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ARTICLE *in* ANALYTICAL CHEMISTRY · JANUARY 2005

Impact Factor: 5.64 · DOI: 10.1021/ac049285e · Source: PubMed

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Anal. Chem. **2004,** *76,* 7143−7148

Quantitation of Lysergic Acid Diethylamide in Urine Using Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Ion Trap Mass Spectrometry

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A quantitative method was developed for analysis of lysergic acid diethylamide (LSD) in urine using atmospheric pressure matrix-assisted laser desorption/ionization ion trap mass spectrometry (AP MALDI-ITMS). Following solid-phase extraction of LSD from urine samples, extracts were analyzed by AP MALDI-ITMS. The identity of LSD was confirmed by fragmentation of the $[M + H]^+$ ion using tandem mass spectrometry. The quantification of LSD was achieved using stable-isotope-labeled LSD (LSD-d_3) as the internal standard. The $[M + H]^+$ ion fragmented to produce a dominant fragment ion, which was used for a selected reaction monitoring (SRM) method for quantitative analysis of LSD. SRM was compared with selected ion monitoring and produced a wider linear range and lower limit of quantification. For SRM analysis of samples of LSD spiked in urine, the calibration curve was linear in the range of 1−100 ng/mL with a coefficient of determination, r^2, of 0.9917. This assay was used to determine LSD in urine samples and the AP MALDI-MS results were comparable to the HPLC/ESI-MS results.

Lysergic acid diethylamide (LSD) is a hallucinogenic drug. Identification and quantitation of LSD in biological samples is of significant importance in forensic and clinical toxicology laboratories as death attributed to the direct effects of LSD has been reported.[1,2] Conventional methods for the determination of LSD include radioimmunoassay,[3,4] HPLC with fluorescence detection,[5,6] capillary electrophoresis,[7] GC/MS,[8,9] and HPLC/MS.[10−12] Of these, GC/MS and GC/MS/MS are most often used for LSD screening or confirmation in biological samples because of their specificity and selectivity. However, because LSD is irreversibly adsorbed on GC columns, prior derivatization is needed.[8,9] LC/ESI-MS and LC/ESI-MS/MS are becoming increasingly popular for the quantitative analysis of LSD without derivatization,[10−12] but complex separation procedures are needed prior to ESI-MS analysis.

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has rapidly developed into one of the most effective qualitative techniques for the determination of high molecular weight compounds such as proteins, oligosaccharides, oligonucleotides, and synthetic polymers. Because of problems associated with MALDI quantitation, this technique has not been extensively applied in quantitative analysis, especially for small molecules. MALDI-MS, however, has several attractive features compared with the popular quantitative technique ESI-MS, such as ease of operation, high speed of analysis, and high-throughput capacity. It also has much greater tolerance for sample concomitants, e.g., salts and buffers, which often impair analysis by ESI-MS.[10−12] Therefore, development of MALDI-MS as a quantitative tool has become a subject of increasing interest.

The major problem associated with MALDI quantitation is irreproducible signal intensities. As well, application of MALDI-MS for analyses of small molecules is more complicated than for large molecules because of complex matrix ion interference in the m/z range below 500. Efforts have been made to eliminate matrix ion interference at low-mass range by using different matrix substances, such as sample substrates,[13,14] high-mass molecules,[15] surfactant-suppressed matrixes,[16] and inorganic materials.[17] In addition, the use of tandem MS for quantification may improve signal-to-noise ratios and eliminate the problem of interference from high-background ions.[18] Careful use of internal standards[18−23] can compensate for variation in sample preparation

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10.1021/ac049285e CCC: $27.50 Published 2004 Am. Chem. Soc.
Published on Web 10/15/2004

and analysis and improve signal ratio reproducibility. Duncan and co-workers[23] compared chemical analogues and stable-isotope-labeled analogues as internal standards for quantitative analysis by MALDI and found that the latter approach can improve reproducibility of analyte signal ratios and provide better linearity and correlation coefficients for standard curves. Successful examples of the application of MALDI-MS to the quantitation of small molecules have recently been published,[18−26] but have all been achieved using vacuum MALDI-MS. To our knowledge, there have been no published accounts of the quantitative analysis of small molecules using atmospheric pressure (AP) MALDI-MS.

