Stephen A. White,[1] *B.Sc.; Alexandra S. Kidd,*[2] *M.Sc.; and Kenneth S. Webb,*[1] *Ph.D.*

The Determination of Lysergide (LSD) in Urine by High-Performance Liquid Chromatography-Isotope Dilution Mass Spectrometry (IDMS)*

REFERENCE: White SA, Kidd AS, Webb KS. The determination of lysergide (LSD) in urine by high-performance liquid chromatography-isotope dilution mass spectrometry (IDMS). J Forensic Sci 1999;44(2):375–379.

ABSTRACT: The use of isotope dilution mass spectrometry (IDMS) has been investigated for the forensic confirmation of lysergic acid diethylamide (LSD) in urine by LC-MS. The advantages of using a deuterated analog of LSD as an internal standard over methysergide are discussed. This study includes a comparison of the electrospray mass spectra of LSD, LSD-d_3 and methysergide, and discusses the choice of suitable ions for use in selected ion monitoring (SIM) mode. An IDMS method is presented for the LC-MS confirmation of LSD in urine, with a limit of quantification (LOQ) of 0.5 ng/mL, reflecting the forensic requirement at this laboratory. Under some circumstances the LOQ can be improved to 0.1 ng/mL. This method is linear in the range tested (up to 10 ng/mL LSD in urine) and has been validated in terms of accuracy and precision.

KEYWORDS: forensic science, forensic toxicology, lysergic acid diethylamide, urine, high performance liquid chromatography-mass spectrometry, electrospray ionization, isotope dilution mass spectrometry

Lysergide (lysergic acid diethylamide, LSD) is an extremely potent hallucinogenic drug with a long history of abuse. A typical dose of LSD is only around 100 μg and it is rapidly and extensively metabolized with only 1% being excreted unchanged in 24 h (1,2). Consequently typical concentrations of LSD in urine are only 1 to 20 ng/mL within 24 h of such a dose.

Analysis of LSD in biological specimens, for example, urine by gas chromatography-mass spectrometry (GC-MS), can be quite exacting due to the irreversible adsorption of LSD on GC columns, together with its low volatility and thermal instability. Some work has been carried out on the use of derivatives for determining low levels of LSD, particularly the tri-methyl silyl (TMS) derivative (3,4), and using this approach body fluid analysis for LSD and related analogs has been carried out using tandem mass spectrometry (5) to obtain sensitivities in the 0.1 ng/mL range. Incorporation of a derivatization step between the extraction and analysis stages lengthens the analytical procedure, introducing more scope for problems. Even chromatography of the TMS derivatives can be difficult, requiring frequent column deactivation. Because of these factors, this laboratory has sought an alternative to the use of GC-MS for the confirmation of LSD (6).

The use of high-performance liquid chromatography (HPLC) enables LSD analysis to be carried out without derivatization and this approach has been used in the investigation of the metabolic products of LSD (2,7). Previous work in this laboratory (8,9) has led to the development of an LC-MS procedure for the confirmation of LSD in urine. This method employed electrospray ionization with methysergide as the internal standard, and was suitable for the baseline separation of LSD and lysergic acid N-methyl, N-n-propylamide (LAMPA). Ideally the most suitable internal standard is an isotopic analog of the analyte since the analog behaves identically to the analyte during extraction and clean-up stages of the analysis, which is the basis of isotope dilution mass spectrometry (IDMS). The use of LSD-d_3 as an alternative internal standard to methysergide has been investigated. Since the electrospray mass spectra (following post-ionization fragmentation) of LSD and LSD-d_3 exhibit similar fragment ions, it became necessary to alter the ions monitored in the selected ion monitoring (SIM) mode when using LSD-d_3 rather than methysergide. An overriding consideration during development was that no compromise in detection limit could be made; neither was it acceptable to monitor less ions in SIM mode, thus reducing selectivity and confidence in the mass spectral confirmation of LSD.

