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Determination of lysergide in urine by high-performance liquid chromatography combined with electrospray ionisation mass spectrometry

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Abstract

A quantitative method which avoids derivatisation is described for the determination of lysergide (LSD) levels in urine. Sample preparation included addition of methysergide as an internal standard followed by solid-phase extraction. LSD was analysed on a system consisting of a C_{18} stationary phase and a mobile phase of 0.1 *M* acetate buffer pH 8.0–acetonitrile–triethylamine (75:25:0.25, v/v). LSD was detected by electrospray ionisation mass spectrometry with selected ion monitoring. The quantification limit was 0.5 ng/ml and the method was linear up to 10 ng/ml of LSD in urine.

Keywords: Lysergic acid diethylamide; Electrospray ionisation

1. Introduction

Lysergic acid diethylamide (LSD) is a psychoactive drug with a long history of abuse. It is generally impregnated on small paper squares containing 50–100 μg of the substance, sufficient to produce a "trip". In this laboratory alone, several thousand doses are received for examination every year.

Confirmation of a controlled drug in a biological sample is ideally performed by a hyphenated mass spectrometric technique because this affords both retention time and spectral information. Confirmation of LSD is not trivial, not least because the isomeric compound lysergic acid N-methyl, N-*n*-propylamide (LAMPA), which is itself a controlled drug, has a virtually identical mass spectrum. LSD and LAMPA can be separated by GC [1], and GC–MS has been applied to non-biological samples [2].

Urine samples are screened in this laboratory for controlled drugs by the enzyme multiplied immunoassay technique (EMIT) [3] or by radio immunoassay (RIA) [4] and most classes, for example opiates, can then be confirmed by GC–MS. LSD, however, is highly metabolised, generally only about 1% being excreted unchanged in urine [5,6]. Positive urine samples therefore typically contain the analyte at only nanogram/ml levels. The cut-off level for the detection of LSD by RIA applied in this laboratory is 1 ng/ml, with positive samples requiring confirmation, although the technique can detect lower levels (down to 0.5 ng/ml).

LSD has limited stability to heat, light and acid and is readily adsorbed onto glass. Furthermore, the compound exhibits a degree of irreversible adsorp-

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tion on GC columns and so direct GC–MS is not capable of reaching the necessary detection limits. Some studies have indicated that LSD can be determined at low levels by GC–MS of the trimethylsilyl (TMS) derivatives [7,8]. Clearly, incorporation of a derivatisation step between the extraction and the analysis is not ideal. Even chromatography of the TMS derivatives can be difficult, requiring frequent column deactivation [7,8]. Because of these factors, this laboratory does not recommend the use of GC for the confirmation of LSD [9].

HPLC is by far a more suitable separation technique for trace LSD samples. Fluorescence detection is capable of meeting the detection limits required, but is much less specific than mass spectrometry and a fluorimetric response is subject to interference by other compounds [10]. An effective confirmatory method based on LC–MS, would need to allow elution of the non-derivatised drug, separation of it from LAMPA and specific detection at concentrations down to the cut-off of the screening technique or below. The most promising work so far published has involved atmospheric pressure ionisation mass spectrometry [6,11–13], with the earlier work [11,12] being performed on one-off modified instruments representing investigation of modern LC–MS techniques rather than the analysis of LSD.

This paper presents an LC–MS method for the routine detection and quantification of LSD in urine down to 0.5 ng/ml, using the $[M+1]^+$ ion (m/z 324) for quantification, based on positive ion electrospray ionisation (ESI) mass spectrometry. The target detection limit was 1 ng/ml. This was the level specified by the laboratory's customers and broadly corresponds to the level found in urine 12 h after a typical dose [5]. Choice of chromatographic system, sample handling and mass spectral conditions are discussed, along with the choice of internal standard and the results of a blind trial.

2. Experimental

The chromatographic separation was developed on an LC system including a Perkin-Elmer LS-4 fluorescence spectrometer, which was operated with excitation and emission wavelengths of 330 and 420 nm, respectively. All LC and LC–MS work employed Hypersil C_{18} columns; initially a 10 cm×3 mm column packed with 5 μm phase proved suitable. For later work when methysergide was employed as an internal standard, 12.5 cm×3 mm columns packed with 3 μm phase were substituted to provide increased resolution of it from LSD. All solvents employed for HPLC analyses were HPLC grade.

