Quantification of LSD and *N*-Demethyl-LSD in Urine by Gas Chromatography/Resonance Electron Capture Ionization Mass Spectrometry

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Demethylation of lysergic acid diethylamide (LSD) in the human has been demonstrated, both in vitro and in vivo, and aromatic hydroxylation at positions 13 and 14 has been tentatively identified. A gas chromatography/resonance electron capture ionization mass spectrometry (GC/MS) assay for LSD and *N*-demethyl-LSD in urine has been developed, in which the drug and its metabolite are converted to their *N*-trifluoroacetyl derivatives prior to GC/MS analysis. Linear and reproducible calibration curves have been obtained for LSD concentrations from 0.05 to 5.0 ng/mL, and for *N*-demethyl-LSD concentrations from 0.03 to 5.0 ng/mL. The assay was used to determine the urinary concentrations of LSD and *N*-demethyl-LSD following administration of a single oral dose of the drug (1 μg/kg) to an adult volunteer. The rates of excretion of LSD and *N*-demethyl-LSD reached maxima in urine collected at time intervals of 4–6 and 8–10 h after administration, respectively. The elimination half-lives for LSD and *N*-demethyl-LSD were 3.6 and 10.0 h, respectively.

LSD was one of the 20 controlled substances most commonly encountered in emergency rooms across the nation in 1985 (*1*), reflecting continuing abuse and trafficking of this illicit drug. Despite the long history of abuse associated with LSD, little is known concerning the disposition of LSD in humans. The lack of pharmacokinetic data on LSD is partly due to the technical difficulty of detecting and measuring the drug in physiological specimens. LSD is an extremely potent psychedelic drug; oral doses as low as 25 μg can cause central nervous system disturbances (*2*). Therefore, concentrations of LSD and its metabolites in blood and urine are likely to be very low. Furthermore, the drug's low volatility, its thermal instability, and its tendency to undergo adsorptive losses during gas chromatographic (GC) analysis all contribute to the difficulty of developing a method for confirmation of LSD in body fluids.

Reagents for a radioimmunoassay (RIA) for LSD are commercially available (Abuscreen, Roche Diagnostic Systems, Nutley, NJ). The RIA is reported to be capable of detecting concentrations of LSD in urine as low as 25 pg/mL. However, the manufacturer recommends a cutoff of 1 ng/mL.

We recently reported a GC/MS assay for LSD in urine involving conversion of the drug to its *N*-trimethylsilyl derivative and detection by selected ion monitoring using electron ionization (*3*). However, application of the assay to urine specimens found to be RIA-positive for LSD was only partially successful in that many of the specimens contained interferences that prevented conclusive identification of the drug.

High-performance liquid chromatography (HPLC) with fluorescence detection has been used to detect LSD in blood and urine specimens (*4*, *5*), but it is also subject to interferences, so that many experts do not consider it a legally defensible method of confirmation.

The disposition of LSD in animals and man has been recently reviewed (*6*). In animals, LSD undergoes *N*-demethylation, *N*-deethylation, aromatic hydroxylation, and oxidation at position 2, depending on the species (*7–10*). Although no LSD metabolites have heretofore been identified in man, their presence has been inferred from the differences in LSD concentrations determined by RIA and by HPLC within samples of plasma and urine (*4*).

We now report for the first time the identification of *N*-demethyl-LSD as a human metabolite of LSD, both in vitro and in vivo, and the development of a sensitive and specific assay for LSD and *N*-demethyl-LSD in urine. The new assay is based on gas chromatography and resonance electron capture ionization mass spectrometry, often incorrectly referred to as negative ion chemical ionization mass spectrometry. This assay permits measurement of lower concentrations of LSD than can be determined by the previously reported electron ionization method (*3*). In a pilot disposition study involving administration of a single oral dose of LSD to an experienced adult volunteer, simple pharmacokinetic parameters for LSD and *N*-demethyl-LSD were calculated from the urinary excretion data. The assay was also applied to the determination of LSD and *N*-demethyl-LSD in urine specimens found to be positive for LSD by RIA.

EXPERIMENTAL SECTION

Instrumentation. Gas chromatographic separations were performed by use of a fused silica capillary column coated with dimethylsilicone (12 m × 0.2 mm i.d., 0.3-μm film thickness, Hewlett-Packard, Palo Alto, CA). Hydrogen was the carrier gas (linear velocity 60 cm/s at 160 °C). The injector and transfer line temperatures were maintained at 260 and 265 °C, respectively. The oven temperature was held at 160 °C for 0.5 min after injection and then programmed to 300 at 20 °C/min.

The gas chromatograph (Finnigan 9610) was coupled to a Finnigan 4500 mass spectrometer, which was operated in the resonance electron capture ionization mode with methane as the reagent gas (ion chamber pressure 90–107 mPa; analyzer manifold pressure 0.005–0.006 mPa). The ionizer temperature setting was maintained at 120 °C during analysis. The electron energy and filament emission current were set at 80 eV and 0.25 mA, respectively.

