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The Analysis of Lysergide (LSD): The Development of Novel Enzyme Immunoassay and Immunoaffinity Extraction Procedures Together with an HPLC-MS Confirmation Procedure

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ABSTRACT: A forensic procedure for the screening and confirmation of the presence of lysergide (lysergic acid diethylamide, LSD) in urine is described together with the evaluation of a novel enzyme immunoassay (EIA) and immunoaffinity extraction procedure. Following initial screening using either an established radioimmunoassay (RIA) or a novel EIA procedure, a quantitative estimate is established using a conventional high performance liquid chromatography-fluorescence (HPLC) technique following solid phase extraction. Final confirmation and quantitation, without derivatization, is established using HPLC in combination with electrospray ionization (ESI) mass spectrometry using methysergide as an internal standard. The detection limit of LSD in urine is 0.5 ng/mL. A blind trial confirmed the validity of the results. The choice of internal standard is discussed. Consideration is given to the photosensitivity of LSD solutions. A study of potential interferants in the HPLC-MS confirmation of LSD is presented and shows that for the wide range of compounds studied, there are none that would interfere with this confirmation technique. A comparison is shown between solid phase and immunoaffinity extraction/clean up procedures, and between RIA and EIA screening procedures.

KEYWORDS: forensic science, forensic toxicology, lysergic acid diethylamide (LSD), urine, high performance liquid chromatography-mass spectrometry (HPLC-MS), electrospray ionization (ESI), solid phase extraction, radioimmunoassay, HPLC-fluorescence, photosensitivity, immunoaffinity, enzyme immunoassay

Lysergide (lysergic acid diethylamide, LSD) is one of the most potent hallucinogenic drugs known. Across the world, it is regarded as a drug of abuse and, in the United Kingdom, it is controlled under the Misuse of Drugs Act (1971). The purpose of this work is to establish a valid procedure for confirming and quantifying LSD in urine for forensic purposes.

Illicit LSD is generally found in tablet form or impregnated on small paper squares containing 50 to 100 μg of the substance. Analysis of such seized substances is relatively straightforward and may be accomplished using thin layer and high performance liquid chromatography (HPLC) to separate LSD from related compounds (1). In the case of the detection of LSD in body fluids, this is an extremely demanding analysis due to the low levels of the drug present in such fluids. A typical dose of LSD is around 100 μg, and it is extensively metabolized with only 1% being excreted unchanged in 24 h (2). Typical levels of LSD in urine are 1 to 20 ng/mL within 24 h of such a dose. In addition to very low levels, LSD is photosensitive, relatively involatile, thermally unstable at gas chromatographic (GC) temperatures, and very prone to undergo severe adsorptive losses during GC analysis (3,4).

LSD analysis of urine in the typical forensic laboratory initially involves the use of a rapid screening procedure such as radioimmunoassay (RIA). Because the RIA technique cannot be demonstrated as totally specific for LSD, it must be followed by one or more confirmatory stages to investigate samples indicated as positive. Samples are required to be confirmed above 1.0 ng/mL as a Laboratory of the Government Chemist customer requirement. The technique of HPLC combined with fluorescence detection (5,6,7) has been used for confirmation purposes; however, it is now generally recognized that some form of more absolute identification is necessary. Such identification can be provided by chromatography/mass spectrometry.

The direct gas chromatography-mass spectrometry (GC-MS) analysis of LSD cannot reach the required detection limit (1 ng/mL). This is principally due to the irreversible adsorption of LSD on GC columns, and is compounded by its low volatility and thermal instability. Consequently, in GC-MS work, a derivatization stage is essential, and the use of the trimethylsilyl (TMS) derivative has been documented (6,8,9). The incorporation of an additional stage, derivatization between the extraction and the analysis is not ideal. The chromatography of the TMS derivatives themselves can be difficult, requiring frequent column deactivation (6,8). The use of a glass column is recommended to minimize decomposition (10) and to prevent a false negative result.

In view of the above factors, it is considered that HPLC is far more suitable than GC as a separation technique for trace LSD

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samples. Thus the combination of HPLC and mass spectrometry is an ideal method, provided that elution of the nonderivatized drug can be achieved readily, separation can be achieved from potential interferants such as the isomeric compound lysergic acid N-methyl, N-n-propylamide (LAMPA) and detection at concentrations down to the cutoff of the screening technique or below can be achieved.

Some work has been carried out using LC-MS and has involved ion spray (11) and atmospheric pressure chemical ionization (12). This work was, however, performed on purpose-built instruments and represented investigation of modern LC-MS techniques rather than the analysis of LSD. The present work describes a procedure for the routine analysis of LSD in urine using an RIA screening procedure, further screening and quantitation of those positives by RIA using HPLC with fluorescence detection and final confirmation using HPLC-MS.

The innovative immunochemical techniques of enzyme immunoassay (EIA) and affinity chromatography have several advantages over their established counterparts. For screening, EIA is safer, no special laboratory is required, there is no environmental waste disposal problem, it has a greater shelf life (six months) and is potentially much cheaper per test than RIA. For extraction and cleanup, affinity chromatography is simple to use, yet is able to produce higher purification factors and therefore better instrumental detection limits than are achieved following conventional solid phase extraction; also, efficient sample preconcentration from very low levels is feasible. New methods have been developed as part of this comprehensive approach to the analysis of LSD in urine, and are here compared, as preliminary steps to HPLC-MS confirmation, with the more widely used RIA and solid phase extraction techniques.

Methods

LSD, LAMPA, LSD-d_3, and methysergide were obtained from Alltech Applied Science Laboratories, State College, Pennsylvania, USA. All solvents used for HPLC analyses were HPLC grade. All other chemicals were analytical reagent grade. An LSD stock solution was prepared at a strength of 300 ng/mL in methanol containing 0.05% (v/v) triethylamine. The internal standard solution comprised 0.225 ng/μL of methysergide in methanol containing 0.001% (v/v) triethylamine. LSD calibration standards were prepared by spiking blank urine with varying levels of LSD and with the internal standard at a level of 2 ng/mL, and subjecting the urines to the solid phase extraction procedure described below.

Radioimmunoassay—A commercially available RIA kit for the detection of LSD in urine was used in this study: Coat-A-Count^R LSD (Diagnostic Products Corporation). Reagents, standards, and controls were prepared in accordance with the manufacturer's instructions and the analysis of urine samples was performed accordingly. The cutoff level used for this test was 0.5 ng/mL.