The LC–MS system comprised a Finnigan SSQ7000 mass spectrometer fitted with an electrospray ionisation interface and coupled to a Waters LC system (Waters 600S controller, 616 pump and 717 autosampler). All LC–MS work was performed with a flow-rate of 0.5 ml/min and, with the mobile phase selected, an ESI voltage of 4 kV produced an acceptable ESI current of about 80 μA.

For SIM work, mass windows of 0.3 a.m.u. were employed with a cycle time of 0.5 s. For scanning work, the instrument was scanned between m/z 400 and 150, with a cycle time of 0.5 s.

LSD, LSD-d_3 and LAMPA were obtained from Alltech Applied Science Laboratories (State College, PA, USA). Methysergide (as the maleate salt) was from Sandoz Pharmaceuticals (Camberley, UK). N-Demethyl LSD (as the fumarate salt) was from Salford Ultrafine Chemicals and Research (Manchester, UK). The solid-phase extraction cartridges were Bond Elut from Varian (Walton-On-Thames, UK).

Samples were treated using the manufacturer's recommended procedure for basic drugs, as follows: Urine samples were refrigerated until immediately before extraction. An aliquot (5 ml) of each was then treated with a 0.1 *M* phosphate buffer and internal standard (10 ng) was added. The pH was then adjusted, if necessary, to lie in the range pH 5–7. The liquid was transferred to a solid-phase extraction cartridge, pre-treated with methanol and 0.1 *M* phosphate buffer. The cartridge was then rinsed with acetic acid and methanol. LSD was eluted with a 2% solution of ammonia in ethyl acetate. The eluent was evaporated to dryness in silanised amber glass vials at room temperature under a stream of nitrogen. Samples were reconstituted with 250 μl of mobile phase shortly before 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, which were injected

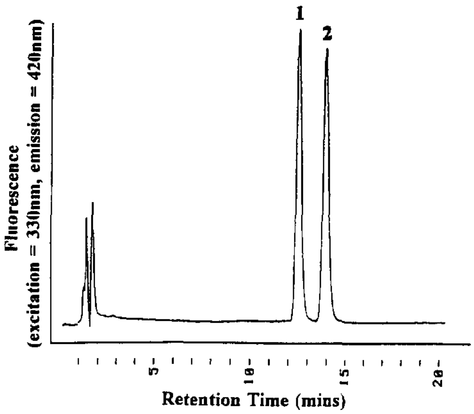

Fig. 1. Separation of LSD and LAMPA (fluorescence detection). Peak identities: 1=LSD; 2=LAMPA.

before and after sample extracts to provide duplicate calibration points for each standard.

3. Results and discussion

Several LC methods incorporating phosphate buffers have previously been employed to separate LSD and LAMPA [14–16]. However, an overriding consideration for most LC–MS interfaces, including ESI, is that the mobile phase should not include involatile salts. Phosphates are always avoided and acetates are generally preferred. Another system [7] referred to a mobile phase consisting of 0.25% triethylamine added to a 70:30 mixture of 0.1 *M* ammonium acetate buffer (pH 8.0) and acetonitrile. Fine tuning of this mobile phase with the make of ODS packing employed in the present study showed that a 75:25 mixture of buffer and acetonitrile gave the optimum separation, with LSD and LAMPA being baseline resolved in 15 min. Fig. 1 illustrates the separation of the two compounds on an HPLC-fluorescence system. In any HPLC analysis, samples are ideally dissolved in the mobile phase. If LSD extracts were reconstituted in methanol or ethyl acetate, the reduction in peak efficiency and symmetry was particularly severe.