The mass spectrometer was tuned prior to analysis by adjustment of ion source voltages so that the m/z 414 negative ion peak (from perfluorotributylamine) displayed a maximum ion current while retaining unit mass resolution and peak symmetry. Derivatized standards (LSD and *N*-demethyl-LSD, 1 ng each) were injected to verify the sensitivity of the GC/MS system. The negative ion currents at m/z 419, 429, 501, and 531 were monitored, each with a sampling time of 0.055 s; the total cycle time was 0.2295 s.

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Materials. Lysergic acid diethylamide (LSD) was purchased from Alltech-Applied Science (Deerfield, IL). Lysergic acid [$^2H_{10}$]diethylamide (LSD-$^2H_{10}$) was obtained from Dr. David Kidwell of the Naval Research Laboratory, Washington DC. *N*-Demethyl-LSD and deuterium-labeled LSD (LSD-$^2H_3$) were obtained from Dr. Colin Pitt (Research Triangle Institute, Research Triangle Park, NC). Methysergide was provided by Sandoz Pharmaceuticals (East Hanover, NJ). All standards were checked for purity by HPLC with fluorescence detection and by GC/MS. All solvents were HPLC grade, glass distilled, obtained from Burdick and Jackson (Muskegon, MI). The following reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI): sodium acetate trihydrate (99+%), sodium bisulfite, sodium hydroxide (97+%), ammonium carbonate, anhydrous sodium sulfate (99+%), 1,4-dimethylpiperazine (98+%), tributylamine (99+%), potassium chloride (99+%), anhydrous dibasic potassium phosphate, anhydrous monobasic potassium phosphate (99+%), and magnesium chloride hexahydrate (99%). β-Glucuronidase (*Helix pomatia*, type H-1), D-glucose-6-phosphate (monopotassium salt), β-nicotinamide adenine dinucleotide phosphate (monosodium salt), and glucose-6-phosphate dehydrogenase (*Torula* yeast, in ammonium sulfate solution) were purchased from Sigma Chemical Co. (St. Louis, MO). Trifluoroacetylimidazole, pentafluoropropionylimidazole, and heptafluorobutyrylimidazole were obtained from Pierce Chemical Co. (Rockford, IL).

Rat Liver Perfusion. Isolated rat liver perfusion experiments were performed, and LSD metabolites were isolated by thin-layer and column chromatography as described by Siddik et al. (*9*, *10*).

Rat Liver in Vitro Metabolism. The procedure used in the investigation of rat liver in vitro metabolism of LSD was identical with that reported by Niwaguchi et al. (*7*). The incubation mixture was saturated with ammonium carbonate and the metabolites were then extracted with ethyl acetate.

Human Liver in Vitro Metabolism. Human livers were obtained after autopsy from the Department of Pathology of the University of Utah Hospital and from Holy Cross Hospital, Salt Lake City, UT. The livers were cut into 1 cm thick slices, rinsed with chilled 1.15% potassium chloride solution to remove traces of blood, dried with paper towels, and quickly frozen in liquid nitrogen. The liver specimens were stored at −80 °C until required. The human liver in vitro procedure described by Jurima et al. (*11*) was adapted to the in vitro metabolic study of LSD. To a 10-mL Teflon-lined, screw-capped test tube were added 30 μM LSD, 120 mM phosphate buffer (pH 7.4), 46 mM potassium chloride, 2 mM magnesium chloride, 0.4 mM β-nicotinamide adenine dinucleotide phosphate ($NADP^+$), 4 mM glucose-6-phosphate, 0.4 units of glucose-6-phosphate dehydrogenase, and 0.2 mL of 9000*g* human liver supernatant. The total volume was 1 mL. After incubation for 2 h at 37 °C in an open tube, the incubation was terminated by addition of 4 mL of chilled acetonitrile. The tube was vortexed and then allowed to stand in an ice bath for 15 min. After centrifugation at 800*g* for 5 min, the supernatant was transferred to a clean test tube and evaporated under a gentle stream of air at 60 °C. The residue was dissolved in 200 μL of methanol and the metabolites of LSD were then separated by thin-layer chromatography (TLC) with a solvent system consisting of chloroform and methanol 4:1 (v/v) (*10*).

Preparation of Perfluoroacyl Derivatives of LSD and *N*-Demethyl-LSD. Methanolic solutions containing approximately 2 μg each of LSD and *N*-demethyl-LSD were evaporated to dryness. The tubes containing the residues were chilled in an ice bath followed by addition of 10 μL of a perfluoroacylimidazole (trifluoroacetylimidazole, pentafluoropropionylimidazole, or heptafluorobutyrylimidazole) and 10 μL of 10% 1,4-dimethylpiperazine in toluene. The tubes were then heated at 80 °C for 20 min.