AP MALDI was invented by Laiko et al.[27,28] This technique has been applied to the analyses of proteins/peptides,[29−32] oligosaccharides,[33,34] and polymers.[35] These studies have indicated that AP MALDI not only has ionization processes similar to conventional vacuum MALDI but also possesses a few notable advantages as compared with vacuum MALDI: sample manipulation at atmospheric pressure, eliminating the complications of introducing the sample into the vacuum of mass spectrometer, softer ionization technique, and mass accuracy and resolution dependent on parameters of the mass analyzer rather than the matrix and sample preparation.[27,28] When AP MALDI is coupled with ion trap mass spectrometry (ITMS), it allows MS/MS to be performed on MALDI-generated ions and offers both speed and specificity of analysis of compounds. This configuration can be quickly interchanged with conventional atmospheric pressure ionization sources such as ESI and APCI on mass spectrometers.

The purpose of our study was to develop an AP MALDI-MS method for the quantitative analysis of LSD in urine. Samples were precleaned using solid-phase extraction (SPE) and then directly analyzed by AP MALDI-ITMS without a time-consuming derivatization or chromatographic separation. Quantification of LSD in urine was achieved by spiking the samples with deuterated LSD. This method was evaluated in detail. To the best of our knowledge, this is the first paper to report a quantitative analysis in biological samples using atmospheric pressure MALDI-MS.

EXPERIMENTAL SECTION

Materials. The LSD and LSD-d_3 standards were purchased from Alltech-Applied Science (State College, PA) and Cambridge Isotope Laboratories, Inc. (Andover, MA), respectively. Ammonium acetate was purchased from Anachemia (Montreal, Canada). α-Cyano-4-hydroxycinnamic acid (HCCA) was obtained from Fluka (Buchs, Switzerland), and the acetonitrile (HPLC grade) and 1% trifluoroacetic acid were from Sigma Aldrich Co. (St. Louis, MO). The urine toxicology negative control was purchased from Bio-Rad Diagnostics Group (Irvine, CA). HPLC-grade methanol was purchased from Fisher Scientific Co. (Fair Lawn, NJ).

AP MALDI-ITMS Analysis. The MALDI-MS experiments were performed on a Finnigan LCQ Deca ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with a commercial atmospheric pressure MALDI source (Masstech Inc, Columbia, MD). The AP MALDI source has been described thoroughly in the literature.[27,28] Samples were mixed with HCCA in methanol at 1:1 ratio. A 1.0-μL aliquot of sample/matrix mixture was deposited on the plate. Samples were irradiated with a pulsed nitrogen laser operated at 337 nm with a repetition rate of 10 Hz. The MALDI interface and mass spectrometer parameters were optimized to obtain maximum sensitivity. The voltage on the MALDI plate was set at 1.0 kV, and the temperature of the LCQ capillary was held at 250 °C. The automatic gain control was on. The sheath gas, which is used in ESI experiments, was turned off for all AP MALDI measurements. Sample acquisition was performed in automated mode using a spiral pattern controlled by Masstech's Target software. Mass spectra were obtained by averaging 30 scans per target spot for aqueous solutions and 50 scans per target spot for urine samples. The number of scans averaged was chosen by balancing speed of analysis and reproducibility of the ion signal ratios of analyte to internal standard.

The mass spectrometer was operated in positive ion mode with selected ion monitoring (SIM) or selected reaction monitoring (SRM) acquisition. In SIM mode, the ions monitored were at m/z 324 (protonated molecule of LSD) and 327 (protonated molecule of internal standard LSD-d_3). In SRM mode, fragment ions were monitored at m/z 223 from the m/z 324 ion and at m/z 226 from the m/z 327 ion.

