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Review

Recent advances in chromatographic and mass spectrometric methods for determination of LSD and its metabolites in physiological specimens

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Abstract

The detection of LSD use continues to be a challenge for toxicology laboratories due to the very low concentrations of LSD and its metabolites in body fluids. However, significant progress has been made in the development of more sensitive and specific analytical methods. Techniques that have proven particularly effective include: (1) immunoaffinity extraction, (2) gas chromatography coupled with chemical ionization and tandem mass spectrometric detection, and (3) liquid chromatography in combination with electrospray ionization and either single-stage or tandem mass spectrometric detection. In addition, a major metabolite of LSD, 2-oxo-3-hydroxy-LSD, has been identified and found to be present in far higher concentrations than LSD in most LSD-positive urine samples.

Keywords: Reviews; LSD

Contents

1. Introduction

Because lysergic acid diethylamide (LSD) continues to be a significant drug of abuse [1,2], particularly in United States and in Europe, efforts to find more effective analytical methods for detecting LSD use also continue. The purpose of this review is to provide an update of chromatographic and mass spectrometric methods for detection and measurement of LSD and its metabolites in physiological

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specimens. Two earlier reviews covered this topic through 1991 [3,4]. Analyses for LSD have also been discussed in two recent reviews [5,6].

Commercial immunoassays for LSD are in general use for screening body fluid samples for the presence of LSD [7–10]. They offer the advantage of speed and relatively low cost, but do not provide the specificity and quantitative accuracy of a well-designed assay based on the combination of chromatographic separation and mass spectrometric detection [11,12]. Also, LSD concentrations determined by an immunoassay are generally substantially higher than concentrations determined by mass spectrometric methods in the same samples, presumably due to cross-reactivity of the antibodies with LSD metabolites [10,13].

The major difficulty in identifying an LSD user by analysis of body fluids is the very low concentration of LSD in blood and urine following ingestion of the drug. Within the US Department of Defense drug-testing program, a urine sample must be found to contain a concentration of 200 pg/ml or more of LSD to be reported as positive for LSD. Following ingestion of a typical ''street dose'' of LSD consisting of approximately 50 μg of drug, the concentration of LSD in urine will normally drop below the 200 pg/ml cutoff within 12 to 24 h. In order to increase the time window for detection of LSD use by urinalysis, it is necessary either to develop analytical methods capable of reliably detecting and measuring LSD at substantially lower concentrations, or to identify a metabolite that is excreted in urine at higher concentrations for a longer time period. Fortunately, significant progress has been made in both areas.

2. Stability of LSD

Comments in the scientific literature regarding the stability of LSD in solution and in biological samples have been inconsistent and have led to uncertainty as to how LSD samples should be handled and stored. For example, LSD is frequently reported to be light sensitive, but the rate of photodegradation is strongly dependent on the wavelength composition of the light. LSD concentrations in urine stored in polyethylene bottles at room temperature and exposed to normal incandescent room light varied by no more than 10% over a period of four weeks [14]. However, the concentration of LSD in urine stored in glass containers and exposed to sunlight fell to 10% of the original concentration within 13 h [15], and LSD concentrations in plasma and urine samples stored in clear vials on a laboratory bench under normal fluorescent lights decreased by about 50% over a two-week period [16]. LSD is reported to be stable in urine stored at −4°C in the dark for four weeks, or at −20°C for three months [14], or at −16°C for up to 45 days [17]. A recent investigation found that LSD concentrations in urine did not change significantly when stored at 25°C for up to four weeks in amber glass or non-transparent polyethylene containers. However, there was a 30% loss in LSD when the storage temperature was 37°C and up to 40% loss at 45°C over the same time period [18]. The same study found that certain metal ions such as Fe_3^+ are able to catalyze the decomposition of LSD in buffers and in urine.

LSD can undergo isomerization to iso-LSD by epimerization at the C-8 carbon (Fig. 1). The reaction has been studied extensively [18,19]. Under

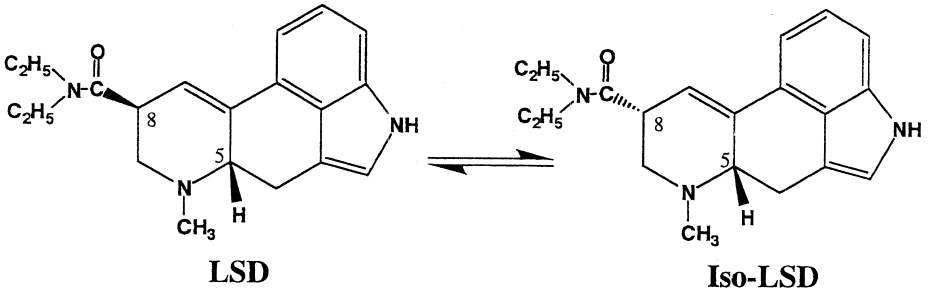

Fig. 1. Base-catalyzed interconversion of LSD and iso-LSD.

basic aqueous conditions at elevated temperatures, the interconversion occurs rapidly and ultimately reaches an equilibrium of approximately 90% LSD and 10% iso-LSD. The epimerization can occur slowly in alcohol even at 0°C. For this reason, reference stock solutions of LSD and iso-LSD should be prepared in acetonitrile rather than in methanol or water [20].

Nearly quantitative conversion of iso-LSD to LSD can be achieved by heating the iso-LSD in an ethanolic solution of 0.5 *M* sodium ethoxide for 10 min at 50°C [19].

3. Metabolism and pharmacokinetics of LSD in humans

Because LSD is rapidly metabolized and only a small fraction of a dose is excreted in the urine as unchanged LSD, there is continued interest in identifying metabolites of LSD that may be detected in urine for a longer time period. The following five metabolites have been identified in urine or blood from human users (Fig. 2): *N*-demethyl LSD (''nor-LSD''), 2-oxo-LSD, 2-oxo-3-hydroxy-LSD, 13-hydroxy-LSD and 14-hydroxy-LSD [4,21–23]. The 13- and 14-hydroxy-LSD metabolites are excreted in urine as glucuronide conjugates [22]. Incubation of LSD with human liver microsomes has permitted identification of lysergic acid monoethylamide and detection of at least five as yet unidentified metabolites of LSD [24]. Additional LSD metabolites have been identified in laboratory animals, but have not yet been conclusively identified in human urine or blood samples [4].