Enzyme Immunoassay—The LSD EIA kit used in this study was developed by The Laboratory of the Government Chemist and Cozart Bioscience Limited (Abingdon, Oxfordshire, United Kingdom), from whom the kit is commercially available. Standards and reagents supplied were used in conjunction with conventional microplate assay equipment. The manufacturer's instructions were followed, and an assay cutoff level of 0.5 ng/mL was adopted.

HPLC-Fluorescence—Extraction: the following solid phase extraction procedure was developed–to 5-mL urine was added 2 mL of 0.1 M phosphate buffer, pH 6.0 (13.61 g potassium dihydrogen ortho phosphate dissolved in 900-mL deionized water, pH adjusted to 6.0 ± 0.1 with 1-*M* potassium hydroxide solution, and made up to 1000 mL with deionized water) and 2 ng/mL of internal standard. The sample was mixed thoroughly and the pH checked. If not between 5.0 and 7.0, it was adjusted appropriately. Methanol (2 mL) was passed through a solid phase extraction column (Bond Elut Certify, Varian Sample Preparation Products, Cambridge, United Kingdom) followed by 2 mL 0.1-*M* phosphate buffer (pH 6.0). The sample was then passed through the column, ensuring that the elution time was not less than 2 min. The column was then rinsed by passing 1 mL of 1-*M* acetic acid (aqueous solution) through the column, drying the column under vacuum for 5 min, passing 6 mL of methanol through the column, and finally drying the column under vacuum for 2 min. The LSD was eluted from the column by passing 2.5 mL of a 2% ammonia solution (2 mL of ammonia solution, specific gravity 0.88, made up to 100 mL with ethyl acetate) through it and collecting the eluate in an amber vial. The eluate was evaporated to dryness at room temperature under a slow flow of nitrogen. To the dry extract was added 250 μL of methanol containing 0.001% (v/v) triethylamine. The vials in which the final extracts were evaporated were pretreated with silanizing agent.

HPLC-Fluorescence—Analysis: A Shimadzu liquid chromatograph was used in combination with a Perkin Elmer LS-4 fluorescence spectrophotometer. Analyses were performed on a Spherisorb ODS column (250 mm by 4.6 mm, 5-μm particle size). The solvent system was prepared by adding 7.7-g ammonium acetate to 900 mL of deionized water, adding 2.5-mL triethylamine and adjusting the pH to 8 (±0.1) with glacial acetic acid. The solution was made up to 1000 mL. From this solution, 700 mL was taken and added to 300 mL of acetonitrile. For fluorescence detection, a flow rate of 2 mL/min was used, the excitation wavelength was 330 nm, and the emission wavelength 420 nm. The monochromator slit width was 10 nm. An injection volume of 10 μL was used.

LC-MS-Extraction: Solid Phase Extraction—Urine samples were extracted as for the HPLC-fluorescence procedure except that 250 μL of the HPLC mobile phase was added to the dry extract. Aliquots of blank urine spiked with 0.5, 1, 2.5, 5, and 10 ng/mL of LSD were taken through the same procedure to provide quantitation standards.

Immunoaffinity Extraction/Cleanup—Affinity gel was prepared in bulk from Protein A Sepharose CL-4B (Pharmacia, St. Albans, Herts, United Kingdom) and polyclonal antiserum against LSD (Cozart Bioscience, Abingdon, Oxfordshire, United Kingdom), then stored for up to six months in the refrigerator before the preparation of individual cleanup cartridges (in press). To each 0.36-g cartridge of affinity gel, 0.2 mL of urine sample was applied and LSD was then allowed 30 min to bind. The unbound material was washed away with 4 mL of phosphate-buffered saline, pH 7.4, followed by 4 mL of water, and then 0.5 mL of absolute ethanol, which was discarded. Finally, LSD was eluted with 1.5 mL of ethanol, the eluate was dried by unassisted evaporation at its boiling point, then reconstituted with 0.1 mL of the HPLC mobile phase. HPLC-MS was performed as with the routine extraction/cleanup method. The internal standard solution was added after the cleanup, because methysergide is not retained by the affinity gel.

LC-MS—The LC-MS system comprised a Finnigan SSQ 7000 mass spectrometer fitted with an electrospray ionization interface (ESI) and coupled to a Waters LC system (Waters 600S controller, 616 pump and 717 autosampler). A Hypersil C_{18}column (125 mm by 3 mm, 3-μm particle size) was used with a flow rate of 0.5 mL/

min. The choice of mobile phase is described in the results and discussion section. An injection volume of 20 μL was used. With the mobile phase selected, an ESI voltage of 4 kV produced an ESI current of about 80 μA. When using the LC-MS instrument in selected ion monitoring (SIM) mode, mass windows of ±0.3 daltons were used with a cycle time of 0.5 s. In scanning mode, the instrument was scanned between 400 and 150 daltons with a cycle time of 0.5 s.

Results and Discussion

RIA and EIA Screening

The RIA screening procedure is reported to show very little cross-reactivity to other drugs, although cross reactivity is likely to occur for metabolites of LSD. A cross-reactivity of 1.5% occurs for LAMPA. This would represent a level of 67 ng/mL of LAMPA in urine at the LSD cutoff level of 1 ng/mL. Such a level of LAMPA in urine is unrealistically high, but to ensure that this is not detected in error, the differentiation of LSD from LAMPA in the subsequent confirmation and quantitation tests is of importance. In the case of EIA screening, extensive cross reactivity testing has been performed with a number of compounds (paper in preparation). This showed that the EIA was comparable with the RIA for cross reactivity (including LAMPA).

The validity of the RIA and EIA procedures was established by means of a blind trial using spiked urine samples. In practical use, the RIA and EIA screens were used in a qualitative mode (positive or negative); however, in this trial, they were tested quantitatively. Figures 1 and 2 shows the RIA and EIA calibration curve respectively. The results of the blind trial are shown in Table 1 from which it can be seen that, for forensic purposes, the screening procedures give satisfactory results in terms of not giving rise to false positive indications, or to false negative indications when used at or above the cutoff level of 0.5 ng/mL for the RIA and EIA screens.

In practical use in this laboratory, over 10,000 urine samples have been screened using the RIA procedure, of which one was positive for LSD (above the cut off level used here of 1 ng/mL). The presence of LSD was confirmed using HPLC-fluorescence followed by HPLC-MS. This very low incidence of positives (1 in 10,000) is not unexpected because LSD is ingested in very small quantities, and its elimination half-life is relatively short [ca. 3.6 h (2)]. The concentration of LSD in the urine of an LSD user generally drops to the sub-ng/mL level within a few hours after ingestion. Extensive metabolism also occurs so that only about 1% of the dose is excreted unchanged (2). These samples were not clinical samples from drug users but were from a population that would be expected to be drug free.