HPLC/ESI-MS Analysis. An Agilent 1100 liquid HPLC system was used in this study (Agilent Technologies Canada Inc.). The chromatography was performed at room temperature using a Zorbax Eclips XDB C8 column (4.6 × 150 mm) with a 5-μm particle size (Agilent Technologies Canada Inc.). The mobile phase was with 2 mM ammonium acetate in acetonitrile/water (70:30, v/v) at a flow rate of 0.8 mL/min. A 1-μL aliquot of sample was injected.

ESI-MS experiments were also performed on a Finnigan LCQ Deca ion trap mass spectrometer equipped with a commercial ESI source. Nitrogen was used as sheath gas at a flow rate of 80 arbitrary units and as an auxiliary gas at a flow rate of 20 arbitrary units. An electrospray voltage of 4.5 kV and a capillary temperature of 350 °C were used. The ESI interface and mass spectrometer

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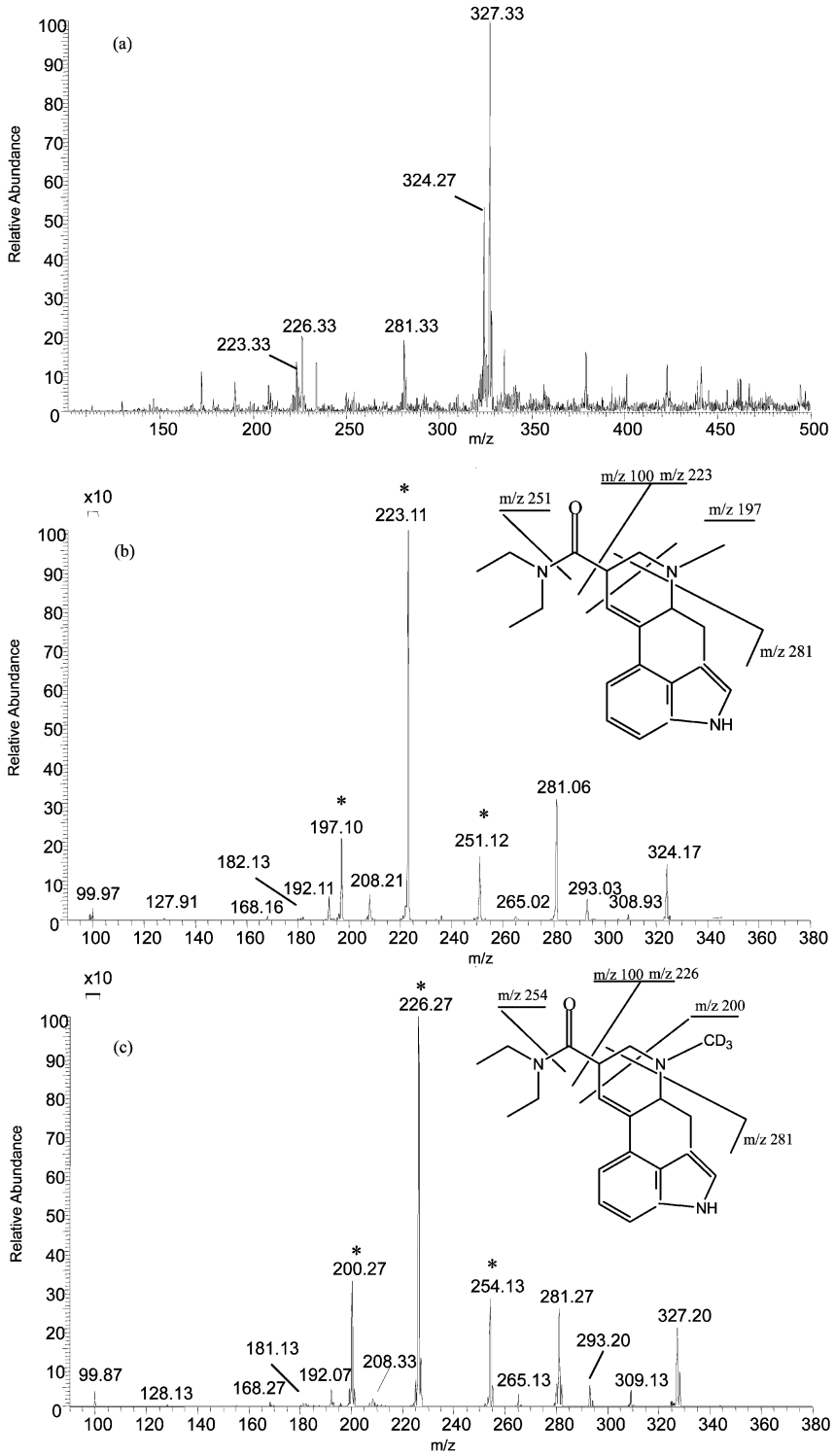