2-Oxo-LSD has been reported as the major human metabolite of LSD, but the experimental basis for this claim is unclear. Evidence from several laboratories suggests that 2-oxo-LSD concentrations in body fluids from LSD users are lower than those of the parent drug [22,23]. However, 2-oxo-LSD may be an intermediate in the formation of 2-oxo-3-hydroxy-LSD, which has been found in much higher concentrations than LSD in urine from LSD users [21,23,25–27]. Fig. 3 compares the concentrations of LSD and 2-oxo-3-hydroxy-LSD determined by liquid

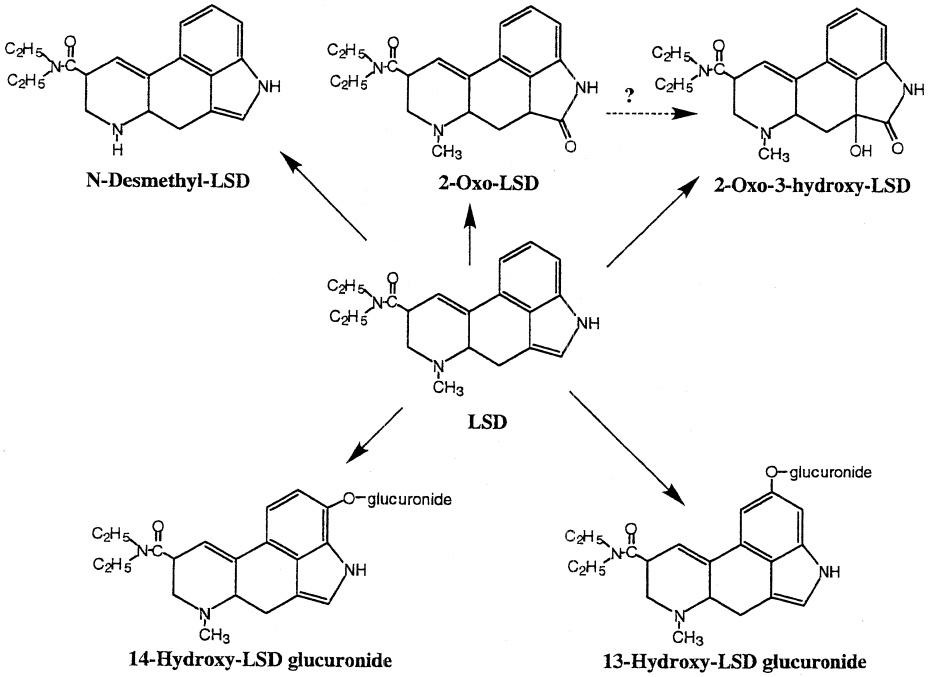

Fig. 2. Known human metabolites of LSD.

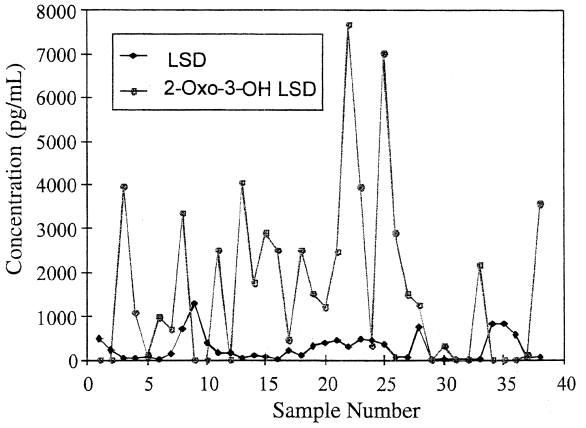

Fig. 3. Comparison of concentrations of LSD and 2-oxo-3-hydroxy-LSD in LSD-positive urine samples.

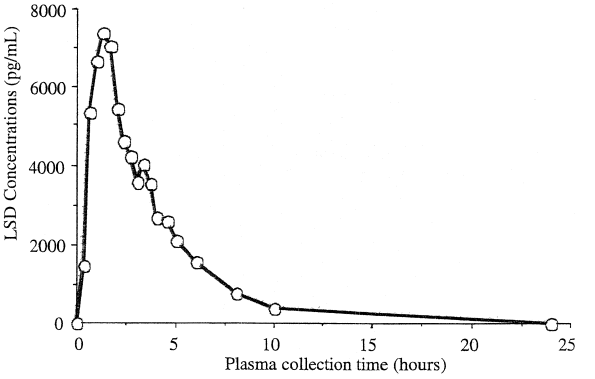

Fig. 5. Plasma concentrations of LSD following oral administration of a 4-μg/kg dose to the female subject.

chromatography–tandem mass spectrometry (LC–MS–MS) in 38 urine samples previously screened positive for LSD by a radioimmunoassay [22]. The samples containing LSD but no 2-oxo-3-hydroxy-LSD may have been blind quality control samples submitted to the laboratory as part of a proficiency-testing program.

Although iso-LSD is not a metabolite of LSD, it is frequently detected in urine and other body fluids from LSD users because it is a major contaminant in many illicit LSD preparations.

Because of legal and ethical restrictions on administration of LSD to humans, only a few well-controlled studies have been directed at determining the pharmacokinetics of LSD in humans. One recent study conducted at the University of Berne in Switzerland involved administration of an oral dose of LSD (4 μg/kg) to two volunteers, one male and one female. Urine and blood samples were collected at intervals for four days following drug administration. Figs. 4 and 5 show plots of the LSD concentrations in these samples versus time of collection [25]. It should be kept in mind that the oral dose in this study was substantially higher than is generally taken by ''recreational'' users.

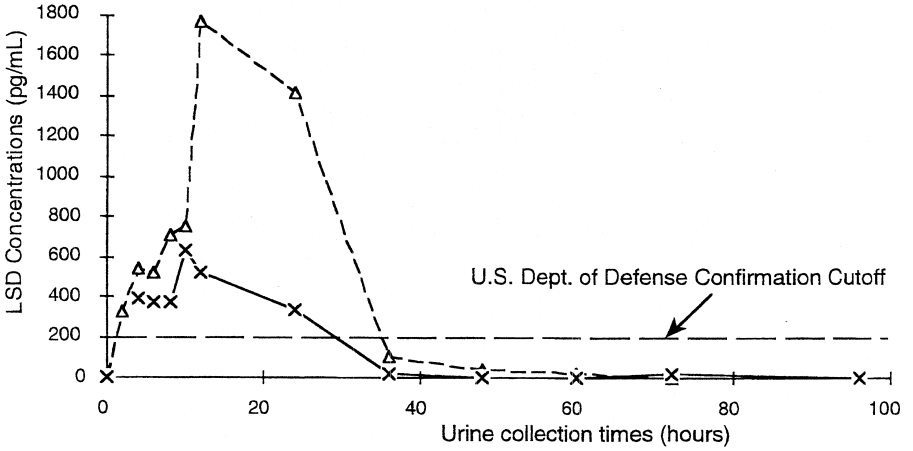

Fig. 4. Concentrations of LSD in urine following oral administration of a 4-μg/kg dose to a female subject (△) and a male subject (×).