GC/MS Assay Procedure. A drug-free urine pool was fortified with LSD and *N*-demethyl-LSD to give a concentration of 100 ng/mL of each. Urine samples containing various concentrations of LSD and *N*-demethyl-LSD were prepared by serial dilution of the spiked urine with drug-free urine. Methanolic stock solutions of LSD-$^2H_{10}$ and methysergide were prepared to give concentrations of 1 and 50 μg/mL, respectively.

Teflon-lined, screw-capped test tubes were washed with chromic acid and then rinsed thoroughly with tap water. The tubes were neutralized with detergent and again rinsed successively with tap water, deionized water, acetone, and methanol. The tubes were then oven-dried and subjected to vapor-phase silylation (*12*).

To a 25-mL Teflon-lined, screw-capped test tube were added 8 mL of urine, 1000 ng of methysergide, and 40 ng of LSD-$^2H_{10}$. The tube was capped, vortexed, and then allowed to equilibrate at room temperature for 1 h. β-Glucuronidase (8000 units) in 1.6 mL of 1 M sodium acetate buffer (pH 4.8) containing 160 mg of sodium bisulfite was added to the tube. Enzymatic hydrolysis of the urine sample was allowed to proceed for 3 h at 37 °C and terminated by adjusting the pH of the incubation mixture to between 2 and 3 by adding 0.6 mL of 6 N hydrochloric acid. Extraction with 10 mL of ethyl acetate was carried out with gentle rocking for 10 min. The mixture was centrifuged at 800*g* for 5 min and the organic phase was removed by aspiration. The remaining aqueous phase was adjusted to pH 9 by addition of 0.62 mL of 6 M sodium hydroxide and 0.5 mL of saturated ammonium carbonate. The sample was then extracted into 10 mL of toluene/methylene dichloride, 7:3 (v/v), with gentle rocking for 15 min, followed by centrifugation at 800*g* for 10 min. The organic layer was transferred to a 25-mL glass tube and washed with 10 mL of 0.1 M ammonium hydroxide for 10 min with gentle rocking. After centrifugation, the aqueous layer was removed with a Pasteur pipet. The organic layer was subsequently dried over anhydrous sodium sulfate. Following centrifugation, the organic layer was transferred to a disposable, 10-mL conical glass tube. Ten percent glacial acetic acid in methanol (200 μL) was added to the tube and the extract evaporated under a gentle stream of air at 50 °C.

The tube containing the residue was capped and cooled in an ice bath for 15 min. Derivatization of the LSD and *N*-demethyl-LSD was achieved by heating the residue with 20 μL of 30% trifluoroacetylimidazole in toluene and 10 μL of 1% 1,4-dimethylpiperazine in toluene for 20 min at 80 °C. The tube was allowed to cool to room temperature and 1 μL of the mixture was coinjected into the GC/MS along with 0.5 μL of 1% tributylamine in toluene.

Calibration Curves. Calibration curves for LSD and *N*-demethyl-LSD were constructed by analyzing serially diluted urine samples in the concentration ranges of 0.05–5.0 and 0.03–5.0 ng/mL, respectively. The samples were then processed as described. Three samples were analyzed at each concentration.

Recovery Studies. Serially diluted samples were processed as described, except that the internal standard was added to the extract rather than to the urine sample. The peak area ratios obtained from these samples were compared to those obtained when equal amounts of LSD and *N*-demethyl-LSD were derivatized without prior extraction.

Human Subject Study. A healthy adult male volunteer (70.5 kg), after an overnight fast, was administered LSD orally at a dose of 1 μg/kg. Urine was collected at 2-h intervals for the first 24 h, and then at 30, 36, 42, 72, and 96 h after administration. The urine samples were stored in polyethylene bottles at −20 °C until analyzed.

RESULTS AND DISCUSSION

Identification of Metabolites. In order to obtain gas chromatographic and mass spectrometric data on LSD metabolites known to be generated in the rat, we reproduced previously reported experiments on in vitro metabolism of LSD, in 9000*g* rat liver supernatant (*7*) and by a perfused rat liver (*10*). We used similar procedures to demonstrate in vitro metabolism of LSD in a 9000*g* human liver supernatant. The GC/MS data obtained from metabolites generated in these studies facilitated identification of LSD metabolites in human urine samples.

We initially identified *N*-demethyl-LSD in products of rat liver metabolism by comparison of the gas chromatographic retention time and the resonance electron capture ionization mass spectrum of the metabolite's trifluoroacetyl derivative with those of the trifluoroacetyl derivative of synthetic *N*-demethyl-LSD. The metabolite was subsequently also found to be formed by human liver enzymes. The concentrated supernatant from the human liver incubate was spotted on a TLC plate along with a reference sample of synthetic *N*-