Figure 1. MALDI mass spectrum of 0.25 μg/mL LSD in aqueous solution with 0.5 μg/mL LSD-d_3 (a) and MS/MS spectra of the $[M + H]^+$ ions of LSD at *m*/*z* 324 (b) and LSD-d_3 at *m*/*z* 327 (c).

parameters were optimized to obtain maximum sensitivity. Quantitation of LSD in urine was achieved with internal standard LSD-d_3 by SRM. The *m*/*z* 223 ion from the *m*/*z* 324 ion and the *m*/*z* 226 ion from the *m*/*z* 327 ion were monitored. All reported data are based on ratio measurements of peak areas of the analyte to the internal standard.

Preparation of LSD Standards in Aqueous Solution. Stock solutions of LSD and LSD-d_3 were prepared at a concentration of 25 μg/mL in methanol and in acetonitrile, respectively, and then stored at 4 °C. LSD calibration standards were prepared in methanol/water (80:20, v/v) at concentrations of 10, 5, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025, and 0.0125 μg/mL with the LSD-d_3 internal standard at a concentration of 0.5 μg/mL.

Preparation of Urine Sample and Extraction. LSD working solutions, at concentrations of 10, 1, and 0.1 μg/mL, were prepared daily by diluting the stock solutions.

A 1-mL volume of negative control urine was spiked with a mixture of LSD working solution and internal standard, LSD-d_3,

to produce urine samples containing 100, 50, 20, 10, 5, 2.5, and 1 ng/mL LSD and 5 ng/mL LSD-d_3. The urine samples were extracted on an Abselut SPE column obtained from Varian (Harbor City, CA). A urine sample was loaded onto the column and passed through it using mild vacuum aspiration. The column was then washed with 1 mL of water and dried under high vacuum for 1 min. The analytes were eluted at 1−2 mL/min using 1 mL of methanol. The eluent was evaporated to dryness under a gentle stream of nitrogen and was reconstituted in 10 μL of acetonitrile immediately before MS analysis.

RESULTS AND DISCUSSION

Mass Spectra of LSD and LSD-d_3. The LSD standards in aqueous solution were characterized by AP MALDI-ITMS. HCCA was selected as the MALDI matrix in this study as preliminary investigations showed that it provides more efficient ionization for LSD at low concentrations than DHB. Another reason to prefer the HCCA as the matrix is that the matrix ions do not interfere with components to be analyzed.

The molar matrix-to-analyte ratio is an important factor in analysis using MALDI-MS technique. Deficiency of matrix or overloading of matrix causes low intensity of analyte signal and complex mass spectra with high interference ions. The optimum molar matrix-to-analyte ratio for analysis of LSD was 200:1. This ratio produced the lowest variability in signal intensity. Figure 1a shows a typical MALDI-MS spectrum of 0.25 μg/mL LSD in aqueous solution with 0.5 μg/mL LSD-d_3. The abundant MH^+ ions of LSD at m/z 324 and of LSD-d_3 at m/z 327 were used for SIM. Low-abundance fragment ions can also be seen in the spectrum.