4. Extraction of LSD and metabolites from biological matrices

To reliably detect and quantify the very low concentrations of LSD it is necessary to use a highly selective extraction procedure or a very sensitive and selective method of detection, or a combination of both. Assays employing electron ionization mass spectrometry generally do not provide adequate selectivity unless they are combined with a highly selective extraction procedure. For example, Clarkson et al. [19] used a combination of liquid–liquid and solid-phase extractions in a gas chromatography–electron ionization mass spectrometry (GC–EI-MS) assay that included conversion of iso-LSD to LSD by treatment with ethanolic sodium hydroxide. Urine samples were made basic with NH_4OH, saturated with NaCl and extracted with 1-chlorobutane. The dried extracts were then dissolved in ethanolic sodium hydroxide (0.5 *M*) and heated at 50°C for 10 min. After addition of water and solid NaCl the solutions were again extracted with 1-chlorobutane. The organic supernatants were evaporated to dryness, reconstituted in isooctane–methylene chloride–triethylamine (50:50:0.1) and poured into solid-phase extraction (SPE) tubes containing a silica-based propylamine stationary phase. After the columns were washed with methylene chloride–triethylamine (100:0.1), the LSD was eluted with methanol–methylene chloride–triethylamine (0.2:10:0.01). Finally, the extracts were evaporated to dryness, reconstituted in 1-chlorobutane, and further purified by extraction into phosphate buffer (pH 4.5). After basification with NH_4OH and addition of solid NaCl, they were back-extracted into 1-chlorobutane. The overall recovery of LSD was reported to be 69%. By including the conversion of iso-LSD to LSD and then measuring the total LSD concentration, the authors showed that they could significantly increase the percentage of urine samples that would be confirmed positive by gas chromatography (GC)–MS at a cutoff concentration of 200 pg/ml [19].

Several laboratories have developed immunoaffinity extraction procedures as an alternative to a time-consuming, multi-step extraction procedure. Francis and Craston [28] used an antiserum raised against LSD in rabbits to prepare an LSD affinity gel. Extraction of urine samples by means of the affinity gel followed by high-performance liquid chromatography (HPLC) with fluorescence detection permitted quantitation of LSD concentrations as low as 0.5 ng/ml. The same laboratory used a similar immunoaffinity extraction in combination with HPLC and electrospray ionization mass spectrometry (ESI-MS) and achieved a comparable lower limit of quantitation [15].

Cai and Henion [29] combined an on-line immunoaffinity extraction with capillary LC–MS–MS for trace analysis of LSD analogs and metabolites in human urine. This array of state-of-the-art techniques permitted detection of LSD analogs at the low pg/ml level.

Until recently the major limitation to immunoaffinity extraction was the absence of a commercial source of an LSD immunoaffinity resin. However, Microgenics Corporation has developed an affinity resin that is specific for LSD and some of its metabolites. The resin is now commercially available[1]. Extraction of LSD-positive urine samples using this affinity resin permits measurement of LSD concentrations in urine by GC–EI-MS at concentrations as low as 61 pg/ml [30].

Laboratories using a highly selective method of detection, such as tandem mass spectrometry, have successfully extracted LSD from urine using standard solid-phase procedures for extraction of basic drugs [15,21].

5. Newer methods of analysis for LSD and its metabolites

During the time period covered by this review (1992–1999) important advances have occurred in development of chromatographic and mass spectrometric methods for detection and measurement of LSD and certain of its metabolites in various biological matrices. Methods based on GC–MS continue to be developed and used, primarily because that is the instrumentation most widely available within toxicology laboratories. However, MS–MS is

[1]LSD ImmunElute Sample Extraction Kit, Microgenics Inc., Pleasanton, CA, USA.

receiving increasing attention, as is the combination of liquid chromatography and atmospheric pressure ionization mass spectrometry (LC–API-MS).

5.1. Methods based on GC–MS or GC–MS–MS

Several variations of previously reported GC–MS assays for LSD have appeared in the recent literature. Bukowski and Eaton [32] used the method reported by Paul et al. [31] to detect LSD concentrations as low as 10 pg/ml. Analysis of serum samples collected from persons under the influence of LSD was reported by Musshoff and Daldrup [33]. In their procedure 1 ml of serum was made basic and extracted with *n*-butyl chloride. The organic layer was dried; the residue was taken up in phosphate buffer (pH 4.5) and washed with *n*-butyl chloride–cyclohexane (1:1). The acidic aqueous layer was then made basic and re-extracted with *n*-butyl chloride. The extracted LSD was derivatized by treatment with *N*-methyltrimethylsilyltrifluoroacetamide (MSTFA)–pyridine (1:1) and analyzed by GC–MS using electron ionization. LSD-d_3 served as the internal standard. The recovery of LSD from the overall extraction procedure was 66%. The assay showed good linearity over the range 0.1 to 10 ng/ml.

Nakahara et al. [34] applied GC–MS with electron ionization to the detection of LSD and nor-LSD in rat hair and in human hair. The rats were administered LSD intraperitoneally once a day for 10 days with doses ranging from 0.05 to 2.0 mg/kg. Newly grown hair was collected four weeks later. The human hair was collected from 17 persons who self-reported recent use of LSD. Hair samples (20 mg each) were washed sequentially with 0.1% sodium dodecyl sulfate and water, then extracted with methanol–5 *M* HCl (20:1). An internal standard consisting of either LSD-d_{10} or lysergic acid methylpropylamide (LAMPA) was added; the extracts were filtered and the filtrates were neutralized with 28% ammonium hydroxide. After drying, the hair extracts were partitioned between dichloromethane and 0.1 *M* NaOH. The organic layers were dried and heated for 1 h (90°C) with a trimethylsilylating reagent consisting of trimethylsilylimidazole–bis - (trimethylsilyl)acetamide–trimethylchlorosilane (3:3:2, v/v/v). The following ions were monitored during the GC–MS analysis: m/z 395, 293, 268 and 253 (LSD-TMS); m/z 381, 279 and 254 (nor-LSD-TMS); m/z 405 (LSD-d_{10}-TMS); and m/z 395 (LAMPA-TMS). Mean LSD concentrations in the rat hair were 0.29 ± 0.1 ng/mg following the 0.05-mg/kg dosing, and 10.6 ± 3.8 ng/mg following the 2-mg/kg dosing. LSD was detected in hair from only two of the 17 human subjects. The LSD concentrations in those two samples were about 10 pg/mg.

Christophersen et al. [35] at the National Institute of Forensic Toxicology in Oslo, Norway, made use of the high selectivity afforded by immunoaffinity extraction combined with GC–MS–MS with negative ion chemical ionization of trifluoroacylated LSD and nor-LSD. This combination permitted detection of LSD and nor-LSD in urine at low-pg/ml concentrations. Blood samples were analyzed in the same way after precipitation of red cells with cold ethanol and incubation of the supernatant with the immunoaffinity resin. The procedure is not effective for detection of iso-LSD due to its low affinity to the LSD ImmunElute resin.