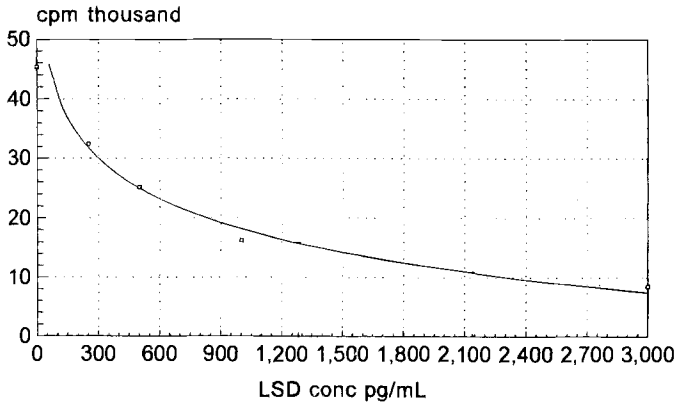

FIG. 1—*RIA calibration curve.*

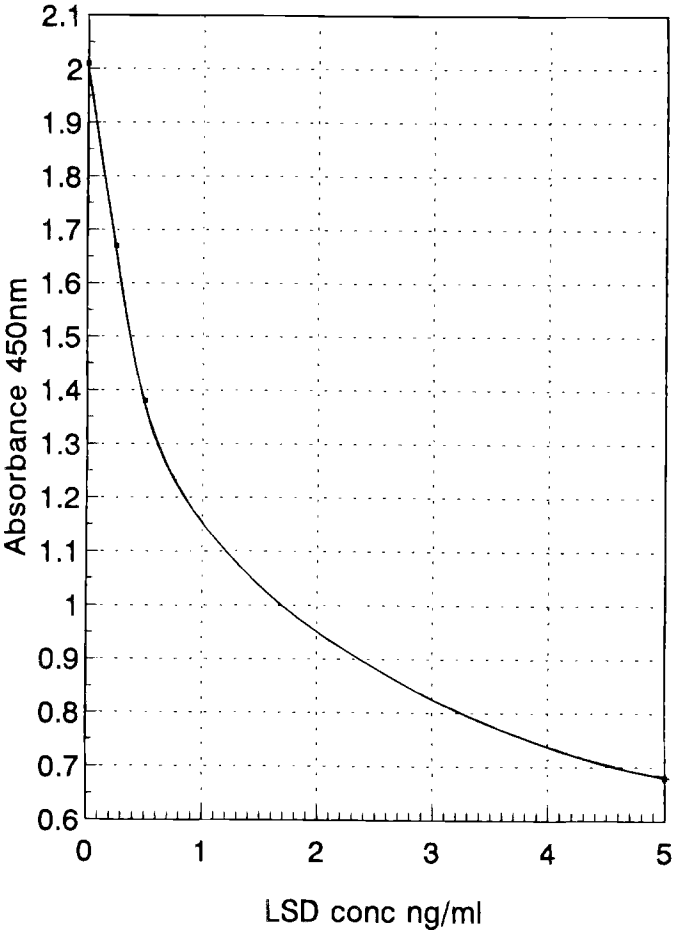

FIG. 2—*EIA calibration curve.*

TABLE 1—*Blind trial results—RIA, EIA, and HPLC-fluorescence.*

Sample Number	Target level LSD ng/mL	RIA, observed Level LSD ng/mL	EIA, observed Level LSD ng/mL	HPLC-fluorescence Observed level LSD ng/mL
1	5.0	>3.0	5.0	4.8
2	0.0	N/D*	N/D*	N/D*
3	1.0	1.0	0.9	1.0
4	0.0	N/D*	N/D*	N/D*
5	1.0	1.0	0.9	1.0
6	3.0	>3.0	2.6	2.7
7	0.0	N/D*	N/D*	N/D*
8	0.3	0.3	0.3	N/D*
9	0.8	0.8	0.7	0.5
10	0.5	0.5	0.5	0.5

*N/D = Not detected.

The EIA has not as yet been used for the routine screening of urine samples, as it is still in validation trials, but approximately 500 urines found to be negative by RIA have also proved negative by EIA. In addition, the urine screened as positive by RIA and confirmed by HPLC-MS was found to be positive by EIA above the cutoff level of 0.5 ng/mL.

The EIA has several advantages over the RIA procedure. Because the technique is nonradio-isotopic, there is a reduced safety risk, and no requirement for disposal of hazardous radio-isotopic waste. The EIA is also suitable for use on a large or small scale, as basic equipment is relatively inexpensive, and automation with sample processors is straightforward. The microplate format means that any number of samples can be run at any one time, up to the capacity of the available equipment. In addition to urine analysis, the EIA kit has potential for use with other forms of body fluids, such as blood and stomach contents. It is anticipated that the EIA method will replace the RIA in routine screening for LSD in this laboratory in the near future.

HPLC-Fluorescence

HPLC-fluorescence is a useful screening technique and can readily be used to give a quantitative estimate of LSD. It is, however, prone to interference from other compounds that possess fluorescent properties, and is considered as a nonspecific technique.

The validity of this technique for quantitative estimation was established using the same solutions as for the RIA and EIA blind trial detailed above. The calibration curve is shown in Fig. 3. The results of the HPLC-fluorescence blind trial are shown in Table 1 from which it can be seen that the use of this technique for screening purposes is satisfactory.

LSD Photosensitivity Study

To further investigate the photosensitive nature of LSD solutions (6), a study was carried out in which solutions of LSD were diluted in urine, placed in clear glass vials and exposed to sunlight and to ordinary (fluorescent) laboratory lighting conditions for varying times. Analysis of these solutions following exposure was carried out by HPLC-fluorescence. The results are shown graphically in Figs. 4 and 5 from which it can be seen that after 13 h exposure to sunlight, the LSD concentration is less than 10% of the original value, whereas those in laboratory (artificial) light were relatively unaffected. This illustrates the need for care in ensuring that LSD solutions are not exposed to sources of ultraviolet light during collection. It is recommended that such solutions are stored in the dark in amber glass containers for maximum stability.

HPLC-MS—Choice of Solvent Systems

In developing a HPLC solvent system for the LC-MS confirmation, it was necessary to ensure that no involatile salts, such as phosphate buffers, were present in the mobile phase because the use of such buffers would cause the LC-MS interface to block. The use of acetate buffers is generally preferred. A published system (6) using 0.25% of triethylamine added to a 70:30 mixture of 0.1-*M* ammonium acetate buffer (pH 8.0) and acetonitrile was initially used. Some modification of this mobile phase resulting from chromatographic tests with the brand of ODS packing employed in the present study showed that a 75:25 mixture of buffer and acetonitrile gave the optimum separation as is shown in Fig. 6. Methysergide, LSD and, LAMPA showed baseline resolution within 15 min. All LSD extracts were reconstituted with the mobile phase rather than with methanol or other solvent. This considerably improved peak efficiency and symmetry.

