSTFA and trifluoroacetylimidazole, respectively, at 80°C for 30 min. The GC was operated in the splitless mode with an oven gradient from 175 to 298°C, using hydrogen as carrier gas. A Finnigan TSQ-70 MS–MS was operated in the selected-reaction monitoring mode using argon as collision gas with 35 eV collision energy (laboratory frame of reference).

The high level of specificity that can be achieved using MS–MS is illustrated in Fig. 9 which shows a comparison of selected-ion current profiles from analysis of a derivatized extract of a urine specimen from a suspected LSD user. Three methods of analysis are compared: (1) GC–MS with electron ionization and selected-ion monitoring of the ion current corresponding to the molecular ion (*m/z* 395); (2) GC–MS with ammonia positive-ion CI and monitoring of the ion current corresponding to the protonated molecule (*m/z* 396); and (3) GC–MS–MS with ammonia positive-ion CI and monitoring of the daughter ion (*m/z* 295) formed by CID of the protonated molecule (*m/z* 396). In this example, interfering peaks make it difficult to clearly identify the peaks due to LSD and LAMPA in the ion current profiles from GC–MS analysis with either method of ionization. In contrast, the only chromatographic peaks observed in the ion current profile from the GC–MS–MS analysis are

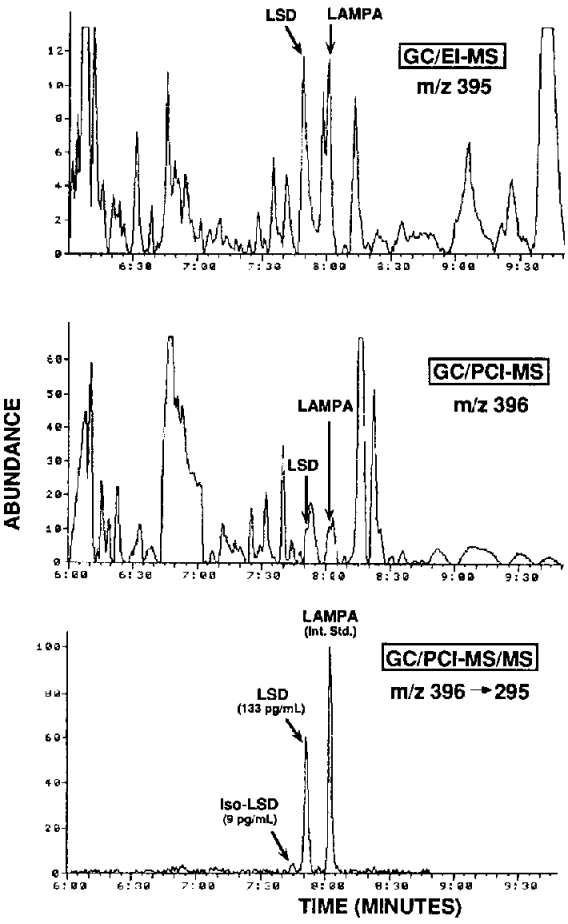

Fig. 9. Comparison of selected-ion current profiles from analysis of a derivatized extract of a urine specimen from a person suspected of using LSD. The urine extract was trimethylsilylated and analyzed by three different methods: (top) electron ionization with monitoring of the ion current corresponding to the molecular ion (*m/z* 395); (middle) ammonia positive-ion CI with monitoring of the ion current corresponding to the protonated molecule (*m/z* 396); (bottom) ammonia positive-ion CI with monitoring of the ion current corresponding to the daughter ion (*m/z* 295) resulting from CID of the protonated molecule (*m/z* 396).

due to iso-LSD, LSD, and LAMPA. The concentrations determined by the GC–MS–MS analysis were LSD, 133 pg/ml and iso-LSD, 9 pg/ml; LAMPA was added to the sample as the internal standard at a concentration of 400 pg/ml.

Duffin *et al.* [40] at Cornell University recently explored the use of both LC-MS and LC–MS–MS for the analysis of underivatized LSD in urine. The LC–MS analyses were performed on a

modified Hewlett-Packard mass-selective detector connected to a 1 mm I.D. HPLC column via an ion-spray interface (pneumatically assisted electrospray). Even though the protonated molecule of LSD was the primary ion formed within the ion spray interface, application of an additional voltage within the interface caused CID of the MH^+ to daughter ions. The resulting daughter ion mass spectrum was very similar to that obtained by CID analysis of LSD on a Sciex TAGA 6000E triple–quadrupole mass spectrometer system. However, lower concentrations of LSD could be detected in biological extracts with the MS–MS system due to its additional stage of mass analysis [41]. For LSD analyses performed on the triple–quadrupole, the chromatographic system consisted of a 600 MS pump (Waters Chromatography Division, Milford, MA, USA) connected to a 50 mm × 2.1 mm I.D. LC8-DB column (Supelco, Bellefonte, PA, USA) and operated in the isocratic mode with a flow-rate of 0.1 ml/min of 35:65 acetonitrile–water containing 5 m*M* ammonium formate. Under these conditions LSD and iso-LSD were well separated and detectable at urine concentrations as low as 50 pg/ml.

4. CONCLUSIONS

To date, only methods based on chromatographic separation and MS detection have provided the sensitivity and specificity required for identification and measurement of LSD in physiological specimens where the analytical results have the potential for legal and punitive consequences. Several GC–MS and GC–MS–MS assays have been developed and used effectively for the determination of LSD and LSD-related compounds in body fluids at sub nanogram/milliliter concentrations. However, laboratories considering implementing a GC–MS or GC–MS–MS assay for LSD in biological specimens should be aware of the difficulty of conducting these assays on a routine basis due to the very high sensitivity that needs to be maintained and the propensity for LSD to undergo adsorptive losses during analysis. These factors have made it very difficult to alternate analyses for LSD with analyses for other drugs on the same GC–MS system. RIAs and chromatographic methods employing fluorescence detection are generally less expensive and are often suitable for analysis of non-forensic specimens.

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