A GC–MS–MS assay developed at Northwest Bioanalytical (Salt Lake City, UT, USA) and reported in 1992 [36] has been used with slight modification for analysis of more than a thousand urine samples. The method consists of addition of a methanolic solution containing 1.2 ng of the internal standard (LAMPA) to 4 ml of urine. The urine is made slightly acidic (pH 6) by addition of 0.1 *M* phosphate buffer, and extracted with a Bond Elut Certify I column (Varian, Harbor City, CA, USA). The SPE column is first conditioned by sequential washing with methanol and 0.1 *M* phosphate buffer (pH 6). After addition of the urine sample, the column is washed sequentially with 1.0 *M* acetic acid and methanol, and the analytes are eluted with ethyl acetate containing 4% ammonium hydroxide. It is important to prepare the 1.0 *M* acetic acid and the 4% ammonium hydroxide fresh each day. The extract is evaporated to dryness and derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane. The GC–MS–MS analyses are performed on a Finnigan TSQ7000 instrument fitted with a well-deactivated 5% phenylmethylsilicone-coated capillary GC column. Ionization is achieved by positive ion chemical ionization

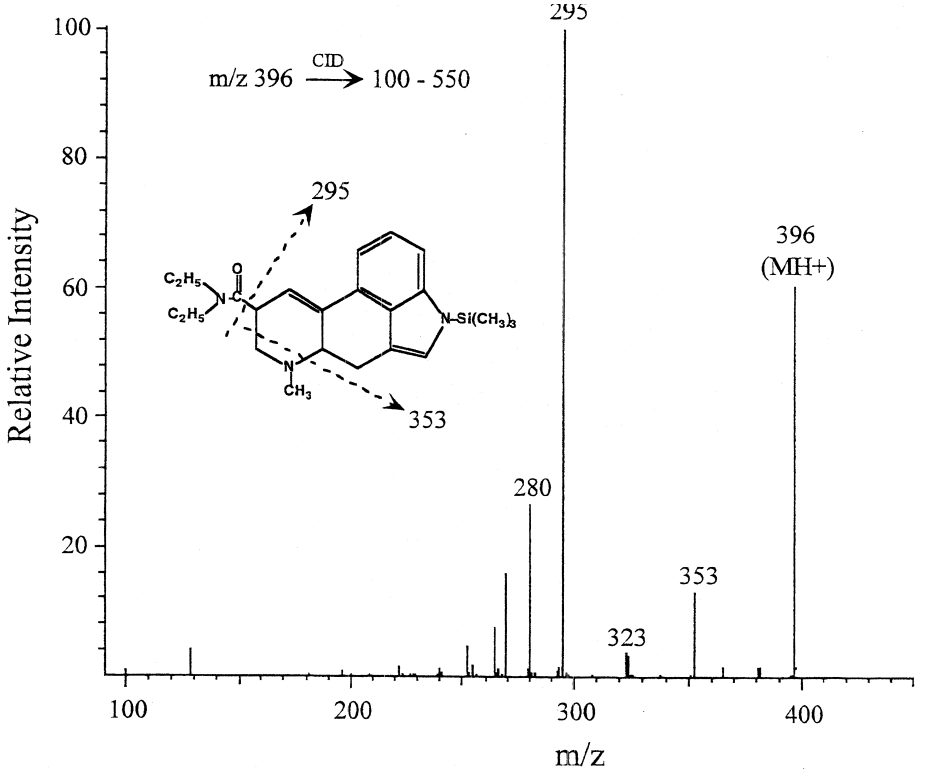

Fig. 6. The product ion spectrum from collision-induced dissociation of the protonated molecule (MH^+) of the trimethylsilyl derivative of LSD.

using ammonia as the reagent gas. Under these conditions the trimethylsilyl derivatives of LSD, iso-LSD and LAMPA are chromatographically separated. They form abundant protonated molecule ions (MH^+) which can be collisionally dissociated to prominent product ions that are selected in the second mass analyzer. Fig. 6 shows the product ion spectrum from collision-induced dissociation of the MH^+ (m/z 396) of trimethylsilylated LSD. The corresponding product ion spectra for iso-LSD and LAMPA are very similar, differing only in the relative intensities of the major product ions.

The same analytical procedure can be easily modified to permit simultaneous determination of the major known metabolite of LSD, 2-oxo-3-hydroxy-LSD [21]. Fig. 7 shows the product ion spectrum from the collision-induced dissociation of the MH^+ (m/z 500) of the bis-(trimethylsilyl) derivative of 2-oxo-3-hydroxy-LSD. The recent commercial availability of reference standards of 2-oxo-3-hydroxy-LSD and its internal standard, 2-oxo-3-hydroxy-LAMPA, has facilitated development of high-sensitivity quantitative assays for this important metabolite[2]. Ion current profiles from GC–MS–MS analysis of a urine sample fortified with LSD and 2-oxo-3-hydroxy-LSD at the lower limit of quantitation of 10 pg/ml are shown in Fig. 8. The high concentration of 2-oxo-3-hydroxy-LSD relative to LSD shown in the ion current profiles from an sample of urine from an LSD user (Fig. 9) is representative of the relative concentrations often found when both compounds are quantitatively determined. Quantitative analysis of 49 urine samples previously shown to contain LSD showed an average LSD concentration of 357 pg/ml and an average

[2]Radian Corp., Austin, TX, USA.

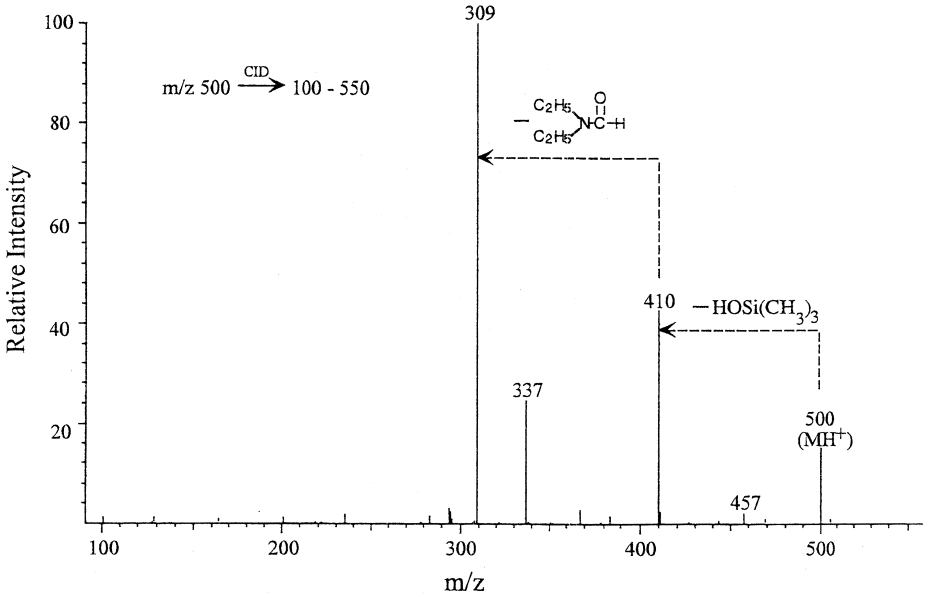

Fig. 7. The product ion spectrum from collision-induced dissociation of the protonated molecule (MH^+) of the bis-(trimethylsilyl) derivative of 2-oxo-3-hydroxy-LSD.