LSD Ion Monitoring

The ESI mass spectrum of LSD (Fig. 7a) is essentially composed of only one ion, the protonated molecular ion $[M + H]^+$ at 324 daltons. Although monitoring this single ion would provide a sensitive means of detecting LSD, there is a lack of specificity in that

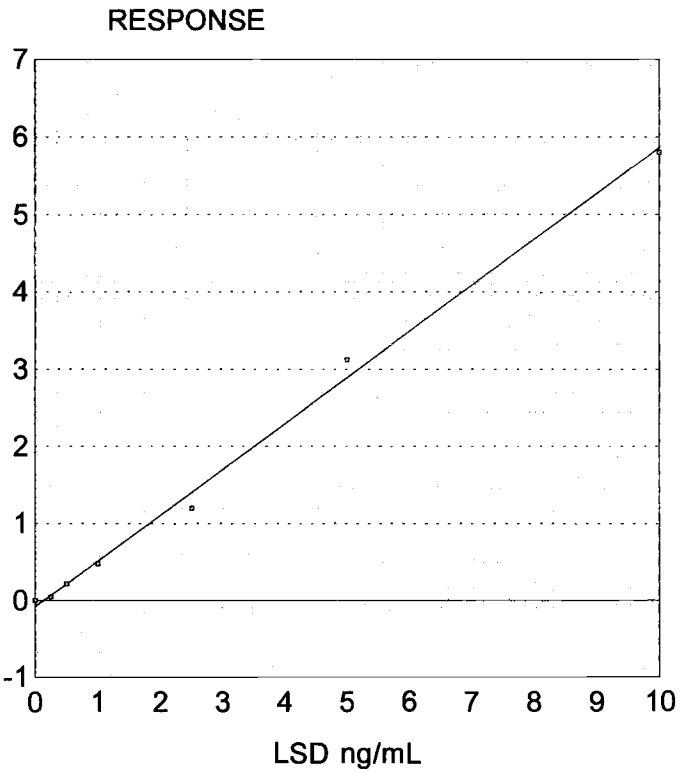

FIG. 3—*HPLC-fluorescence calibration curve.*

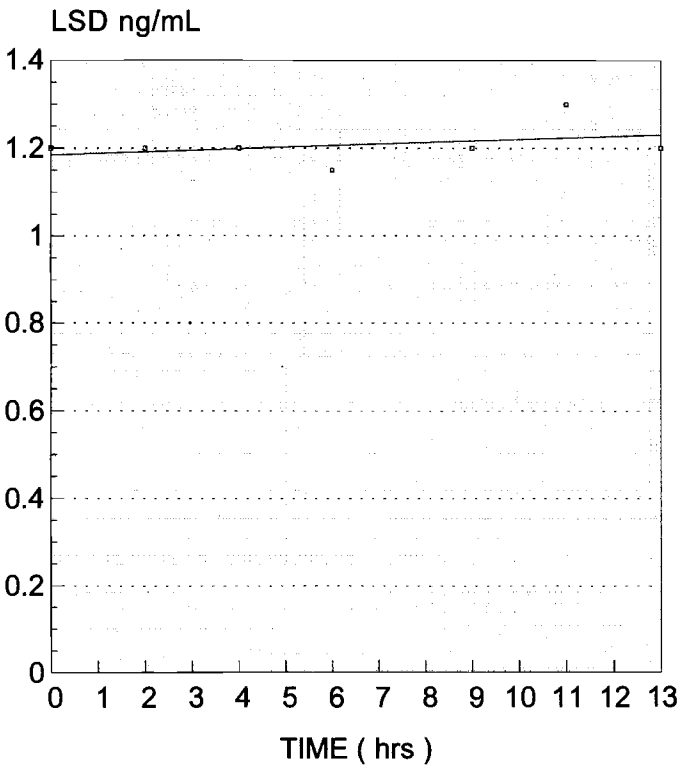

FIG. 4—*LSD in urine photosensitivity study—1.2 ng/mL LSD in urine stored in glass—laboratory lighting.*

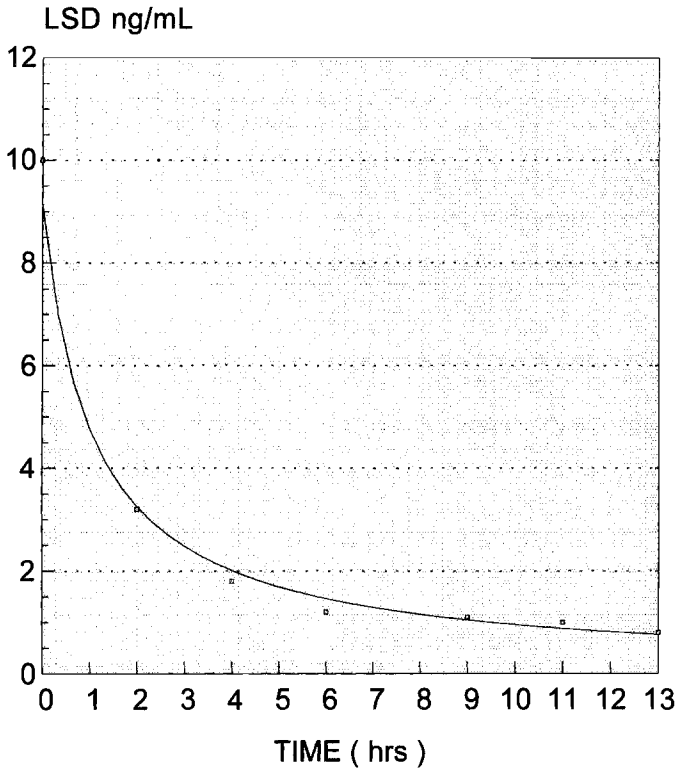

FIG. 5—*LSD in urine photosensitivity study—10 ng/mL LSD in urine stored in glass—sunlight.*

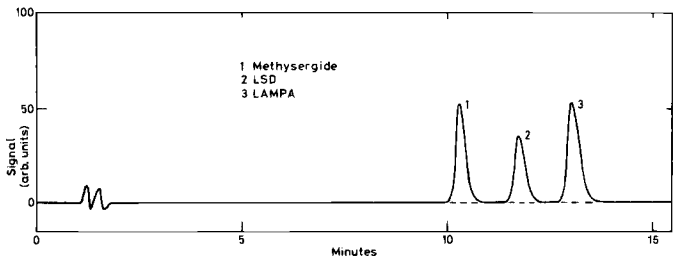

FIG. 6—*HPLC separation of methysergide, LSD, and LAMPA (UV detection).*

other compounds producing an ion at 324 daltons and co-eluting with LSD could lead to false positive results. The mass spectrometer used for this work, in common with other ESI instruments, has a means of inducing post ionization fragmentation. This is achieved, on this instrument, by applying a voltage to an octapole rod assembly between the ESI source and the quadrupole analyser.