Methods

Instrumentation

A Waters (Watford, UK) LC system, comprising a 600S system controller, 616 gradient pump and 717*plus* autosampler was connected to a Finnigan SSQ7000 mass spectrometer (Finnigan MAT, Hemel Hempstead, UK) fitted with an electrospray ionization source. A spray voltage of 4.5 kV and a multiplier voltage of 1300 V were found to be suitable. The heated capillary located at the exit from the ionization chamber was held at 300°C. For scanning work, a mass range of 150 to 450 daltons was employed, with a scan rate of 0.5 s. For SIM work, mass windows of 0.3 daltons were employed with a cycle time of 0.5 s. By applying a voltage (around 20 V) to a set of octapole rods, situated between the ionization chamber and the quadrupole analyzer, a degree of fragmentation can be induced. This voltage (octapole offset) was optimized at the time of analysis to give a spectrum for LSD exhibiting an $[M + 1]^+$ ion (324 daltons) as the base peak and a 223 daltons fragment ion with 60 to 80% relative intensity. Under these conditions the relative intensity of the third ion monitored for LSD in

[1] Laboratory of the Government Chemist, Queens Road, Teddington, Middlesex, England.
[2] Biomedical Science Division, Kings College London, Strand, London, England.

* The work described in this paper was supported under contract with the Department of Trade and Industry as part of the National Measurement System Valid Analytical Measurement Programme.
Received 2 June 1998; and in revised form 14 Aug. 1998; accepted 17 Aug. 1998.

SIM mode (either 197 or 281 daltons) is approximately 10%. The limiting factor for the LOQ is that the S/N ratio for this third (weakest) ion is 3/1.

A stainless steel column (125 × 3 mm) packed with 3 μm Hypersil ODS (Hewlett Packard, Stockport, UK) was employed with a mobile phase flow rate of 0.5 mL/min and an injection volume of 20 μL.

Reagents

Acetonitrile (HPLC grade), methanol (HPLC grade), ethyl acetate (HPLC grade), glacial acetic acid (AR grade) and ''880'' ammonia solution (AR grade) were obtained from Fisher Scientific (Loughborough, UK). Triethylamine (HPLC grade), sodium azide (AR grade) and ammonium acetate (HPLC grade) were obtained from Merck Ltd. (Lutterworth, UK).

LSD, LSD-d_3, and LAMPA (25 μg/mL solutions in acetonitrile) and methysergide maleate (British Pharmacopoeia standard) were obtained from Promochem Ltd. (Welwyn Garden City, UK).

The Bond Elut Certify solid-phase extraction (SPE) cartridges were obtained from Varian (Walton-on-Thames, UK).

The mobile phase employed consisted of 0.25% triethylamine in 0.1 M aqueous ammonium acetate (pH 8.0)/acetonitrile (75/25) at a flow rate of 0.5 mL/min.

Extraction Protocol

Samples were extracted by SPE using the manufacturer's recommended procedure for basic drugs as recently described (9) with the LSD fraction being collected in silanized amber glass vials. The eluent was evaporated to dryness at room temperature under a stream of nitrogen and the residues reconstituted with 250 μL of mobile phase and stored at 4°C prior to analysis. Aliquots of blank urine and urine spiked with 0.5, 1, 2.5, 5 and 10 ng/mL LSD were taken through the same procedure to provide quantification standards.

Internal Standard Comparison

Blank urine samples were obtained from staff volunteers (male and female), combined and screened by radioimmunoassay (RIA) to confirm the absence of LSD, and 0.05% sodium azide added. This bulk urine was then used to prepare spiked samples containing either 1 ng/mL or 5 ng/mL LSD. Some blank urine was also retained for use in preparing the standard curves.

Multiple extractions of the 1 ng/mL and 5 ng/mL spiked samples were made using either methysergide (2 ng/mL of urine) or LSD-d_3 (2.5 or 1 ng/mL of urine) as the internal standard. For each set of sample extracts (3 sets for each LSD level) multiple injections of one extract were made onto the LC-MS, while for the remainder single injections were made, thus providing within-extraction variation and between-extraction variation, respectively, for each LSD level with each internal standard.

Standard Curve Reproducibility

Once the conditions for the IDMS technique had been established (see Results and Discussion section), extractions on different days of LSD-spiked urine (zero, 0.5, 1.0, 2.5, 5.0 and 10.0 ng/mL), using different blank urine samples each time, were used to produce the standard curves. On each occasion duplicate extractions of a 1.2 ng/mL quality control (QC) spiked urine were made.

Blind Trial

The use of IDMS was validated by means of a blind trial. Eight urine samples spiked at different levels and ten extracts of a ninth spiked sample were extracted and submitted as unknowns for LSD confirmation using LC-IDMS, by two independent analysts. In addition, duplicate extractions of a 1.2 ng/mL QC spiked urine and a calibration curve were provided.