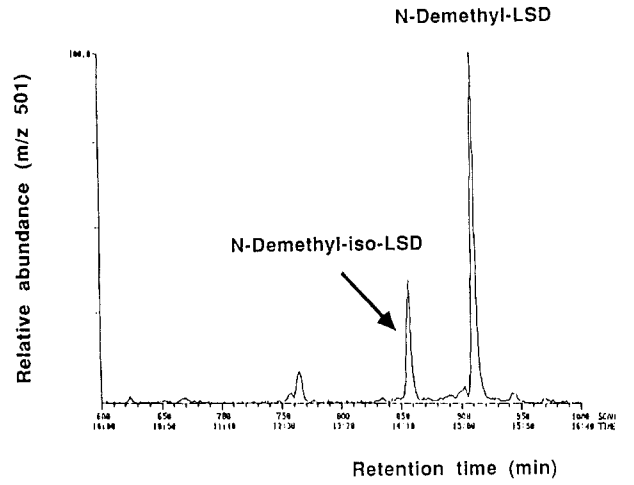

Figure 1. Ion current profile (m/z 501) resulting from trifluoroacetylation of *N*-demethyl-LSD isolated by TLC from human liver incubate.

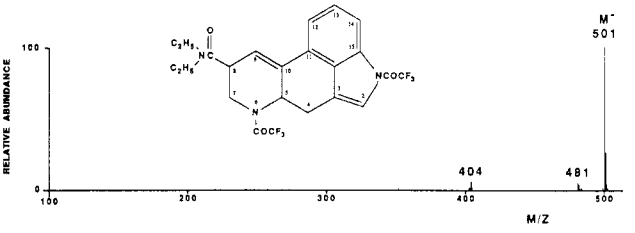

Figure 2. Resonance electron capture ionization mass spectrum of the bis(trifluoroacetyl) derivative of synthetic *N*-demethyl-LSD.

demethyl-LSD. After development of the TLC plate, the adsorbent in the region of the R_f of the *N*-demethyl-LSD was removed from the plate and extracted with methanol. The methanol extract was then evaporated to dryness, derivatized by treatment with trifluoroacetylimidazole, and analyzed by GC/MS. The resulting total ion current chromatogram showed two major peaks (Figure 1), both of which gave mass spectra consistent with the bis(trifluoroacetyl) derivative of *N*-demethyl-LSD (Figure 2). We surmised that the two peaks corresponded to the diastereoisomers, *N*-demethyl-LSD and *N*-demethyl-iso-LSD and that formation of the *N*-demethyl-iso-LSD had occurred during the derivatization step. This conclusion was based upon the following observations: (1) The two chromatographic peaks were formed in the same relative proportion when synthetic *N*-demethyl-LSD was derivatized under the same conditions. (2) The relative sizes of the two peaks were affected by changes in the derivatization conditions. For example, the relative size of the *N*-demethyl-iso-LSD peak decreased when the residue containing the metabolite was cooled just prior to addition of the trifluoroacetylimidazole. (3) The relative size of the N-demethyl-iso-LSD peak was lower when the compound was converted to its pentafluoropropionyl derivative, as compared to formation of the trifluoroacetyl or heptafluorobutyryl derivatives.

It has been reported that LSD can be converted to iso-LSD by epimerization at carbon-8 under basic conditions (*5*, *13*). Therefore, abstraction of the mildly acidic proton at the chiral carbon-8 of *N*-demethyl-LSD by 1,4-dimethylpiperazine may be responsible for initiation of epimerization of *N*-demethyl-LSD to *N*-demethyl-iso-LSD during derivatization. Such a mechanism has been suggested for racemization of *N*-trifluoroacetyl-L-prolyl chloride in the presence of triethylamine (*14*).

Extraction, derivatization, and GC/MS analysis of urine from a volunteer after oral administration of LSD (1 μg/kg) gave the ion current profiles shown in Figure 3. The retention

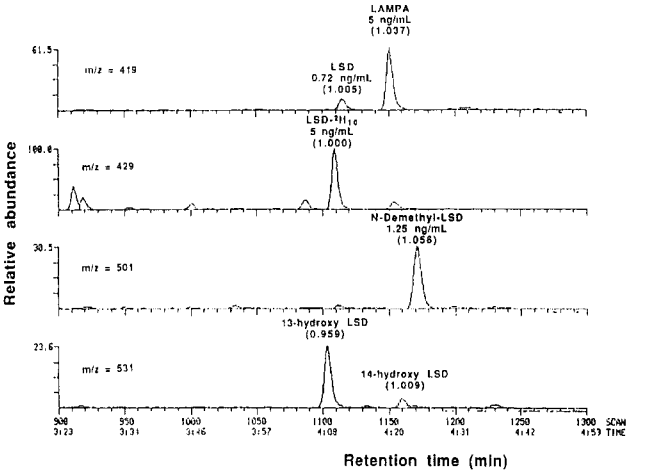

Figure 3. Selected ion current profiles from the gas chromatographic/resonance electron capture ionization mass spectral analysis of a volunteer's urine collected 8–10 h postadministration of LSD (1 μg/kg). The relative retention times are given in parentheses. LSD-$^2H_{10}$ and LAMPA were added to the urine specimen as internal standards.

time of the peak in the m/z 501 ion current profile identified as the trifluoroacetyl derivative of *N*-demethyl-LSD is identical with that obtained for the trifluoroacetyl derivative of synthetic *N*-demethyl-LSD. So far, every specimen that we have analyzed and found to contain LSD has also given a m/z 501 ion current profile peak at the relative retention time corresponding to *N*-demethyl-LSD.