Identification of LSD and LSD-d_3 was confirmed by tandem mass spectrometry. Panels b and c of Figure 1 show full-scan AP MALDI-MS/MS spectra of the m/z 324 ion and the m/z 327 ion, respectively. Note that these spectra of LSD and LSD-d_3 are consistent with the fragmentation pathways shown in Figure 1b and c. The same pattern was also obtained by ESI ion trap tandem mass spectrometry (data not shown). In Figure 1b and c, there were three pairs of fragment ions observed with a mass difference of 3 Da, at m/z 197/m/z 200, m/z 251/m/z 254, and m/z 223/m/z 226 (indicated with *), because three hydrogen atoms on the *N*-methyl group in LSD were replaced by deuterium atoms. As the m/z 223/226 ion pair was the base peak of the tandem mass spectra; they were selected for the SRM transition that was used for quantitative purposes.

Comparison of SRM with SIM for Detection of LSD in Aqueous Solution. To date, because of the predominant use of TOF mass spectrometry with vacuum MALDI sources, most MALDI quantitative analyses have utilized molecular ions. In this study, an atmospheric pressure MALDI source was combined with an ion trap mass spectrometer and the fragment ions of AP MALDI-generated molecular ions were determined operated in MS/MS mode. In principle, SRM can produce higher signal-to-noise ratios and lower detection limits than SIM, so SRM and SIM were compared for the quantification of LSD in aqueous solution.

LSD standard solutions were analyzed by AP MALDI-MS in both SIM and SRM modes. Each concentration was determined using four replicates, and relative standard deviations (RSDs) of signal intensities of LSD and signal intensity ratios of LSD to internal standard were calculated (shown in Table 1). The RSD data for both modes of operation indicate that the introduction of the deuterated internal standard leads to good signal ratio reproducibility. The improvement in RSDs obtained using the deuterated internal standard varied between 4- and 20-fold. With addition of deuterated internal standard, in SIM mode, the RSD values ranged from 4.4 to 16.5% and, in SRM mode, from 1.8 to 10.1%. As expected, the SRM mode provides more stable signal ratios than the SIM mode.

Table 1. Reproducibility of MALDI Signal Intensities for LSD Standards in Aqueous Solution (n = 4)[a]

concn (μg/mL)	SIM mode (%)		SRM mode (%)	
	LSD/LSD-d_3[b]	LSD[c]	LSD/LSD-d_3[b]	LSD[c]
10	6.3	30.3	4.3	33.0
5	6.2	34.6	4.8	32.6
2	7.8	38.0	3.2	29.3
1	4.4	33.4	2.3	31.1
0.5	3.5	44.5	1.8	38.5
0.25	5.9	26.4	2.1	32.9
0.1	11.7	29.6	5.7	41.8
0.05	16.5	44.2	8.9	45.6
0.025			9.1	24.2
0.0125			10.1	41.1

[a] Based on four replicate measurements using a spiral laser illumination pattern on the AP MALDI-MS target. [b] RSD of ratio of signal intensities of LSD to LSD-d_3. [c] RSD of signal intensities of LSD.

The calibration curve plotted the average ratio of intensities of the analyte to internal standard (y) as a function of the concentration of LSD (x). In SIM mode, the line of best fit was $y = 1.878x + 0.048$ over the range 0.05−10 μg/mL with a correlation coefficient $r^2 = 0.9993$. In SRM mode, the line of best fit was $y = 2.019x + 0.020$ over the range 0.0125−10 μg/mL with a correlation coefficient $r^2 = 0.9994$. It is obvious that SRM provides a wider linear range and lower limit of quantification than SIM.

The above data show the analytical power and the utility of SRM as compared to SIM for the quantitative analysis of LSD by AP MALDI-MS; therefore, SRM was used for the quantification of LSD in urine samples to complete this study.

Quantitative Analysis of LSD in Urine. LSD standards were prepared in 1 mL of drug-free urine at concentrations ranging from 1 to 100 ng/mL. Figure 2a shows a typical AP MALDI-MS spectrum of a urine sample spiked with LSD. Despite the chemical background from the urine sample, the analyte at m/z 324 was resolved from the major interferences. Panels b and c of Figure 2 show the corresponding SRM spectra of the m/z 324 and the m/z 327 ions. Good signal-to-noise ratios were evident.