Results and Discussion

Choice of Internal Standard

Previous work in this laboratory (8,9) employed methysergide as the internal standard. As detailed in the Introduction the ideal internal standard is an isotopically labeled analog of the analyte. It was not thought possible to use an isotopically labeled analog of LSD as the internal standard because the ESI fragmentation induced spectrum of the deuterated analog (LSD-d_3) contained a common ion with that of LSD. In order to provide sufficient evidence for forensic purposes it was considered that monitoring the protonated molecular ion $(M + H)^+$ at 324 daltons and the two structurally significant ions at 223 and 281 daltons was necessary. The deuterated analog has ions at 226, 281 and 327 daltons; thus the presence of the common ion at 281 daltons effectively ruled out the use of the analog as an internal standard in this forensic analysis, resulting in the adoption of methysergide. A reexamination of the fragmentation induced spectrum of LSD showed the presence of an ion at 197 daltons of abundance 10 to 15%, as shown in Fig. 1. This ion is not present in the LSD-d_3 spectrum; however, an ion is present at 200 daltons, as shown in Fig. 2, demonstrating that the deuterium atoms are part of that fragment. The fragment ion of LSD at 197 daltons is the protonated addition to the characteristic fragment ion at 196 daltons whose structure has been established (10) and is shown in Fig. 3. Consequently it was considered that monitoring the ions of LSD at 197, 223 and 324 daltons would provide sufficient evidence for forensic purposes to confirm the presence of LSD provided that the correct relationship between these ions is maintained. IDMS quantification of LSD is then carried out based on the ratio of the abundances of the 324 daltons ion of LSD and the 327 daltons ion of LSD-d_3.

The definition of the LOQ of this procedure is that it is the minimum concentration at which a spiked urine specimen satisfies both quantitation and ion ratio requirements. For this procedure the LOQ is 0.5 ng/mL, which also satisfies the requirement of this laboratory of a limit of 1 ng/mL. The 197 daltons ion of LSD has only a 10 to 15% intensity relative to the base peak consequently if only two ions were monitored (223 and 324 daltons) the LOQ would be improved by a factor of five to 0.1 ng/mL (the 223 daltons ion has an intensity relative to the base peak of some five times that of the 197 daltons ion). Such a procedure would be acceptable, for example, in LSD dosing experiments and other nonforensic work where it is clear that LSD is present.

Internal Standard Comparison

Since only a very low number of urine samples were indicated as positive for LSD by this laboratory's routine screening procedure (8), the work described in this paper was carried out using spiked urine in order to generate sufficient samples for a comprehensive study.

A comparison was carried out between the analysis of LSD using methysergide as internal standard and LSD-d_3 as internal standard.