Two other metabolites have been tentatively identified in the volunteer's urine as 13-hydroxy-LSD and 14-hydroxy-LSD. These metabolites are evident in the m/z 531 ion current profile from the gas chromatograph/resonance electron capture ionization mass spectral analysis of the 8–10 h urine, after enzymatic hydrolysis followed by extraction and conversion of the metabolites to their bis(trifluoroacetyl) derivatives (see Figure 3). The concentrations of these metabolites in the volunteer's urine were too low to yield good-quality, full-scan mass spectra. However, we were able to obtain clean, full-scan mass spectra on the hydroxy metabolites generated in the rat liver experiments. The resonance electron capture mass spectra of the trifluoroacetyl derivatives of both metabolites contain only one major peak, the negative molecular ion at m/z 531. Identification of the earlier-eluting metabolite as 13-hydroxy-LSD is based on the following considerations: In a previous study, only the 13-hydroxy metabolite was identified following incubation of LSD with 9000*g* rat liver supernatant (*8*). In our experiments, the predominant hydroxylated metabolite detected in the rat liver incubate had the same relative retention time as that of the earlier-eluting metabolite in the human urine ion current profile. Also, 14-hydroxy-LSD is reported to be less stable than 13-hydroxy-LSD (*10*), consistent with our observation that the relative size of the later-eluting metabolite peak was highly dependent on the conditions of the extraction procedure. For example, addition of sodium bisulfite to the urine during enzymatic hydrolysis inhibited degradation of the metabolite, resulting in a consistently larger chromatographic peak than was obtained when the bisulfite was not added to the urine. Finally, the two hydroxylated metabolites can be separated by thin-layer chromatography and give different colors when visualized with Van Urk reagent (*10*). The thin-layer chromatographic behavior and color reactions of the two hydroxy metabolites that we isolated from rat liver incubate were consistent with those previously reported for 13- and 14-hydroxy-LSD (*10*).

The hydroxylated LSD metabolites appear to be present in the human urine samples as glucuronide conjugates, since

Table I. Significant Ions in the Resonance Electron Capture Ionization Mass Spectra of Perfluoroacyl Derivatives of LSD and *N*-Demethyl-LSD

derivative	mass/charge (% of total ion current)			
	negative molecular ion	fragment ions		
N-trifluoroacetyl-LSD	419 (61)	399 (0.6)	376 (0.6)	322 (1)
N-pentafluoropropionyl-LSD	469 (ND)[a]	449 (19)	147 (40)	
N-heptafluorobutyryl-LSD	519 (ND)	499 (3)	197 (86)	
N,N'-bis(trifluoroacetyl)-*N*-demethyl-LSD	501 (55)	481 (0.3)	404 (0.5)	
N,N'-bis(pentafluoropropionyl)-*N*-demethyl-LSD	601 (7)	581 (13)	453 (8)	434 (6)
		413 (2)	147 (31)	
N,N'-bis(heptafluorobutyryl)-*N*-demethyl-LSD	701 (5)	681 (3)	641 (1)	504 (4)
		484 (5)	197 (46)	

[a] Not detected.

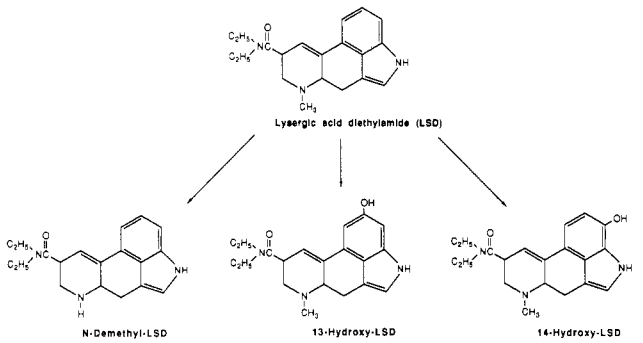

Figure 4. Established and proposed human metabolites of LSD.

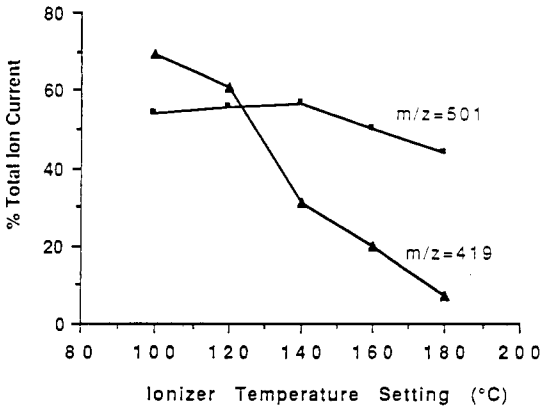

Figure 5. Intensities of negative molecular ions of the trifluoroacetyl derivative of LSD (*m/z* 419) and *N*-demethyl-LSD (*m/z* 501) as a function of the ionizer temperature setting.

they could only be detected if the urine was subjected to β-glucuronidase hydrolysis before extraction and GC/MS analysis.