One feature of ESI is that the spectra produced by most compounds are more like chemical ionisation spectra than electron impact spectra. The technique produces $[M+1]^+$ ions or adduct ions such as $[M+Na]^+$ and $[M+NH_4]^+$. Sometimes, both pseudo-molecular ions and adduct ions are formed, but there

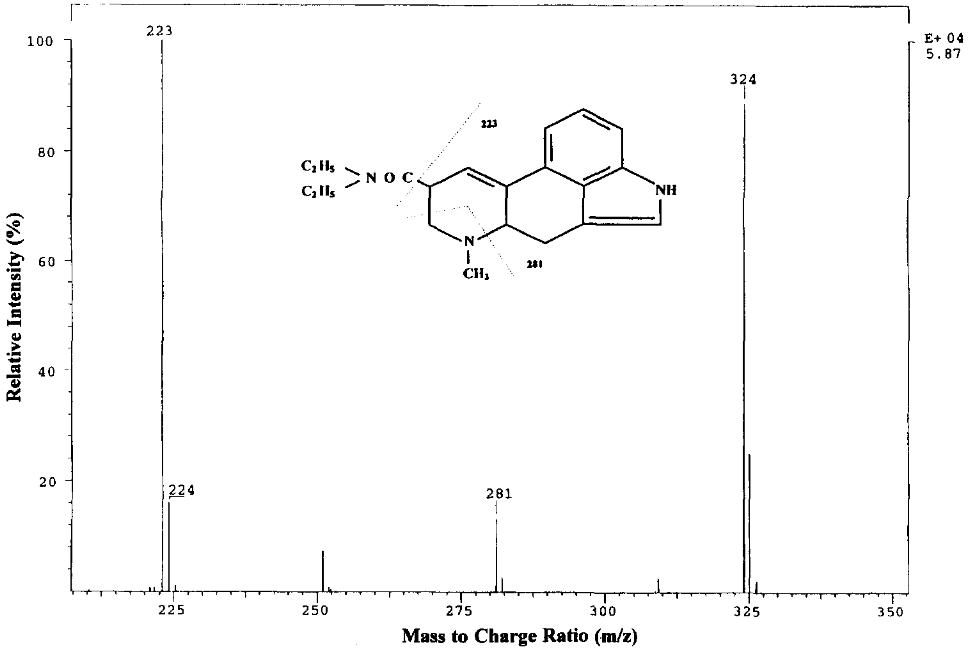

Fig. 2. Electrospray ionisation spectrum of LSD and proposed fragmentations.

are frequently no fragment ions of appreciable intensity. Commercial ESI interfaces therefore incorporate a method of allowing fragmentation to be induced post-ionisation. The instrument employed had a set of octapole rods between the ionisation chamber and the quadrupole analyser rods. If a voltage is applied on the octapole rods, a degree of fragmentation can be induced. For forensic analysis in particular, it is desirable to monitor a number of ions where possible. An LSD standard was injected without application of fragmentation voltage and little was seen apart from the $[M+1]^+$ ion at *m/z* 324. When fragmentation voltages of 10–20 V were applied, the spectrum still had an intense *m/z* 324 as well as two structurally significant ions of high relative intensity at *m/z* 223 and *m/z* 281. Fig. 2 shows the ESI spectrum and the proposed fragmentation with an octapole voltage of 20 V.