It was found that applying 10 to 20 V to this assembly produced a characteristic fragmentation pattern for LSD (Fig. 7b) containing two structurally significant fragment ions of 223 and 281 daltons (see Fig. 8) which are of a relatively high intensity. Examples of other compounds producing an ion at 324 daltons under ESI conditions are Bulan, crotananine, cyamemazine, ethyl p-nitrophenyl benzenethiophosphonate (EPN), lycofawcine, phenylhexylamine, piperidolate, and stylopine. However, even if they were to co-elute with LSD none of them would produce the structurally significant fragment ions at 223 and 281 daltons with the same relationship as in LSD.

It is considered that monitoring the ions of LSD at 223, 281, and 324 daltons provides sufficient evidence for forensic purposes provided that the correct relationship between these ions is maintained. In this context it was observed that the degree of fragmentation was subject to change from day to day (but not during a day). It is thus essential to carry out a daily corrective check of fragmentation voltage versus fragmentation when carrying out LSD analysis to maintain the correct fragmentation pattern. It was also found that the degree of fragmentation varied with different batches of "identical" mobile phase. Hence when using a new batch of mobile phase, the fragmentation voltage must be checked and adjusted appropriately.

Choice of Internal Standard

The choice of internal standard for this analysis was necessarily influenced by the choice of ions to be monitored for LSD. The ideal internal standard is an isotopically labeled analogue of the analyte because its behavior during the analytical process will be identical to that of the analyte. In the case of LSD, the LSD-d_3 analogue has been used in this context (6); however, when carrying out analysis using ESI the fragmentation induced spectrum of the deuterated analogue has ions at 226, 281, and 327 daltons. The presence of a common ion at 281 daltons in both LSD and its deuterated analogue rules out the use of this analogue as an internal standard in forensic analysis. The compound methysergide has been used as an internal standard in the HPLC analysis of LSD (7). The ESI mass spectrum of methysergide produces an intense ion at 354 daltons, $(M + 1)^+$, with no interfering ions at the masses used for monitoring LSD, hence its use was adopted for this work.

Potential Interferants

Although excellent separation was achieved among methysergide, LSD, and LAMPA, it is clear that the potential exists for other compounds to co-elute with LSD together with the possibility that they may have common ions with LSD. Additionally, any compound that interfered with the internal standard would make accurate quantitation of LSD difficult. A study was carried out of compounds that had the potential to interfere with LSD analysis. Clearly, it was impossible to study every compound, and we decided to investigate compounds whose presence was feasible and generally available.

Types of compounds of particular interest included those related to LSD, drugs which are abused by drug addicts or may be used in the treatment of this habit as well as the more common "over the counter" type drugs. The compounds included in the study are shown in Table 2.

The study was carried out by injecting solutions of the above compounds (1 mg/mL) into an HPLC system using the same column type and mobile phase conditions as for the LC-MS studies, but using photodiode array detection. Of major importance were those compounds that co-eluted with LSD (capacity factor[3] (k') 7.64, retention time (t_R) 11.8 min). Those that could interfere with methysergide and LAMPA (k' (relative to LSD) 0.84, 1.13; t_R 10.1 min, 13.1 min, respectively) were also of interest. Any compound with a k' value relative to LSD of ± 0.18 was considered as a potential interferant and was further investigated by LC-MS.

Of the 104 compounds examined, three (methylprednisolone, methaqualone, and oxycodone) fell within this category. A further

[3]The capacity factor (k') is given by the expression $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the compound and t_0 is the retention time of an unretained compound.