The structures of LSD and the metabolites identified in this study are shown in Figure 4.

Development of the Analytical Method. It is well-known that attachment of a perfluoroacyl group increases a compound's electron affinity and can result in greatly increased ionization efficiency under electron resonance capture ionization (*15*). We therefore investigated various methods of attaching perfluoroacyl groups to LSD and *N*-demethyl-LSD. We were unable to achieve efficient derivatization of LSD by treatment with trifluoroacetic anhydride. However, derivatization with perfluoroacylimidazoles in the presence of a tertiary amine catalyst was successful.

The most abundant ions in the resonance electron capture ionization mass spectra of various perfluoroacyl derivatives of LSD and *N*-demethyl-LSD are listed in Table I. The trifluoroacetyl derivatives of LSD and *N*-demethyl-LSD (as well as of the tentatively identified 13- and 14-hydroxy-LSD) give mass spectra containing very intense molecular ions and therefore are well suited for high-sensitivity GC/MS analysis. In contrast, the resonance electron capture ionization mass spectra of the pentafluoropropionyl and heptafluorobutyryl derivatives show little or no molecular ion peaks and are dominated by lower-mass fragment ions unsuitable for selected ion monitoring analysis.

The effect of ionizer temperature on the relative intensities of the negative molecular ions of *N*-trifluoroacetyl-LSD and *N*-bis(trifluoroacetyl)-*N*-demethyl-LSD is shown in Figure 5. In this experiment, 100 ng of each of the trifluoroacetyl derivatives was injected into the GC/MS at various ionizer temperatures, and the percentages of total ion current carried by the molecular ions were determined. The temperatures shown in Figure 5 are the ionizer temperature settings on our Finnigan 4500 mass spectrometer; the actual ionizer temperatures were not measured but are reported by the manufacturer to be higher than the settings. The relative intensity of the molecular ion of *N*-bis(trifluoroacetyl)-*N*-demethyl-LSD is not significantly changed over the temperature range investigated. However, the relative intensity of the molecular ion of the *N*-trifluoroacetyl-LSD is strongly affected by the ionizer temperature; the sensitivity for detection of *N*-trifluoroacetyl-LSD is best at an ionizer temperature setting of 100 °C. However, such a low temperature setting can result in chromatographic peak broadening and rapid contamination of the ion chamber. Therefore, an ionizer temperature setting of 120 °C was selected for subsequent analysis in order to minimize fragmentation of the molecular ions, while providing narrow chromatographic peaks.

The selection of the internal standard can be critical to the successful development of a quantitative GC/MS assay. Lysergic acid *N*-methylpropylamide (LAMPA), LSD-$^2H_3$, and LSD-$^2H_{10}$ were evaluated as internal standards for the assay described here. Of these three compounds, LSD-$^2H_{10}$ proved most satisfactory. The LSD-$^2H_3$ available to us was unsuitable because it was contaminated with a small amount of *N*-demethyl-LSD, and therefore interfered with the determination of the metabolite's concentration. Although LSD-$^2H_{10}$ and LAMPA both proved satisfactory as internal standards, we prefer to use LSD-$^2H_{10}$ because LSD and LAMPA are both detected in the *m/z* 419 ion current profile; since the profile is normalized to the largest peak, a small LSD peak can be difficult to visually detect in the presence of a large LAMPA peak, even when LSD and LAMPA are well separated chromatographically.

Because of LSD's propensity to undergo adsorptive losses during gas chromatography, we investigated the coinjection of chemically similar compounds as a means of reducing adsorptive losses. We found methysergide best suited for this purpose. It is readily available and does not give significant ions at the ion currents monitored for LSD or its metabolites. Coinjection of methysergide does appear to improve the sensitivity and reproducibility of the assay, although the

Table II. Extraction Recoveries of LSD and *N*-Demethyl-LSD from Urine

amt of drug added to urine, ng/mL	% recovered (CV[a]) (no. of replicates = 4)	
	LSD	*N*-demethyl-LSD
2.5	86 (2.2)	62 (7.5)
0.5	77 (5.7)	
0.05		60 (9.9)

[a] CV, coefficient of variation.

Table III. Precision and Accuracy for Determination of LSD and *N*-Demethyl-LSD in Fortified Urine Specimens

target concn, ng/mL	measd concn, ng/mL (CV[a])	
	LSD	*N*-demethyl-LSD
	Intraassay (n = 4)	
0.05	0.046 (13)	0.040 (7.5)
0.50	0.44 (2.8)	0.53 (3.6)
2.00	2.03 (5.0)	2.03 (4.6)
	Interassay (n = 4)	
0.50	0.47 (6.8)	0.50 (5.4)
2.00	2.15 (5.7)	1.95 (3.6)

[a] CV, coefficient of variation.

available evidence is not conclusive.