The linearity of the method was evaluated by analyzing urine samples spiked with LSD. The best linear fit to the calibration data is described by the equation $y = 0.2034x + 0.050$ in the range 1−100 ng/mL for 5 ng/mL internal standard. The correlation coefficient, r^2, was calculated to be 0.9917. By changing the amount of internal standard added, the linear range may be extended.[36]

The LOD, defined as three times the standard deviation of five replicate measurements on a blank urine sample, was calculated

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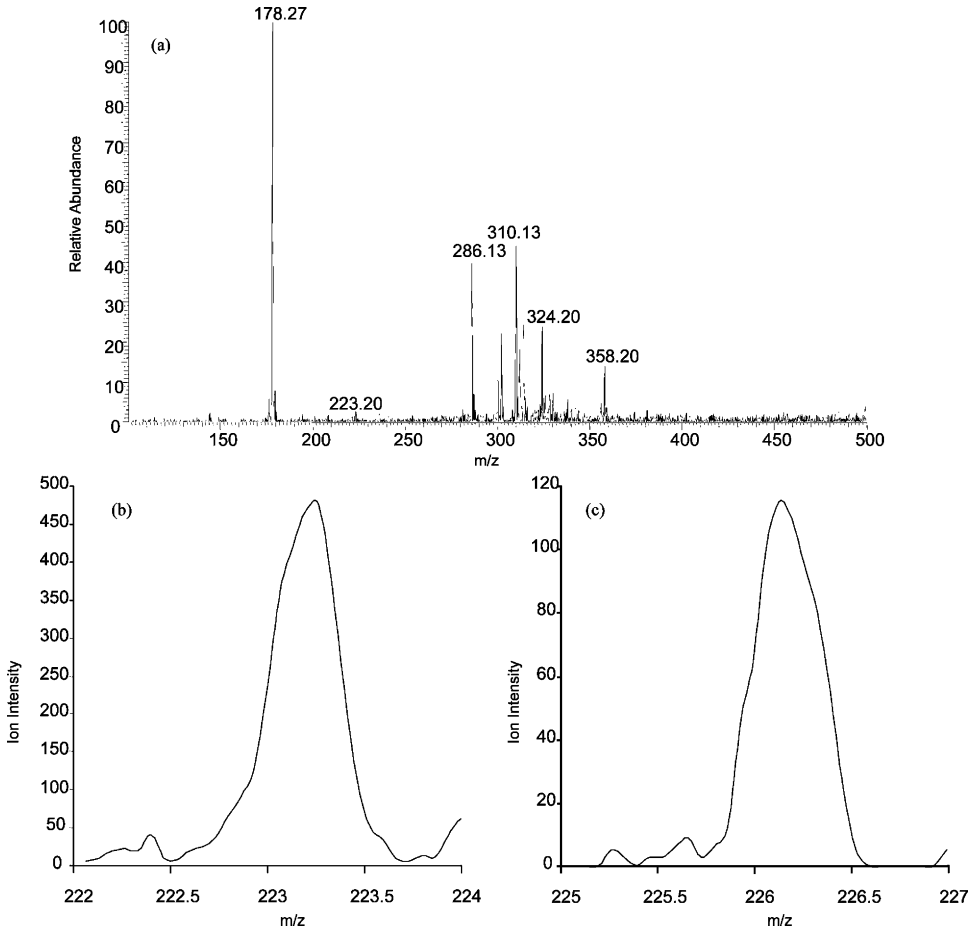

Figure 2. MALDI mass spectrum of LSD (20 ng/mL) in urine sample with LSD-d_3 (5 ng/mL) (a) and the corresponding SRM spectra of the *m*/*z* 223 ion from $[M + H]^+$ ion of LSD (b) and the *m*/*z* 226 ion from the $[M + H]^+$ ion of LSD-d_3 (c).