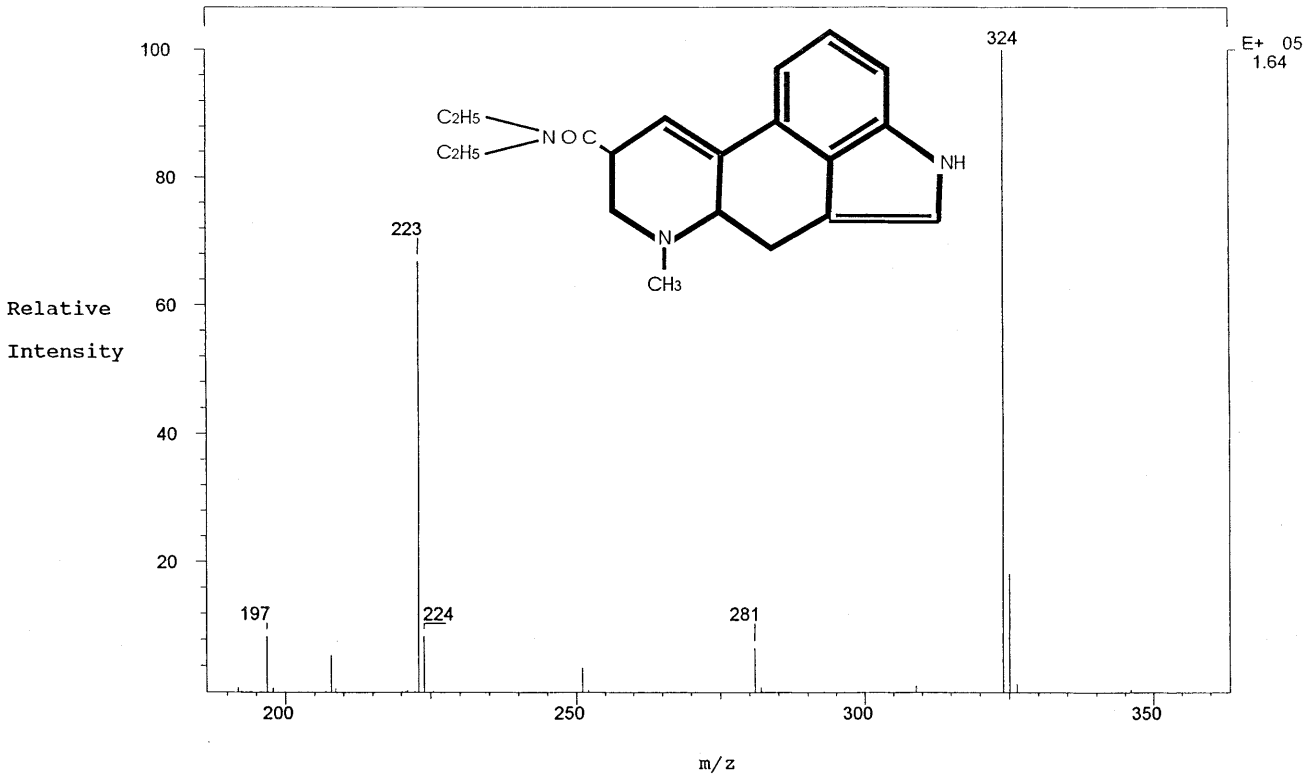

FIG. 1—*Structure and fragmentation induced ESI mass spectrum of LSD.*

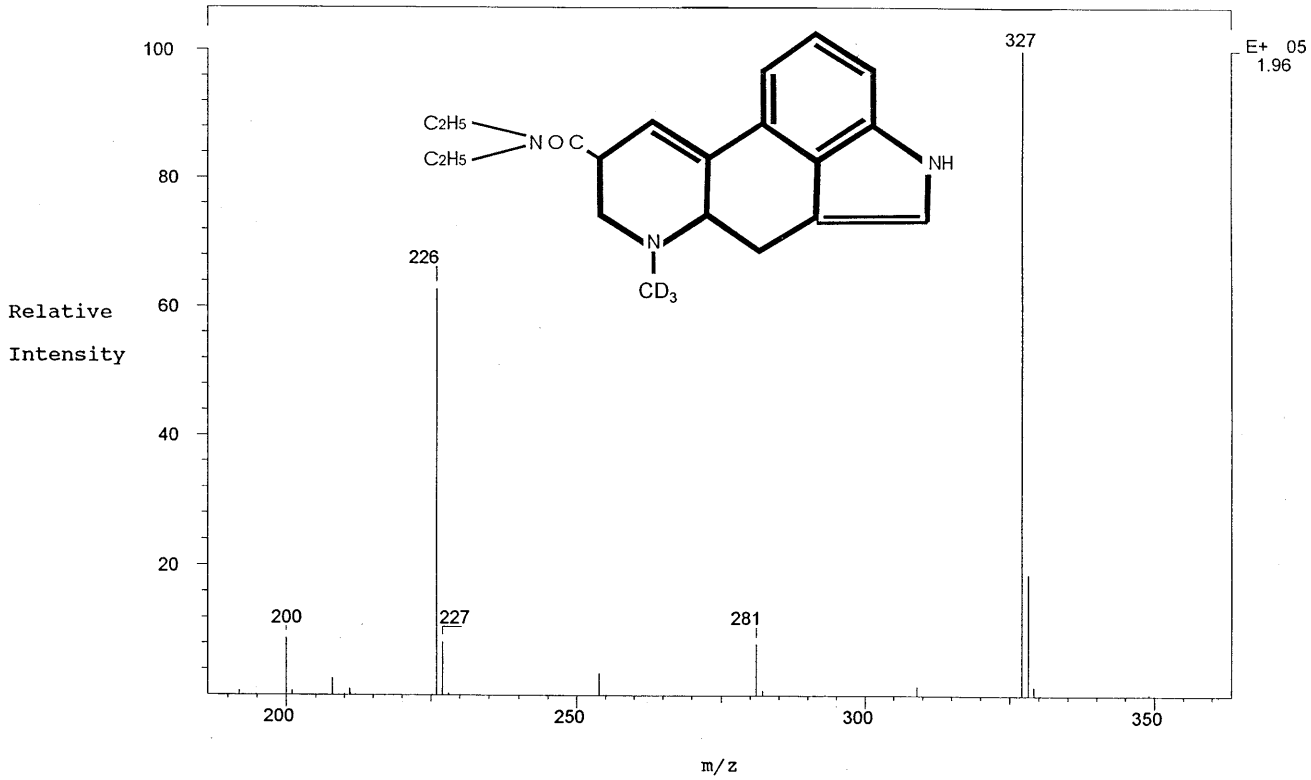

FIG. 2—*Structure and fragmentation induced ESI mass spectrum of LSD-d_3.*

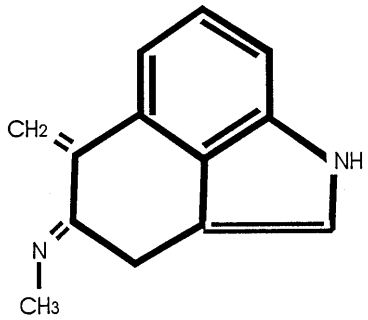

FIG. 3—*Structure of 196 daltons fragment ion of LSD.*

TABLE 1*a*—*Single injection from multiple extracts (n = 10), urine spiked at 1 ng/mL level.*

Internal Standard	Mean LSD, ng/mL	Standard Deviation, ng/mL	RSD%
Methysergide 2 ng/mL	0.79	0.09	11.6
LSD-d_3 1 ng/mL	1.18	0.03	2.4
LSD-d_3 2.5 ng/mL	1.15	0.05	4.0

TABLE 1*b*—*Multiple injections (n = 10) from a single extract, urine spiked at 1 ng/mL level.*

Internal Standard	Mean LSD, ng/mL	Standard Deviation, ng/mL	RSD%
Methysergide 2 ng/mL	1.11	0.19	17.0
LSD-d_3 1 ng/mL	1.16	0.03	2.7
LSD-d_3 2.5 ng/mL	1.12	0.02	2.1

TABLE 2*a*—*Single injection from multiple extracts (n = 10), urine spiked at 5 ng/mL.*

Internal Standard	Mean LSD, ng/mL	Standard Deviation, ng/mL	RSD%
Methysergide 2 ng/mL	4.56	0.30	6.5
LSD-d_3 1 ng/mL	4.98	0.08	1.5
LSD-d_3 2.5 ng/mL	5.11	0.21	4.1

TABLE 2*b*—*Multiple injections (n = 10) from a single extract, urine spiked at 5 ng/mL.*

Internal Standard	Mean LSD, ng/mL	Standard Deviation, ng/mL	RSD%
Methysergide 2 ng/mL	4.92	0.39	8.0
LSD-d_3 1 ng/mL	5.07	0.07	1.3
LSD-d_3 2.5 ng/mL	5.11	0.12	2.4

The comparison was carried out at two levels of LSD (1 and 5 ng/mL) in spiked urine and the results are shown in Table 1 (LSD 1 ng/mL) and Table 2 (LSD 5 ng/mL).