The extraction scheme required enzymatic hydrolysis of the urine prior to extraction in order to hydrolyze the glucuronides of hydroxylated metabolites of LSD. Addition of an antioxidant, such as sodium bisulfite, during hydrolysis improved recovery of the 14-hydroxy-LSD, which has been reported to be susceptible to oxidative degradation (*10*). The recoveries of LSD and *N*-demethyl-LSD were not affected by enzymatic hydrolysis.

The extraction is more complex than the procedure previously described for extraction of LSD from urine (*3*). The additonal cleanup steps provide a relatively clean extract, which is necessary in order to achieve the sensitivities reported here. For example, the 0.1 M ammonium hydroxide wash removes a substance frequently present in urine extracts that can interfere with the LSD peak in the m/z 419 ion current profile.

Recovery data for LSD and *N*-demethyl LSD from urine are shown in Table II. At the concentration range examined, the mean recoveries of LSD and *N*-demethyl-LSD were 81% and 61%, respectively. In each case, the coefficient of variation was less than 10%. The extraction efficiencies were not dependent on concentrations.

Initially, the formation of N-demethyl-iso-LSD during derivatization of the *N*-demethyl-LSD was inconsistent; consequently, the calibration curve for *N*-demethyl-LSD was not linear. However, the amount of *N*-demethyl-iso-LSD formed could be decreased and controlled reproducibly if the residue was cooled to 4 °C prior to addition of the derivatization reagent. When this was done, the calibration curves obtained for LSD and *N*-demethyl-LSD were linear from 0.05 to 5 ng/mL and 0.03 to 5 ng/mL, respectively. Correlation coefficients greater than 0.99 were obtained for both compounds. The mean coefficient of variation over the concentration range was less than 10%.

Precision of the assay at various concentrations is shown in Table III. At each concentration, the intra- and interassay coefficients of variation obtained for LSD and *N*-demethyl-LSD were less than 10%, except at the lowest LSD concentration. In our judgment, the lowest concentrations of LSD and *N*-demethyl-LSD that can be reliably measured by this assay are 0.05 and 0.03 ng/mL, respectively.

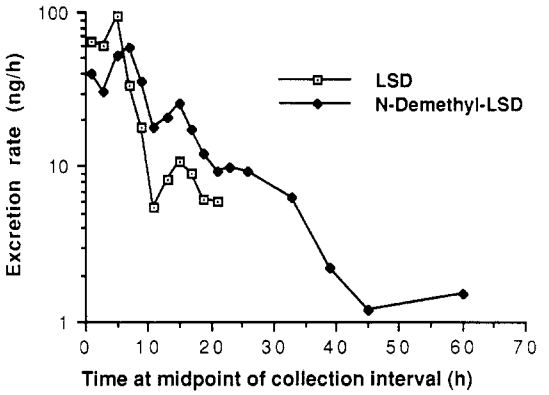

Figure 6. Rates of urinary excretion of LSD and *N*-demethyl-LSD following oral ingestion of LSD (1 μg/kg).

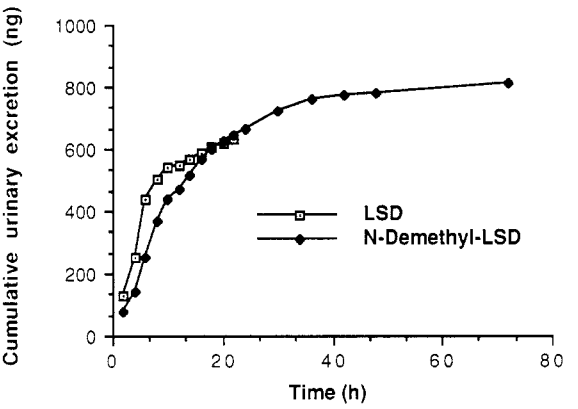

Figure 7. Cumulative urinary excretion of LSD and *N*-demethyl-LSD following oral ingestion of LSD (1 μg/kg).

Table IV. Pharmacokinetic Parameters for LSD and *N*-Demethyl-LSD Following Oral Ingestion of LSD by an Adult Volunteer

parameters	LSD	*N*-demethyl-LSD
elimination half-life, $T_{1/2}$ (h)	3.6	10.0
elimination rate constant, K_e (h^{-1})	0.2	0.1
% of dose excreted in urine	0.9	1.2

APPLICATION OF THE ASSAY

The urinary excretion rates of LSD and *N*-demethyl-LSD following oral ingestion of LSD (1 μg/kg) are shown in Figure 6. The sensitivity of the assay was adequate to permit quantification of LSD and *N*-demethyl-LSD for up to 22 and 72 h, respectively. The rate of excretion of LSD reached a maximum in urine collected from 4 to 6 h after administration, while the maximum rate of excretion of *N*-demethyl-LSD occurred between 8 to 10 h postadministration. Simple pharmacokinetic parameters for LSD and *N*-demethyl-LSD, obtained by analysis of the urinary excretion data using Sigma-minus plots, are shown in Table IV. The elimination half-lives for LSD and *N*-demethyl-LSD were 3.6 and 10.0 h, respectively. Cumulative urinary excretion profiles (Figure 7) show that the amount of LSD and *N*-demethyl-LSD excreted in urine represent 0.9% and 1.2% of the oral dose, respectively.