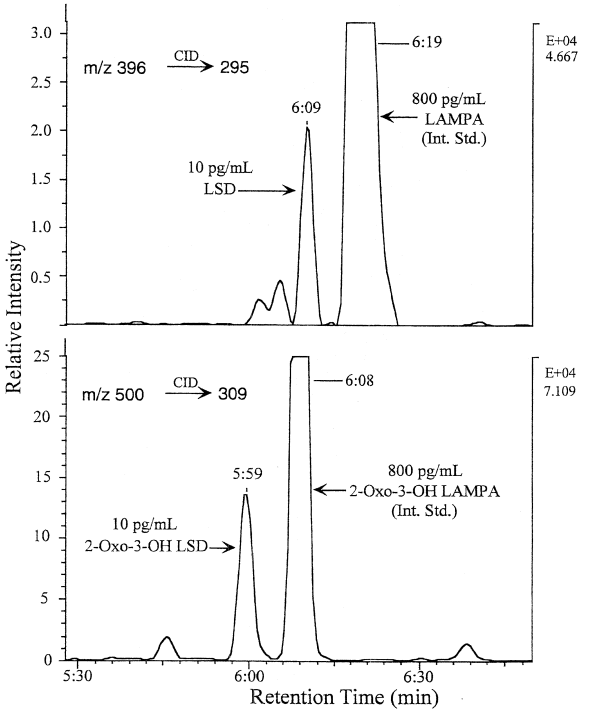

Fig. 8. Ion current profiles from GC–MS–MS analysis of urine containing LSD and 2-oxo-3-hydroxy-LSD at the lower limit of quantitation.

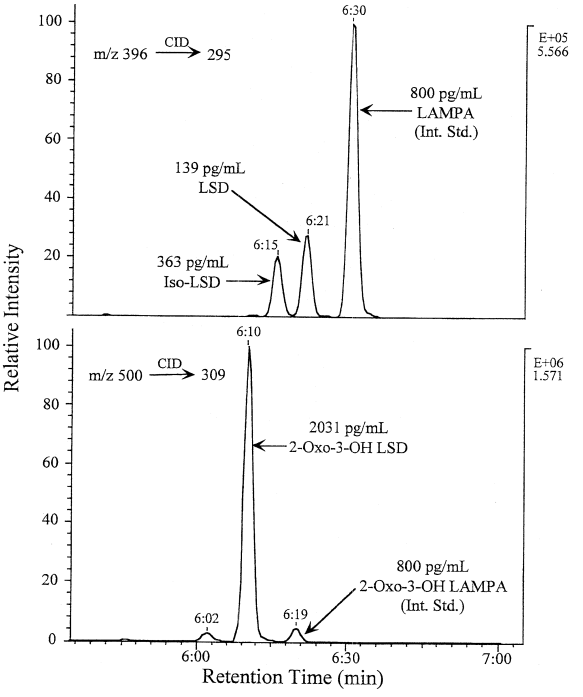

Fig. 9. A reconstructed ion current profile from analysis of urine from a person who had recently used LSD.

2-oxo-3-hydroxy-LSD concentration of 3470 pg/ml [21].

During development of the GC–MS–MS assay for 2-oxo-3-hydroxy-LSD it was found that derivatization with BSTFA resulted in formation of a mixture of the mono- and the bis-(trimethylsilyl) derivatives, and that the two derivatives were difficult to separate chromatographically on a 15 m×0.25 mm I.D., 5% phenylmethyl silicone column. However, the two derivatives could be distinguished by MS–MS analysis, as shown in Fig. 10A. Chromatographic separation required lowering the starting temperature and using a slower temperature gradient (Fig. 10B). Interestingly, the ratio of the mono to the bis derivative was significantly different when the same samples were analyzed on two different GC–MS systems. In spite of this puzzling behavior, the assay provided excellent sensitivity and quantitative accuracy, no doubt due in part to use of the 2-oxo-3-hydroxy-LAMPA as the internal standard.

Two other laboratories have recently described GC–MS–MS assays for determining LSD in physiological specimens. Dalpé-Scott et al. [37] at the Royal Canadian Mounted Police forensic laboratory in Ottawa, Canada, used an automated SPE to prepare urine, blood and liver homogenates for GC–MS–MS analysis. The assay has a reported lower limit of quantitation of 0.1 ng/ml.

A team at the US Armed Forces Institute of Pathology's Division of Forensic Toxicology has developed an assay for LSD in urine that employs MS–MS on an ion trap mass spectrometer [38]. In contrast to other methods, theirs consists of detection of underivatized LSD. This is surprising in view of the common belief that LSD is a ''sticky molecule'' that tends to undergo adsorptive losses during sample preparation and gas chromatography unless it is derivatized and the GC column is well deactivated [15]. Nevertheless, Sklerov et al. [38] reported a lower limit of quantitation of 80 pg/ml for their method. Following SPE the extracts were introduced directly into a temperature-programmable injector. The LSD and the LAMPA (internal standard) were ionized by electron ionization and the molecular ions at m/z 323 subjected to resonant dissociation to produce prominent product ions at m/z 280, 222 and 196. The LSD and LAMPA ions at m/z 222 were used for quantitation of the LSD.

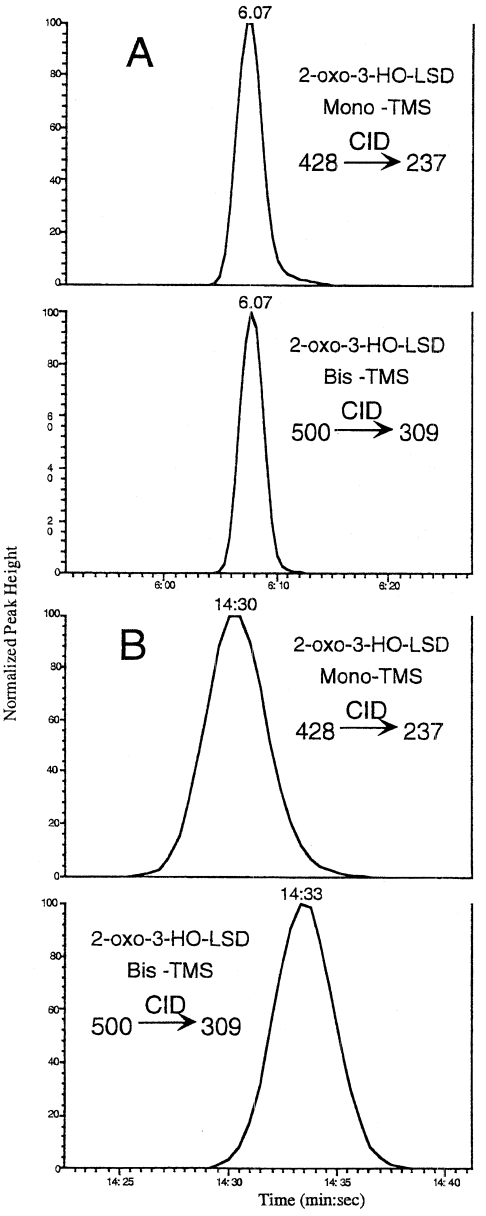

Fig. 10. Ion current profiles from GC–MS–MS analysis of a 2-oxo-3-hydroxy-LSD standard following derivatization with BSTFA. (A) Column temperature programmed from 175°C to 298°C at 20°C/min. (B) Column temperature programmed from 125°C to 298°C at 10°C/min.