It is clear from these results that the adoption of LSD-d_3 as the internal standard improves both accuracy and reproducibility of measurement (by up to a factor of 5) compared to the use of methysergide. The improvement occurred for both the between extraction variation (Tables 1*a* and 2*a*) and within extraction variation (Tables 1*b* and 2*b*). It was hence decided to adopt the use of LSD-d_3 as internal standard, at a level of 1 ng/mL. The level of 1 ng/mL was chosen as this would produce the ideal abundance ratio of 1:1 for the $(M + 1)^+$ ions of LSD and LSD-d_3 at the regulatory cut off level of 1 ng/mL, where measurement is most critical.

Standard Curve Reproducibility

A study was carried out of linearity and reproducibility using LSD-d_3 at a level of 1 ng/mL as internal standard. Spiked urine standards were used in the duplicate LSD determination of a 1.2 ng/mL QC spiked urine. Six standard curves, prepared on different days using different blank urine on each occasion, gave consistent data. Good linearity was obtained within the range tested (0.5 to 10 ng/mL), with a mean correlation coefficient of the six curves of 0.9997 and a mean RSD of less than 4% for the LSD determinations demonstrating the good reproducibility achieved when using LSD-d_3 as internal standard.

Blind Trial

In order to validate the use of LSD-d_3 as an internal standard a comprehensive blind trial was carried out. The results are summarized in Tables 3 and 4. Statistical analysis of the data in Table 3 was carried out and a paired comparison t-test showed that there was no significant difference at the 95% confidence level between:

—the results obtained by Analyst 1 and Analyst 2 for samples A–H.
—the results obtained by Analyst 1 and the target levels for samples A–H.
—the results obtained by Analyst 2 and the target levels for samples A–H.