As a further evaluation of the resonance electron capture ionization method, a group of 32 selected urine specimens from separate individuals was submitted for GC/MS analysis. The group included 10 specimens that were positive for LSD by RIA, using a limit of detection of 0.25 ng/mL, and 22 specimens that showed no detectable LSD-immunoreactive sub-

Table V. GC/MS Detection of LSD and Metabolites in Urine Samples Found to Be RIA-Postive for LSD

sample	RIA measd concn of RIA-reactive substances, ng/mL	GC/MS		
		LSD, ng/mL	*N*-demethyl LSD, ng/mL	13-hydroxy-LSD (peak area rel to int std peak area)
A	2.1	2.1	0.1	ND[a]
B	>2.5	~8.6	0.9	ND
C	1.5	1.3	0.7	ND
D	0.7	0.3	0.3	ND
E	0.8	ND	ND	ND
F	1.0	ND	ND	ND
G	1.8	>2.5	1.2	1.7
H	0.7	0.5	0.9	ND
I	0.7	0.6	1.6	0.1
J	1.5	1.8	0.3	0.4

[a] ND, not detected.

stances. No LSD or LSD metabolites were detected in any of the RIA-negative specimens. The analytical results for the RIA-positive specimens are summarized in Table V. The GC/MS assay detected both LSD and *N*-demethyl-LSD in eight of the RIA-positive specimens, while no LSD or LSD metabolites were detected in two of the RIA-positive specimens. The lack of a reference standard of 13-hydroxy-LSD prevented us from determining the concentrations of this metabolite. Consequently, the numbers listed under 13-hydroxy-LSD in Table V correspond to the area of the peak attributed to 13-hydroxy-LSD relative to the area of the internal standard, LSD-$^2H_{10}$ (5 ng/mL). Chromatographic peaks attributable to 13-hydroxy-LSD were detected in the ion current profiles from only three specimens. However, detection of the 13-hydroxy metabolite was not expected in specimens A and B because those urine samples were not subjected to enzymatic hydrolysis prior to extraction. Only sample G gave ion current profiles that contained a clearly discernible peak attributable to 14-hydroxy-LSD, which appears to be a minor metabolite of LSD in man.

A comparison of the concentrations determined with the resonance electron capture ionization method and a GC/MS assay employing electron ionization (*3*) is shown in Table VI. The urine samples from the volunteer were analyzed by the two methods, each performed by a different analyst. The advantages of the resonance electron capture ionization method over the electron ionization method are that it is more sensitive and it permits simultaneous detection of LSD and three LSD metabolites. However, if the resonance electron capture ionization method is used for confirmation of LSD in urine specimens where there may be legal and disciplinary ramifications, we recommend that a specimen not be reported as positive for LSD unless both LSD and *N*-demethyl-LSD are found to be present. Even though resonance electron-capture ionization is a relatively selective method of ionization, there is a significant potential for interfering peaks in the ion current profiles from urine specimens containing sub-nanogram-per-milliliters concentrations of LSD. The method described here permits simultaneous measurement of both compounds without sacrificing sensitivity or time, and detection of the metabolite provides strong corroboration for identification of the parent drug.

Table VI. Urine Concentrations of LSD Determined by Two Different GC/MS Methods

time interval of urine collection, h	measd LSD concn, ng/mL	
	GC/RECl-MS	GC/EI-MS
0–2	0.75	0.90
2–4	0.43	0.53
4–6	0.58	0.66
6–8	0.72	0.73
8–10	0.35	0.34
10–12	0.14	<0.25
12–14	0.20	*a*
14–16	0.20	*a*
16–18	0.19	*a*
18–20	0.14	*a*
20–22	0.15	*a*

[a] Below the limit of quantitation for the assay.

CONCLUSIONS

We have shown that LSD undergoes metabolic N-demethylation at position 6 in man. Aromatic hydroxylation of LSD at positions 13 and 14 also appears to occur in man. A sensitive gas chromatography/resonance electron capture ionization assay for LSD and *N*-demethyl-LSD in urine has been developed, based on conversion of LSD and *N*-demethyl-LSD to their trifluoroacetyl derivatives. Simple pharmacokinetic data for LSD and *N*-demethyl-LSD, determined by analyzing urine samples from an adult volunteer following administration of 1 μg/kg LSD, suggest that demethylation is a minor metabolic pathway for LSD in man.

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Registry No. LSD, 50-37-3; N-demethyl LSD, 35779-43-2; 13-hydroxy-LSD, 73236-73-4; 14-hydroxy-LSD, 73236-69-8.

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