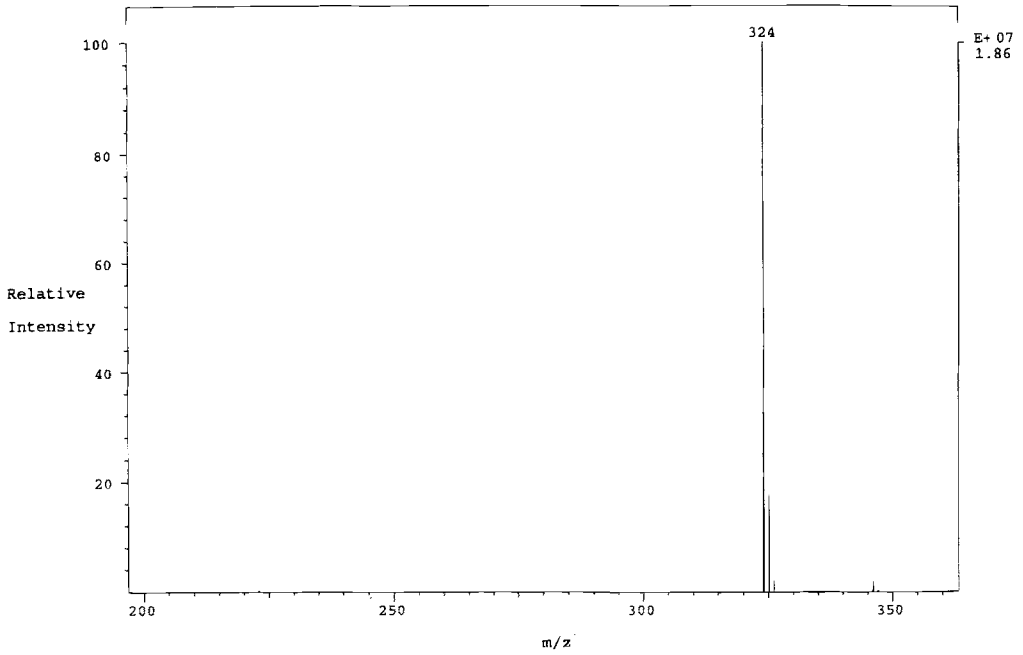

FIG. 7a—*ESI mass spectrum of LSD.*

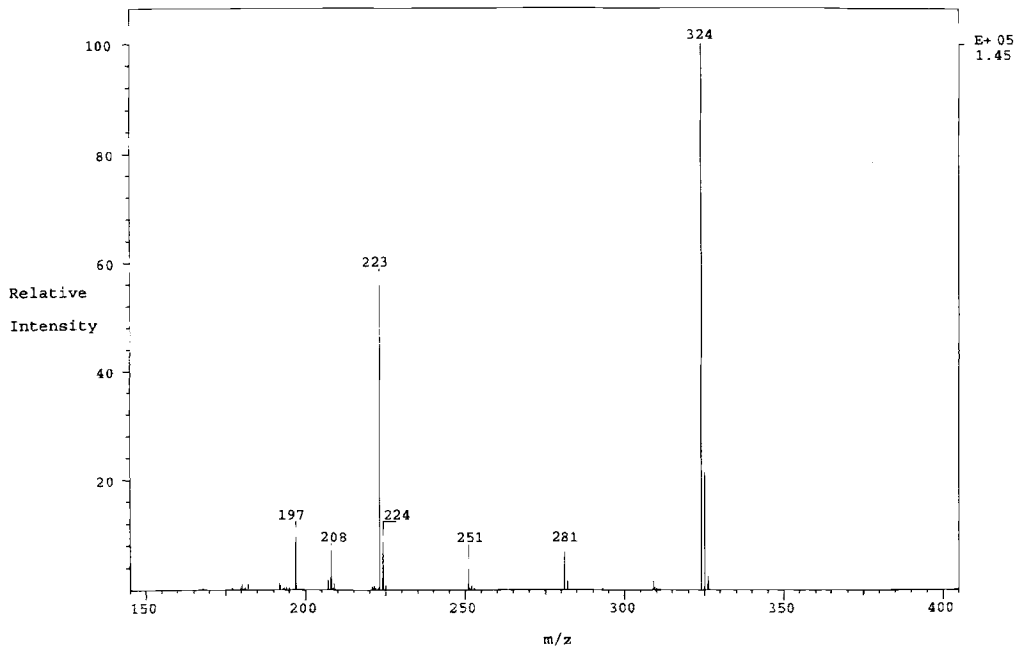

FIG. 7b—*Fragmentation induced ESI mass spectrum of LSD.*

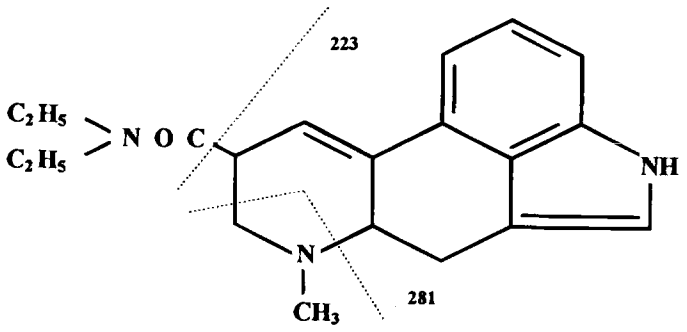

FIG. 8—*Principal fragmentation pathways of LSD under fragmentation induced ESI conditions.*

two (nitrazepam and oxazepam) fell just outside, but were considered of sufficient interest to warrant LC-MS investigation. Table 3 shows the k' (relative to LSD) of the compounds of interest.

The compounds listed in Table 3 were introduced onto the LC-MS system using the same conditions as for LSD. Monitoring the total ion current gave no response for any of these compounds. This suggests that under the conditions used, these compounds are not ionized. It is, hence, concluded that they will not interfere with the detection of LSD using the LC-MS system used for this work. In addition, it should be noted that urines positive for other drugs of abuse do not interfere/cross-react with the screening tests. It should also be noted that this work does not rule out the possibility that the compounds listed in Table 2 might affect the extraction

TABLE 2—*Compounds investigated as potential interferants in the HPLC-MS analysis of LSD.*

Compounds related to LSD	
Lysergol	Ergometrinine
Dihydroergocristine	Ergosine-base
Dihydroergocryptine	Ergosinin-base
Dihydroergotamine	Ergotamine
D-iso-LSD	LSD-S-S-LSD
Ergocristine	LSM oxalate
Ergocryptine	Lysergic acid
Ergocryptinine	
Steroids	
Alphaxalone	Norethandrolone
Alphaxalone acetate	Norethisterone
4-androsten-3,17-dione	Norethisterone acetate
Clobetasol-17 propionate	DL Norgestrol
Deoxycortone	β-Oestradiol
Dexamethasone acetate	Oestriol
Equilin	Oestrone
Ethisterone	Prednisolone
Ethinyl oestradiol	Quinestrol
Ethyloestrone	Stanolone
Fluocontolon capronate	Stilboestrol
Fluoxy-mesterone	Stilboestrol dipropionate
Flurandrenolide	Testosterone
Hydrocort-17-butyrate	Testosterone enanthate
Hydrocortisone	Testosterone cypionate
Mestranol	Triamcimolone hexacetonide
17α-methyltestosterone	Xantinol nicotinate
Methylprednisolone	
Benzodiazepines	
Bromazepam	Nitrazepam
Clobazam	Oxazepam
Diazepam	Oxazolam
Lorazepam	Prazepam
Medazepam	Temazepam
Barbiturates	
Phenobarbitone	Pentobarbitone
Cyclobarbitone	Hexabarbitone
Butabarbitone	Secobarbitone
Other Drugs	
Acetylmorphine	MDMA HCl
Atropine	Methadone
Atropine sulphate monohydrate	Methaqualone
Caffeine	Methylamphetamine
Cannabigerol	Morphine sulphate
Cocaine	N-hydroxy-MDA HCl
Codeine	Nortryptyline
Cyclizine	N-phenyl-1-naphthylamine
Dexedrin	Oxycodone
Dextropropoxyphene	Papaverine
Diethylpropion	Paracetamol
Diphenhydramine	Phenazone
Fentanyl	Phencyclidine
Heroin base	Phentermine HCl
Heroin HCl	Procaine
Hydrocodone bitartrate	Promethazine
Hydromorphone HCl	Tetrahydrocannabinol
Lignocaine	Tropacocaine
MDA	Thebaine base

TABLE 3—*Retention data for possible interferants in LSD analysis.*

Compound	k' (relative to LSD)
Oxycodone	0.90
Methylprednisolone	0.96
Methaqualone	1.18
Nitrazepam	1.29
Oxazepam	1.30

TABLE 4—*Blind trial results: LC-MS (solid phase and immunoaffinity extraction/cleanup).*

Sample Number	Target level LSD ng/mL	Solid phase E/C Observed level LSD ng/mL	Immunoaffinity E/C Observed level LSD ng/mL
1	5.0	4.3	3.2
2	0.0	N/D*	N/D*
3	1.0	1.0	0.9
4	0.0	N/D*	N/D*
5	1.0	1.0	0.7
6	3.0	2.7	2.3
7	0.0	N/D*	N/D*
8	0.3	0.3	0.5
9	0.8	0.7	0.6
10	0.5	0.5	0.6

*N/D = Not detected.

procedure. There is also a possibility that the metabolites of these compounds might affect the extraction or the HPLC detection of LSD.