Statistical analysis of the data summarized in Table 4 was carried out and an F-test showed that there was no significant difference

TABLE 3—*Blind trial results: two independent analyses of spiked urine extracts plus quality control (QC) extracts.*

Sample	Target Level	Analyst 1 Observed Level LSD, ng/mL	Analyst 2 Observed Level LSD, ng/mL
QC Spike Y	1.20	1.30	1.33
A	0	N/D*	N/D*
B	0.90	0.97	0.96
C	1.10	1.35	1.32
D	1.50	1.66	1.66
E	1.00	1.05	1.05
F	9.10	8.52	8.49
G	0.80	0.86	0.93
H	0	N/D*	N/D*
QC Spike Z	1.20	1.30	1.33

* N/D = Not detected.

TABLE 4—*Blind trial results: summary of two independent analyses of ten extracts of a spiked urine at level of 3.5 ng/mL.*

	Analyst 1 Observed Level LSD, ng/mL	Analyst 2 Observed Level LSD, ng/mL
Mean	3.59	3.42
Standard deviation	0.05	0.06
RSD%	1.4	1.7

at the 97.5% confidence level between the two variances calculated from the independent sets of data from Analyst 1 and Analyst 2. These results demonstrate that the use of LSD-d_3 as internal standard for the forensic analysis of LSD in urine provides greater accuracy and better reproducibility when compared with the use of methysergide as internal standard (8,9).

Conclusion

IDMS has been applied to the forensic confirmation of LSD in urine using LC-MS. The use of an isotopic analog (LSD-d_3) as an internal standard has been demonstrated to give a greater precision, both within extraction and between extraction, when compared with the use of methysergide (8,9). Similarly, the repeatability of the standard calibration curve is greatly improved, as are the results obtained by blind trial. The presence of common ions in the spectra of LSD and LSD-d_3 required the reselection of a third ion for measurement in SIM mode. This was achieved without compromising the LOQ of 0.5 ng/mL LSD in urine obtained using methysergide as the internal standard.

Acknowledgments

The authors would like to thank Peter Stokes and David Carter for their valuable contribution to the processing of the experimental data.

References

1. Lim HK, Andrenyak D, Francom R, Foltz R, Jones T. Quantification of LSD and N-demethyl-LSD in urine by gas chromatography/resonance electron capture ionization mass spectrometry. Anal Chem 1988;60:1420–5.
2. Cai J, Henion J. Elucidation of LSD in vitro metabolism by liquid chromatography and capillary electrophoresis coupled with tandem mass spectrometry. J Analyt Toxicol 1996;20:27–37.
3. Francom P, Andrenyak D, Heng-Keang L, Bridges RR, Foltz RL. Determination of LSD in urine by capillary column gas chromatography and electron impact mass spectrometry. J Analyt Toxicol 1988;12:1–8.
4. Paul BD, Mitchell JM, Burbage R, Moy M, Sroka R. Gas chromatographic-electron-impact mass fragmentometric determination of lysergic acid diethylamide in urine. J Chromatogr 1990;529: 103–12.
5. Nelson CC, Foltz RL. Determination of lysergic acid diethylamide (LSD), iso-LSD, and N-demethyl-LSD in body fluids by gas chromatography/tandem mass spectrometry. Anal Chem 1992;64: 1578–85.
6. Gough TA, Baker PB. Identification of major drugs of abuse using chromatography. J Chromatog Sci 1982;20:289–329.
7. Cai J, Henion J. On-line immunoaffinity extraction-coupled column capillary liquid chromatography/tandem mass spectrometry: trace analysis of LSD analogs and metabolites in human urine. Anal Chem 1996;68:72–8.
8. Webb KS, Baker PB, Cassells NP, Francis JM, Johnston DE, Lancaster SL, et al. The analysis of lysergide (LSD): The development of novel enzyme immunoassay and immunoaffinity extraction procedures together with an HPLC-MS confirmation procedure. J Forensic Sci 1996;41:938–46.
9. White SA, Catterick T, Harrison ME, Johnston DE, Reed GD, Webb KS. Determination of lysergide in urine by high-performance liquid chromatography combined with electrospray ionisation mass spectrometry. J Chromatogr B 1997;689:335–40.
10. Bellman SW. Mass spectral identification of some hallucinogenic drugs. J AOAC 1968;51:164–75.

Additional information and reprint requests:
Dr. Kenneth S. Webb
Laboratory of the Government Chemist
Queens Road
Teddington
Middlesex, TW11 OLY
England