5.2. Methods based on LC–MS or LC–MS–MS

The development of atmospheric pressure ionization techniques for coupling liquid chromatographic

systems to mass spectrometers has resulted in a steady flow of published LC–MS drug assays that require less sample preparation and achieve sensitivities equal to or better than those previously achieved by GC–MS analysis. Henion and co-workers [24,29,39,40] at Cornell University were the first to report LC–MS methods for determination of LSD in biological specimens. The Cornell group investigated a variety of instrument designs and procedures for LC–MS analysis of LSD and related compounds. They achieved the best sensitivity with a system consisting of an on-line immunoaffinity extraction combined with two-stage liquid chromatography and tandem mass spectrometry [29]. LC–MS as well as capillary electrophoresis (CE) coupled with MS–MS were also used to detect metabolites formed by incubation of LSD with human liver microsomes [24]. To help identify the metabolites, MS–MS data were acquired for 13 compounds related to LSD and the major common neutral losses were identified in the product ion spectra. Lysergic acid monoethylamide was determined to be the major human liver metabolite of LSD in vitro, although nor-LSD and 2-oxo-LSD were also identified in those experiments. Other in vitro metabolites with molecular masses of 16 or 48 higher than LSD were detected but not identified.

The first LC–MS assay to be used for routine analysis of forensic samples was described by Webb and co-workers [15,41] at the Laboratory of the Government Chemist, Middlesex, UK. Initially, urine samples screened positive by radioimmunoassay or an enzyme immunoassay were subjected to SPE; the extracts were analyzed by LC–MS with ESI. An immunoaffinity extraction/cleanup procedure was also developed as an alternative to the SPE. The HPLC separation was performed isocratically on a C_{18} column with a mobile phase consisting of 0.1 *M* ammonium acetate buffer (pH 8.0)–acetonitrile (75:25). The electrospray mass spectrum of LSD consists of a single major ion, the protonated molecule (MH^+) at *m*/*z* 324. In order to gain additional specificity, the protonated molecule ions were caused to undergo post-ionization fragmentation by applying 10 to 20 V between the electrospray source and the quadrupole analyzer. This resulted in structurally significant fragment ions at *m*/*z* 281 and 223, which were monitored along with the MH^+ ions. The cutoff for confirmation of LSD in urine was set at 1.0 ng/ml. Lower concentrations could be measured, but the assay's sensitivity was primarily limited by the relatively low intensity of the *m*/*z* 281 fragment ion [41]. Methysergide was used as the internal standard for this LC–MS assay; however, a later paper from this laboratory disclosed that quantitative accuracy was improved if LSD-d_3 served as the internal standard [42].

Hoja et al. [43] have developed and validated a LC–MS assay for LSD and nor-LSD in urine. In their procedure LSD-d_3 was added to 2 ml of urine as the internal standard, followed by 1 ml of a saturated solution of ammonium chloride; the pH was then adjusted to 9.5 with ammonia and the mixture was applied to an Extrelut-3 extraction cartridge. After a variety of elution solvents were evaluated, toluene–diethyl ether (60:40) was chosen as giving the best selectivity, with extraction efficiencies ranging from 78 to 98%. Chromatographic separation was performed on a C_{18} (150 mm×1 mm I.D.) reversed-phase column using a mobile phase consisting of 2 m*M* ammonium formate (pH 3)–acetonitrile (70:30). The protonated molecules for LSD and nor-LSD formed by electrospray ionization were partially fragmented by applying orifice voltages of 20 to 70 V. The following ions were monitored: *m*/*z* 324 and 223 (LSD and iso-LSD); *m*/*z* 310 and 209 (nor-LSD); and *m*/*z* 327 (LSD-d_3). The lower limits for quantitation reported for the assay were 0.1 ng/ml for LSD and 0.25 ng/ml for nor-LSD. Iso-LSD was detected but not quantified.

LSD and nor-LSD have been determined in 1-ml samples of blood, serum, plasma and urine by a method that includes automated SPE and LC–MS–MS analysis [16]. After addition of the internal standard (LSD-d_3), the specimens were diluted with 1 ml of water and 2 ml of phosphate buffer (pH 6.0). The samples were then loaded onto a Zymark RapidTrace robotic system and extracted using Bond Elut Certify SPE cartridges. The extracts were chromatographed on a Zorbax SB-phenyl column with a mobile phase consisting of 35% ammonium acetate buffer and 65% acetonitrile–*n*-propanol (20:1). LSD, nor-LSD and LSD-d_3 were ionized by electrospray and detected by selected reaction monitoring of product ions at *m*/*z* 223, 208 and 197 (LSD); *m*/*z* 237 and 209 (nor-LSD); and *m*/*z* 226,

211 and 200 (LSD-d_3). Under the HPLC conditions used, LSD and LAMPA essentially co-eluted. However, because the LAMPA product ion ratios were sufficiently different from the corresponding LSD product ion ratios, it was considered unlikely that LAMPA would be mistaken for LSD. The lower limit of quantitation for LSD in each of the matrices tested was 50 pg/ml. The determination of nor-LSD by this assay was considered to be only qualitative because inter-assay reproducibility for this analyte was poor.

The most recently reported LC–MS assays for LSD have included analysis for 2-oxo-3-hydroxy-LSD which, as mentioned above, should permit determination of LSD use over a longer time period. Slawson and co-workers [26,44] evaluated an LC–MS system for determination of LSD, iso-LSD and 2-oxo-3-hydroxy-LSD in urine. Samples (4 ml) from LSD users were spiked with internal standards (LSD-d_3 and 2-oxo-3-hydroxy-LAMPA) and extracted using a standard solid-phase procedure for extraction of basic drugs. The LC–MS analysis employed a MetaSil basic 100×3 mm I.D. HPLC column with gradient elution. The mobile phase consisted of 0.1% formic acid in water (solvent A) and methanol (solvent B). Solvent B was held at 15% for 1 min, increased to 30% in 1 min, and held at 30% for 6 min. ESI gave abundant protonated molecule ions (MH^+) for each of the analytes and internal standards. Quantitation was based on the MH^+ peak areas of the analytes relative to the MH^+ peak areas of the corresponding internal standards. Measured concentrations of LSD and 2-oxo-3-hydroxy-LSD in urine from users were in good agreement with results from analysis of the same samples by a fully validated GC–MS–MS assay. Calibration curves were linear from 25 to 5000 pg/ml with correlation coefficients greater than 0.99.