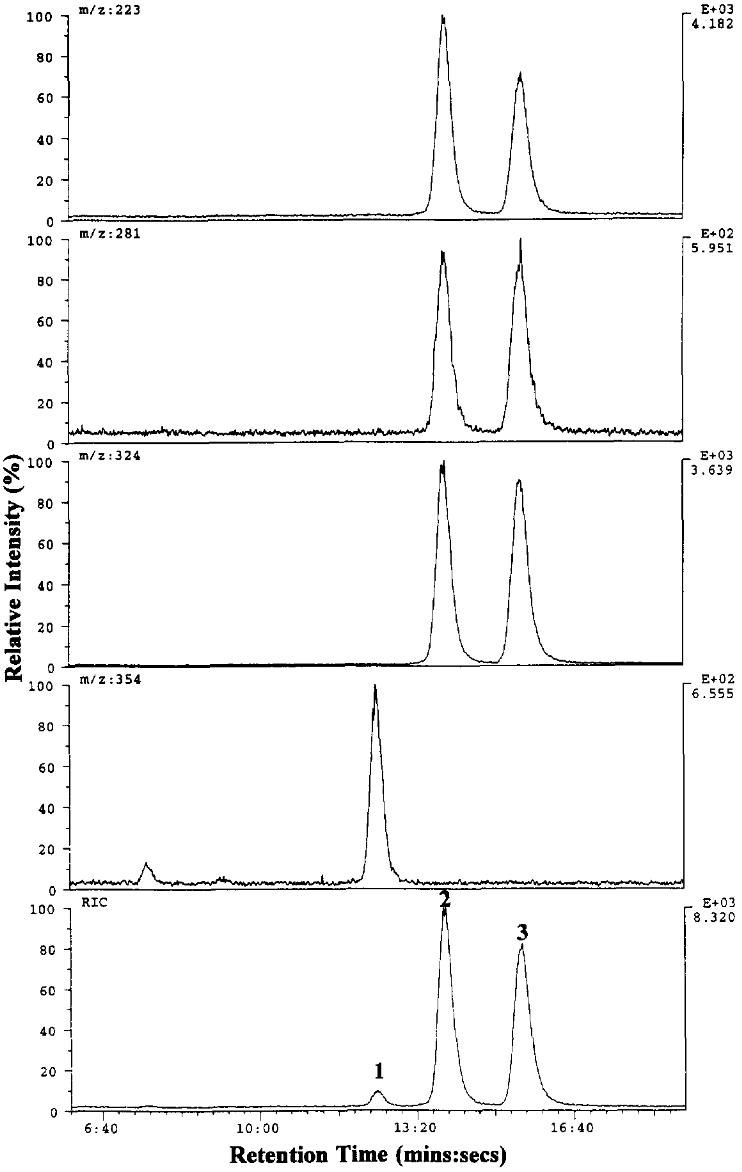

Fig. 3. Ion chromatograms of the $[M+1]^+$ ions of LSD, LAMPA and methysergide plus the fragment ions of LSD and LAMPA. Peak identities: 1=methysergide; 2=LSD; 3=LAMPA.

It is important to avoid excessive fragmentation in this analysis; there is little point in generating a large number of intense fragment ions at the cost of greatly reduced sensitivity through reduction of the $[M+1]^+$ ion. Fragmentation voltage was a critical system variable; with a given voltage, the degree of fragmentation of the $[M+1]^+$ ion of LSD varied significantly with different batches of mobile phase. Fragmentation voltage, therefore, needs checking and adjustment each time a new batch of mobile phase is prepared.

In methods of analysis involving GC–MS or LC–MS, a deuterated analogue of the analyte is often ideal as the internal standard. LSD-d_3 has been successfully employed as the internal standard in methods using GC–MS, even though the EI spectra of LSD and LSD-d_3 have several ions in common [7,8]. The electrospray mass spectrum of LSD has few ions (principally m/z 324, 281 and 223). Since the electrospray spectrum of the deuterated compound had ions at m/z 327, 281 and 226, the potential use of LSD-d_3 was rejected because of the common m/z 281 ion. No LC–MS work referring to the analysis of LSD has yet incorporated an internal standard. Methysergide has been employed as the internal standard in a HPLC assay of biological LSD [17]. Urine samples spiked with LSD and methysergide were taken through the extraction procedure. LC-fluorescence analysis of the extracts appeared promising and when samples were analysed by LC–MS, the internal standard had a pronounced $[M+1]^+$ ion at m/z 354. Fig. 3 shows the mass chromatograms of the pseudomolecular ions from a mixture of LSD, LAMPA and methysergide, plus the fragment ions of LSD and LAMPA. Standards of LSD in urine containing methysergide were taken through the clean-up and analysed.

Standard curves were linear in the range tested (up to 10 ng/ml LSD in urine). A typical standard curve with six data points is specified by the equation $y=2.858x+0.382$ ($r=0.998$), where y denotes the ratio of the peak area of the $[M+1]^+$ ion of LSD to the peak area of the $[M+1]^+$ ion of methysergide, x represents the concentration of LSD in ng/ml and r is the correlation coefficient. For this particular curve, the standard deviations of the slope and the intercept were 0.0604 and 0.0827, respectively. An LSD level of 10 ng/ml in urine corresponded to an ion area ratio of about 28. The correlation coefficients of standard curves obtained over a 6 month period were >0.993 with %R.S.D. less than 10%. A spiked urine sample at the RIA cut-off level tested 5 times over this period gave a consistent LSD quantification with %R.S.D. of 4.9%.