During the course of this work, a number of different blank urines were extracted and examined by LC-MS as above. No compounds were detected within the k' (relative to LSD) range 0.6 to 1.7. It is concluded that here are no endogenous compounds in urine that will interfere with the detection of LSD after it has been subjected to the solid phase extraction procedure described.

System Validation

System calibration displayed good linearity as is shown in Fig. 9. Calibration and quantitation is based on the area of the 324 daltons $(M + 1)^+$ ion of LSD, ensuring that the 223 and 281 daltons ions are present at the correct relative intensity (± 10% of the predicted intensity). The blind trial used to establish the validity of the RIA and EIA procedures (see Table 1) was also used to ensure that valid results could be obtained using this LC-MS procedure. It was conducted on spiked urine samples that were treated as for the LSD standards. As part of the blind trial, a comparison was made between recoveries by the newly developed immunoaffinity extraction/clean-up and the routinely used solid phase procedure.

The results are shown in Table 4 and demonstrate that good agreement is obtained between the measured values, following the routine solid phase extraction/cleanup and the actual concentrations. To illustrate the signal/noise levels achieved on this system, the ion traces of the principal LSD ions (223, 281, and 324 daltons) are shown in Fig. 10a at the cutoff level of 1 ng/mL in a spiked urine standard extracted by the routine procedure. The ion traces of a urine sample that screened positive for LSD using the RIA procedure and indicated by HPLC-fluorescence to contain 9.0 ng/mL LSD are also illustrated in Fig. 10b. LC-MS quantitation gave an LSD content of 9.7 ng/mL in urine.