Poch et al. [23] at the US Navy Drug Screening Laboratory in San Diego, CA, USA, have developed an LC–MS–MS assay for measurement of nor-LSD and 2-oxo-3-hydroxy-LSD in urine. In this procedure urine samples (5 ml) were initially extracted with methylene chloride–isopropanol (95:5). The organic layer was separated, evaporated to dryness, reconstituted in 0.05 *M* phosphate buffer (pH 6.0) and further purified by SPE. Analyses of the resulting extracts were performed on an LC–MS–MS system comprised of a Hewlett-Packard 1050 HPLC system with a 150×4.6 mm I.D. C_{18} column connected to a Finnigan LCQ ion trap mass spectrometer. The mobile phase consisted of 0.01 *M* ammonium acetate buffer (pH 8.0)–acetonitrile (80:20) and 0.02% triethylamine. The protonated molecules formed by electrospray ionization were energized and caused to fragment to product ions at m/z 338, 265 and 237 for 2-oxo-3-hydroxy-LSD and the 2-oxo-3-hydroxy-LAMPA internal standard, and at m/z 237, 209 and 183 for nor-LSD. In nearly all of 74 urine specimens previously found to contain LSD by GC–MS, the nor-LSD concentration was lower than the LSD concentration, but the 2-oxo-3-hydroxy-LSD concentration was far higher.

LC–MS–MS has also been used at Northwest Bioanalytical to study the metabolism of LSD [22]. Figs. 11 and 12 show the product ion spectra resulting from collision-induced dissociation of the protonated molecules formed by electrospray ionization of LSD and one of its metabolites. The major product ions in each spectrum correspond to loss of the diethylamide group ($MH^+ - 101$) and loss of CH_2NCH_3 ($MH^+ - 43$) from the protonated molecules. These fragmentation processes have proven useful in detecting and tentatively identifying other LSD metabolites. Table 1 lists the LSD-related compounds detected in LSD-positive urine samples, along with their HPLC retention times and their major fragmentation processes. For these studies,

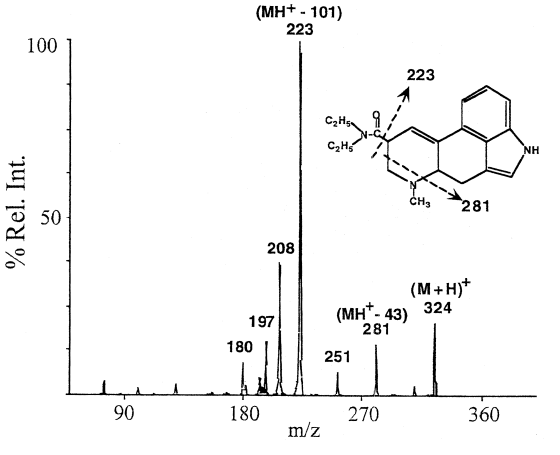

Fig. 11. The product ion spectrum from collision-induced dissociation of the protonated molecule (MH^+) of LSD.

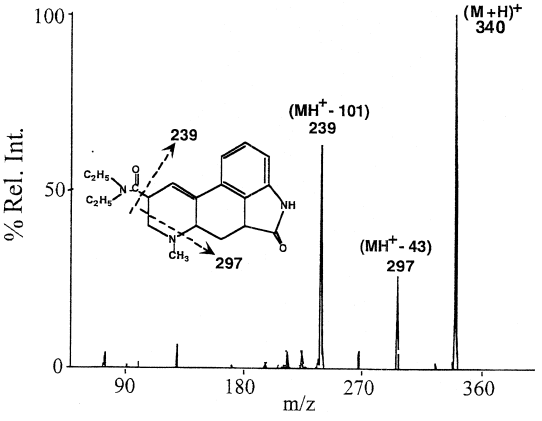

Fig. 12. The product ion spectrum from collision-induced dissociation of the protonated molecule (MH^+) of 2-oxo-LSD.

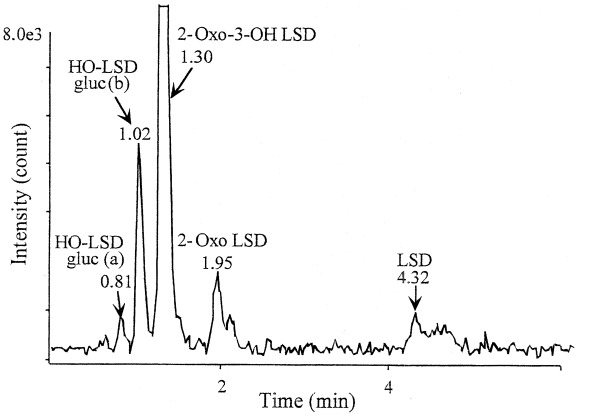

Fig. 13. A reconstructed ion current profile from LC–MS–MS analysis of pooled urine samples from an LSD user.

Table 1
LSD-related compounds detected in LSD-positive urine samples

Compound	MS–MS transition	Approximate retention time (min)
Glucuronide of HO-LSD	516→340	0.81
Glucuronide of HO-LSD	516→340	1.02
2-Oxo-3-hydroxy-LSD	356→237	1.30
HO-LSD	340→239	1.16
HO-LSD	340→239	1.72
2-Oxo-LSD	340→239	1.93
LSD	324→223	4.47
LSD-d_3	327→226	4.47
nor-LSD	310→209	4.84
iso-LSD	324→223	5.23
nor-iso-LSD	310→209	6.08

urine samples from LSD users had been extracted by either solid-phase or immunoaffinity procedures. The retention times shown in the table were obtained using a Prodigy ODS 100 mm×2 mm I.D. column (Phenomenex, Torrance, CA, USA) and isocratic elution, with a mobile phase of 10 m*M* ammonium acetate (pH 3.3)–methanol (3:1), a flow-rate of 0.4 ml/min, and a column temperature of 30°C.

Fig. 13 is a reconstructed ion-current profile resulting from LC–MS–MS analysis of pooled urine samples from an LSD user. The urine was extracted using the LSD ImmunElute immunoaffinity resin. The peaks labeled LSD-O-gluc(a) and LSD-O-gluc(b) correspond to the glucuronide conjugates of hydroxylated LSD. The primary product ion in the MS–MS spectrum of the major glucuronide metabolite corresponds to loss of glucuronic acid from the protonated molecule ($MH^+ - 176$). When the urine was incubated with β-glucuronidase before analysis, the two conjugated metabolite peaks were no longer present; two new peaks appeared which had mass spectral characteristics consistent with monohydroxylated LSD (Fig. 14). These compounds were presumed to be 13- and 14-hydroxy-LSD, each a metabolite that had been previously identified in laboratory animals [45].

5.3. HPLC with fluorescence detection

Because LSD possesses native fluorescence, the drug can be detected with good sensitivity by HPLC using fluorescence detection. However, this technique is prone to interference from other compounds with fluorescent properties. Therefore, recently reported applications of the technique for determination of LSD in biological samples have used it only in combination with other analytical methods [15,28,34,46,47].