A blind trial was conducted on duplicate spiked urine samples taken through the clean-up. The results shown in Table 1 illustrate good agreement between the measured values and the actual concentrations. A paired comparison *t*-test showed that there was no significant difference between the two sets of data at the 95% confidence limit. It is interesting to note that the method was capable of detecting and accurately measuring a level of 0.3 ng/ml of LSD in urine. At this low level, however, the *S/N* ratio of the weakest of the three ions monitored (m/z 281) was approximately 2.5/1, which is why this level is not claimed to be the limit of quantification of the method. For urine spiked at 0.5 ng/ml, the *S/N* ratio of the weakest ion monitored (m/z 281) was 5/1. The *S/N* ratio for the ion used for quantification (m/z 324) was 40/1 for urine spiked at 0.5 ng/ml.

An independent blind trial was conducted on a different set of spiked samples, using a different blank urine, on a TSQ7000 instrument at the Finnigan MAT applications laboratory. This blind trial also gave satisfactory results and the variances on the two independent sets of data were found to have no significant difference at the 95% confidence limit.

A real sample which had tested positive under SIM conditions was subjected to scanning MS. The level of analyte was sufficiently strong to give a full

Table 1
Results of LC–MS LSD blind trial

Sample No.	Concentration of LSD (ng/ml)	
	Added	Measured
1	5.0	4.3
2	0.0	ND
3	1.0	1.0
4	0.0	ND
5	1.0	1.0
6	3.0	2.7
7	0.0	ND
8	0.3	0.3
9	0.8	0.7
10	0.5	0.5

ND=not detected.

HPLC conditions as in text; quantification based on area of m/z 324 ion of LSD (SIM experiment).

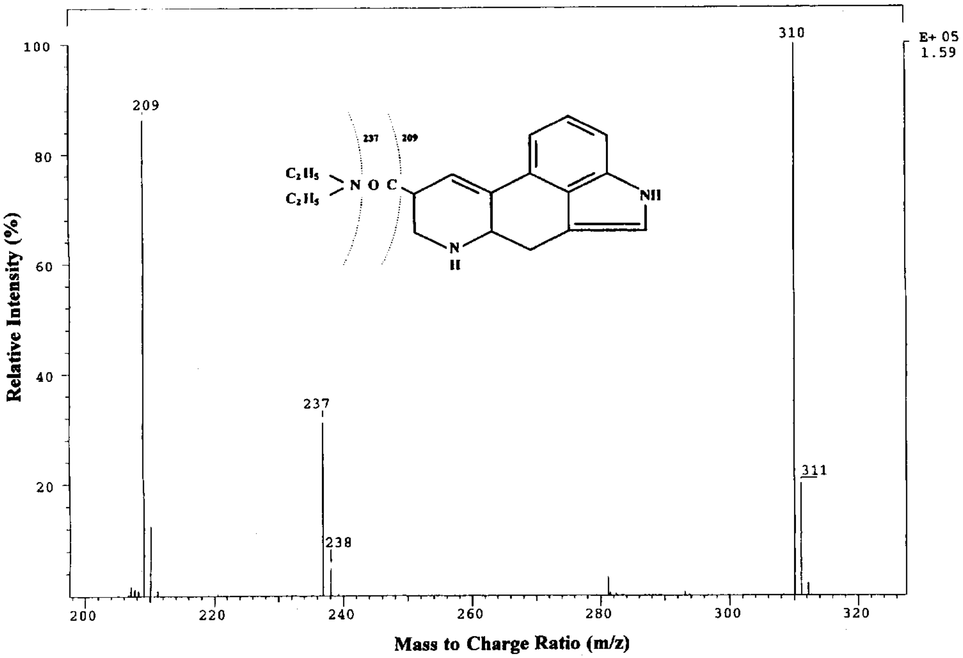

Fig. 4. Electrospray ionisation spectrum of N-demethyl LSD and proposed fragmentations.

spectrum matching the electrospray spectrum of an LSD standard. One of the known metabolites, N-demethyl LSD [5,6,13], was also identified in the sample extract. The spectrum obtained from a standard of N-demethyl LSD and the fragmentations proposed to give the *m/z* 209 and 237 ions are shown in Fig. 4. With the chromatographic system employed, N-demethyl LSD eluted before the internal standard and the two were baseline resolved.

Acknowledgments

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