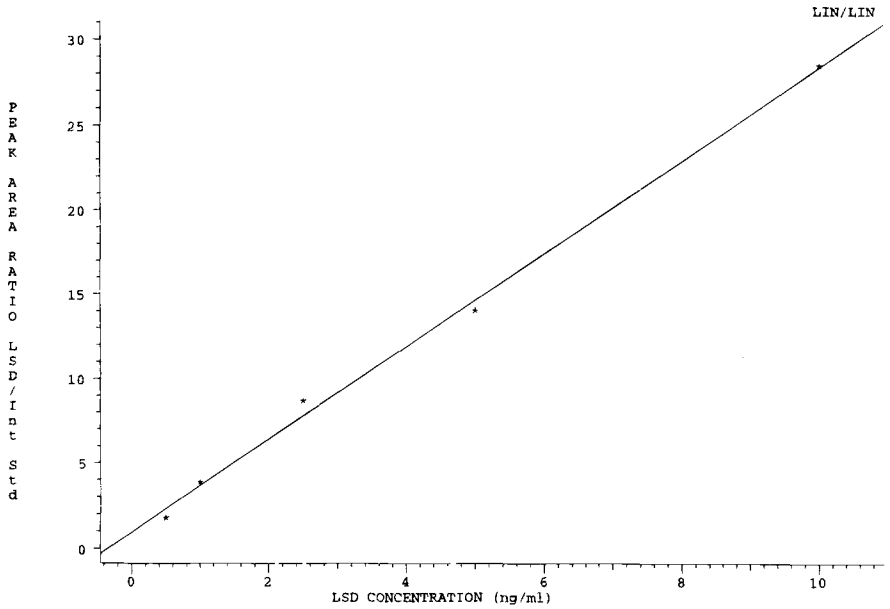

FIG. 9—*LSD calibration graph.*

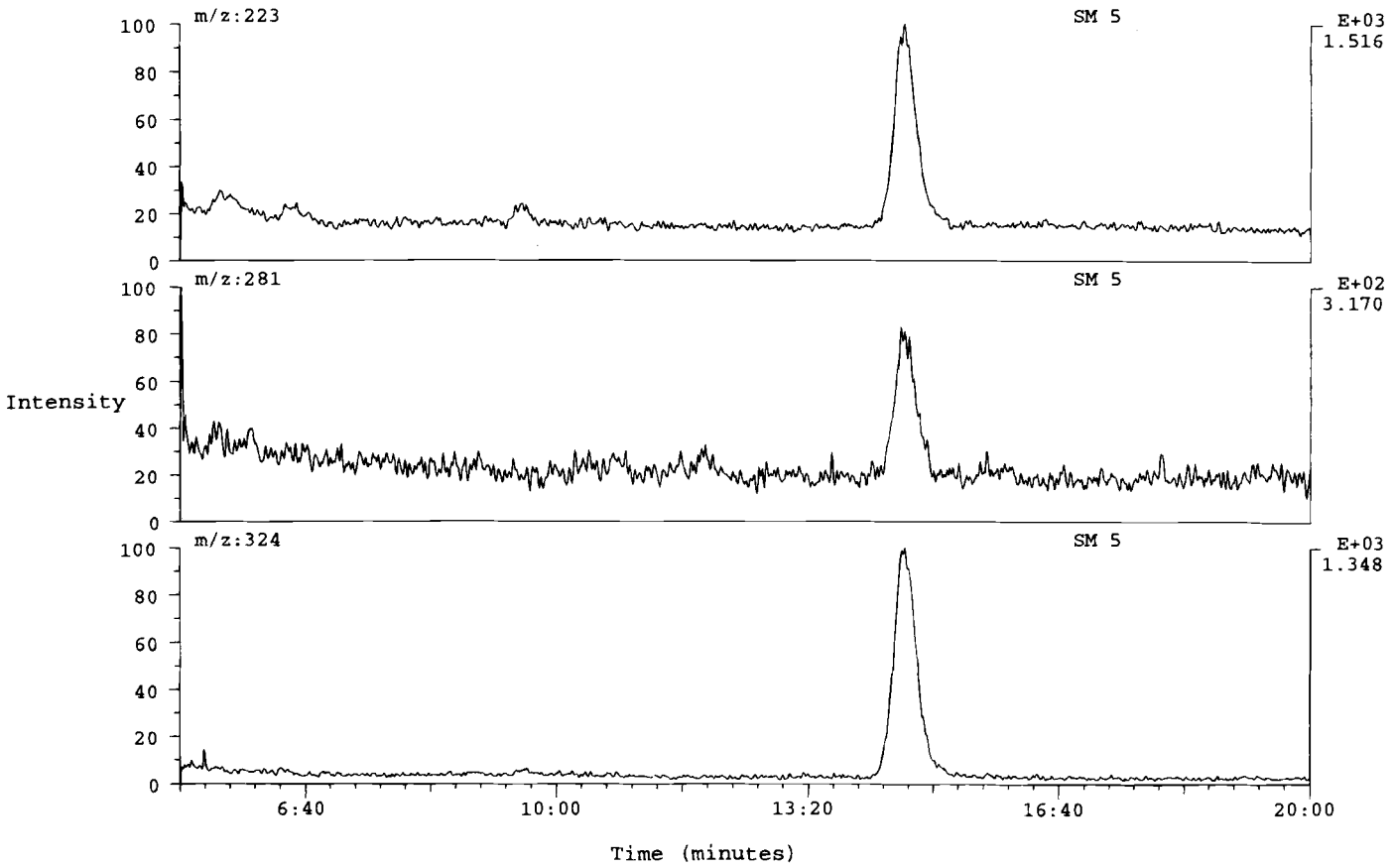

FIG. 10a—*Ion traces of the principal LSD ions at the cutoff level of 1 ng/mL in spiked urine.*

In view of the high concentration of LSD in the sample, the extract was further examined by HPLC-MS with the mass spectrometer recording full spectra. The LSD metabolite N-demethyl-LSD, known to exist in human physiological fluids (2), was identified. The detection of this metabolite provides strong corroboration for the identification of LSD in the sample. Because this metabolite has a greater persistence in body fluids than LSD, then its presence alone could be considered as evidence of LSD use.

The recoveries following the new immunoaffinity extraction/cleanup also correlated well with the actual concentrations of LSD in the spiked urine samples. The method discriminated correctly in all cases between blank, positive, and highly positive (> 1 ng/

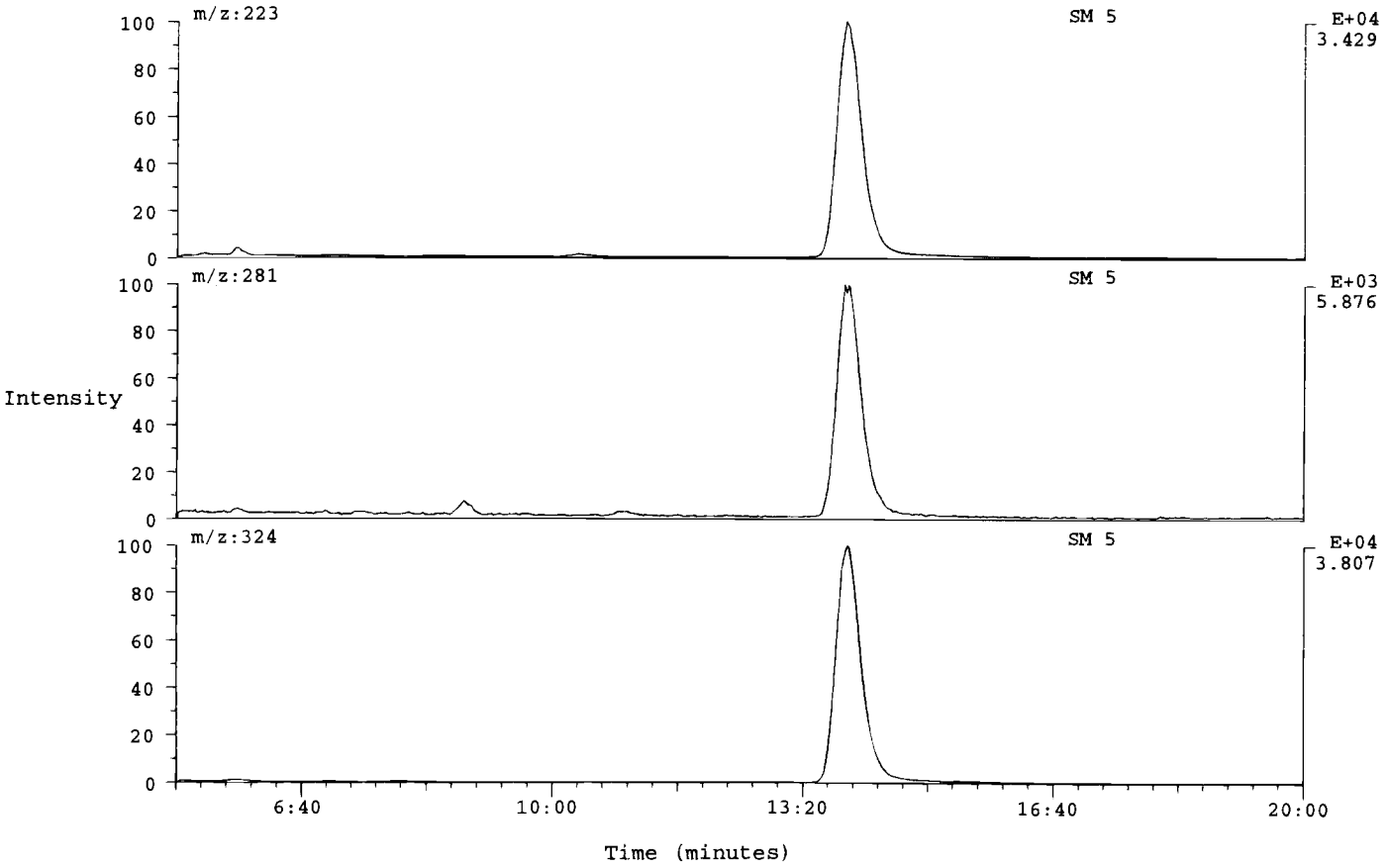

FIG. 10b—*Ion traces of the principal LSD ions in a positive urine sample at a level of 9 ng/mL.*

mL LSD) samples. However, recoveries were less quantitative than with the routine solid phase extraction/cleanup. The fact that recoveries are relatively low for highly positive samples indicates that the limited LSD-binding capacity of the affinity gel is approaching saturation in these cases, which is characteristic of the method. This effect can readily be overcome by increasing the volume of affinity gel in the cartridge, but unless cartridge manufacture is undertaken on a larger scale, in practice, this option is limited by the relatively high cost of immunochemical reagents. Because the highly efficient purification characteristic of immunoaffinity extraction/cleanup can compensate, to some extent, for the limitations of detection techniques, which are less selective than LC-MS, its main application will lie in combination with such techniques.

Conclusion

A forensic procedure for the preliminary screening and for the confirmation of the presence of LSD in urine has been developed. A preliminary evaluation of novel immunochemical screening and sample preparation techniques has also been carried out. The results reported here show that HPLC-MS provides unequivocal identification of underivatized LSD in urine, with quantification down to a detection limit of 0.5 ng/mL.

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