Francis and Craston [28] developed an enzyme-linked immunosorbent assay (ELISA) screening assay for detection of LSD in urine and used HPLC with fluorescence detection to confirm samples that gave positive results with the ELISA assay. The HPLC assay included immunoaffinity extraction and analysis of the extract with a 250 mm×4.6 mm I.D. Hypersil ODS column; the mobile phase consisted of 100 m*M* ammonium acetate buffer–methanol

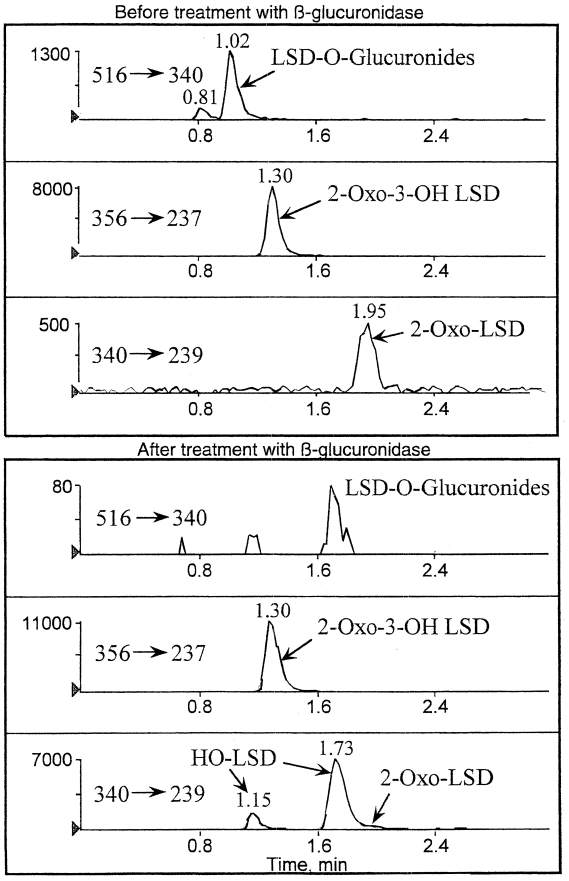

Fig. 14. Ion current profiles from LC–MS–MS analysis an LSD-positive urine sample before (top) and after (bottom) treatment with β-glucuronidase.

(35:65). The excitation and emission wavelengths were 320 and 400 nm, respectively. The lower limit of quantitation was reported to be 0.5 ng/ml. Other investigators at the same institution used SPE and an HPLC assay with fluorescence detection to screen forensic urine samples for LSD and then confirmed positive samples by LC–MS analysis [15].

Both whole-blood and urine samples were analyzed by an HPLC–fluorescence assay developed by Bergemann et al. [47]. Samples that tested positive for LSD by an immunoassay were extracted by solid-phase using DetectAbuse Type R columns (Biochemical Diagnostics, New York, USA); the HPLC analysis was performed with a LiChroSphere 60 RP-Select B column (E. Merck, Darmstadt, Germany). The mobile phase consisted of a triethylammonium phosphate buffer (pH 2.9)–acetonitrile (80:20). Methysergide served as the internal standard. The excitation and emission wavelengths were 320 and 420 nm, respectively. Although the lower limit of detection was reported to be 0.05 ng/ml, the intra- and inter-assay standard deviations reported for analysis of urine spiked with 1.0 ng/ml of LSD were greater than 35%.

Another HPLC–fluorescence assay was developed for determining LSD in post-mortem blood samples [46]. After addition of LAMPA as the internal standard, the blood was extracted by a solid-phase procedure. The extracts were analyzed using a 250 mm×4.6 mm I.D. Hypersil ODS column with a mobile phase consisting of 0.3% triethylamine in phosphate buffer (0.1 *M*, pH 8.0)–acetonitrile (68:32). The excitation and emission wavelengths were 303 and 413 nm, respectively. The lower limit of detection was reported to be 0.2 ng/ml.

Nakahara et al. [34] compared an HPLC–fluorescence assay with GC–MS analysis for determination of LSD and nor-LSD in rat hair following administration of the drug to rats. Both analytical methods were also used for analysis of hair from self-reported users of LSD. After addition of either LSD-d_{10} or LAMPA as internal standards, the hair was extracted with methanol–5 *M* HCl (20:1); the extracts were neutralized, evaporated and purified by partitioning between dichloromethane and 0.1 *M* sodium hydroxide. Analysis by HPLC with fluorescence detection (excitation, 315 nm; emission, 420 nm) involved a 250 mm×4.6 mm I.D. Puresil C_{18} column (Millipore, Bedford, MA, USA) and gradient elution. Solvent A consisted of 4 m*M* pentane sulfonate–methanol with 1% acetic acid (1:9), and solvent B contained acetonitrile–0.5% phosphoric acid (1:1). During the gradient elution, solvent B was increased from 20 to 40%. The calibration curves for HPLC analysis of 20-mg human hair samples were linear from 5 to 50 pg/mg, with correlation coefficients (*r*) greater than 0.999.

5.4. Capillary electrophoresis analysis

Cai and Henion [24] employed CE in combination with ESI and selected ion monitoring to investigate the in vitro metabolism of LSD. The electrophoretic

separation of metabolites was performed using a 100 cm×50 μm I.D. capillary column. The voltage applied across the column was 25.5 kV and the electrolyte was 80 m*M* ammonium acetate (pH 4.5) containing 20% methanol. Injection of a synthetic mixture containing LSD and six analogs, each corresponding to approximately 50 pg, gave an electropherogram showing an intense, well-resolved peak for each compound.

A major limitation of CE for analysis of drugs in body fluids is the restricted volume of sample that can be injected into the narrow capillary columns. However, Frost and co-workers [48,49] employed CE with laser-induced fluorescence for determination of LSD and nor-LSD in blood at concentrations as low as 0.1 ng/ml. Their method included addition of methylergometrine (internal standard) to the blood sample and precipitation of proteins with acetonitrile. After evaporation of the supernatant, the residue was taken up in acetate buffer (pH 4) and washed with hexane. The aqueous phase was then made basic with ammonia and extracted with methylene chloride. Following evaporation of the methylene chloride, the extract was reconstituted in methanol and electrokinetically injected into the CE column. A He–Cd laser with a wavelength of 325 nm excited the fluorescence, which was detected at 435 nm. Efforts to apply the method to the analysis of urine samples were unsuccessful because a large matrix peak interfered with detection of the LSD.

6. Conclusions

Significant advances continue to be made in developing more sensitive and specific methods for determination of LSD and its metabolites in physiological specimens. In particular, the recent identification of 2-oxo-3-hydroxy-LSD as a major human metabolite of LSD promises to facilitate the detection of LSD use by analysis of body fluids. Preliminary methods have been reported for measurement of this metabolite, but additional development and validation is needed before the methods will be widely adopted by forensic toxicology laboratories.

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