Table 2. Repeatability and Reproducibility Study for LSD Standards Spiked in Urine Samples

concn (ng/mL)	repeatability (%)		reproducibility (%)	
	LSD/LSD-d_3[a]	LSD[b]	LSD/LSD-d_3	LSD
2.5	11.3	34	19.9	38.2
10	6.9	26.1	4	28.4
50	8	29.2	10.4	34.3

[a] RSD of ratio of signal intensities of LSD to LSD-d_3. [b] RSD of signal intensities of LSD.

Table 3. Comparison of LSD Concentration in Urine Samples as Measured by HPLC/ESI-MS and AP MALDI-MS

HPLC/ESI-MS		AP MALDI-MS	
concn (ng/mL)	RSD[a] (%)	concn (ng/mL)	RSD[a] (%)
1.68	7.4	1.83	14.3
1.96	6.5	2.27	13.5
2.61	4.3	2.99	10.7
3.43	3.1	3.37	4.8
7.76	3.6	8.64	8

[a] Based on four replicate measurements at each sample.

to be 0.7 ng/mL or the equivalent of 35 pg of LSD deposited on the target spot.

Repeatability was determined by analyzing five replicates at each of three different concentrations (50, 10, and 2.5 ng/mL) on the same day. The calculated relative standard deviations ranged from 6.9 to 11.3% as shown in Table 2.

Reproducibility was examined by analyzing the urine samples spiked with LSD at three different concentrations (50, 10, and 2.5 ng/mL) on five consecutive days. Four replicates for each concentration were analyzed per day. Table 2 lists the RSD values within the range 4−19.9%.

Recovery was tested by analyzing 1-mL urine samples spiked with LSD at three different concentrations (50, 10, and 2.5 ng/mL). For these experiments, the internal standard (LSD-d_3) was added to the extract after SPE. Standard aqueous solutions (1 mL) of LSD at these three identical concentrations, together with internal standard, were prepared. All samples were dried and reconstituted in acetonitrile. The recovery, determined by comparing the ratios of intensities of the analyte and internal standard between urine and aqueous solutions, averaged 87%.

Comparisons of Analytical Methods. A comparison was made with results obtained by AP MALDI-MS and HPLC/ESI-MS. In a blind trial, five urine samples were analyzed by the two methods. Table 3 shows the comparison of LSD concentration in urine samples as measured by HPLC/ESI-MS and AP MALDI-MS, respectively. As can be seen in Table 3, there is a good correlation between the results obtained by the two techniques (a correlation coefficient of 0.9967). The two methods were statistically compared using the paired *t*-test. This analysis showed that the *p*-value is 0.1012 and the *t*-value is 2.12. Therefore,

the two sets of data obtained are not significantly different at the 95% confidence level. A detection limit of 0.77 ng/mL was calculated (defined as three times the standard deviation of five replicate measurements on a blank urine sample) for LSD using HPLC/ESI-MS, which is virtually identical with that obtained with AP MALDI (0.7 ng/mL).

CONCLUSIONS

It has been shown that atmospheric pressure MALDI ion trap mass spectrometry combined with a simple solid-phase extraction cleanup provides a rapid and sensitive tool for confirmation and quantification of LSD in urine samples without derivatization and chromatographic separation. Addition of a stable-isotope-labeled analyte as internal standard was a very effective method for reducing the effects of variation in sample handling, preparation, and analyte ionization and improving reproducibility of the MALDI-generated signal ratios. The use of SRM further improved the selectivity and sensitivity of the method for low concentrations of LSD in urine samples. Calibration curves were developed with a linear correlation coefficient of >0.99. For repeatability and reproducibility, RSD values ranged from 4 to 20%. Quantitative analysis by AP MALDI-MS yielded results comparable to those obtained by HPLC/ESI-MS. This work demonstrates the potential usefulness of AP MALDI-MS/MS for rapid screening for drugs in biological samples.

ACKNOWLEDGMENT

M. Cui would like to thank NRC and NSERC for financial support.

Received for review May 15, 2004. Accepted August 26, 2004.

AC049285E