Rapid drug-screening and quantitation of 3,4-methylenedioxymethamphetamine in urine by MALDI-TOF mass spectrometry

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Abstract

A method for the rapid screening of the drugs, 3,4-methylenedioxymethamphetamine (MDMA), in urine by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) is described. In this method, α-cyano-4-hydroxycinnamic acid (CHCA) is used as the matrix for the MALDI samples. The identity of MDMA was confirmed by flight time of the fragmentation ($[M+H]^+$ ion) using a mass calculation curve, the quantification of MDMA was achieved using stable-isotope-labeled MDMA (MDMA-D$_5$) as the internal standard. For the analysis of MDMA/MDMA-D$_5$-spiked urine samples, the calibration curve was linear in the range of 0.1–100 ppm with a coefficient of determination, r^2, of 0.9993. This assay was used to determine MDMA in actual urine samples. The MALDI-TOFMS results were comparable to the GC/MS results. No significant bias is shown between the two methods by t-test at 95% confidence level. We conclude that MALDI-TOFMS can serve as a reliable and complementary method to GC/MS for use in forensic analysis.
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Thus far, GC/MS is the officially prescribed method and constitutes the most popular and powerful technique for the analysis of illicit drugs such as amphetamine and analogs thereof [1–8]. Because of this, GC/MS analysis has dominated the field of "drug screening" for many years, and as a result, a huge database is typically available in commercial libraries. However, the sample preparation for GC/MS analysis is complicated and time consuming. Typically, urine samples must be centrifuged, filtered, extracted (by either liquid–liquid or solid-phase extraction methods), and then derivatized prior to their injection into the GC system. In addition, each compound has unique properties and the conditions required for the subsequent individual derivatization are also different. In the case of 3,4-methylenedioxymethamphetamine (MDMA), it has

been reported that MDMAs provide only an imine fragment ($m/z = 58$), which is a major peak in the electron impact mass spectrum of MDMAs. An optimum derivatization of MDMA can be performed via reaction with acetic anhydride, which provided specific fragments ($m/z = 58$, 100 and 162) permitted its characterization of a derivatized-MDMA. However, while such a specific derivative-reagent is suitable for one compound, it may not be suitable for others. It has recently been found that urine samples of suspects and clandestine tablets that contain multi-components, including methamphetamines, MDMAs, ketamine as well as other so-called "designer drugs" such as phenethylamine. For an analysis of such complicated mixtures, a specific reagent is wholly insufficient for these needs. Thus, a rapid and complementary method would be highly desirable, especially when it is necessary to analyze thousands of samples. A number of analytical methods have been commercially developed for this, including a fluorescence polarization

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immunoassay [9], an immunochromatographic assay [10] and thin layer chromatographic analysis [11]. Each of these methods has unique advantages and disadvantages with respect to sensitivity, precision and simplicity of use. More simple methods, such as the use of the drug/narcotic detection kits, aerosol sprays/cans or collection paper dispensers are also commercially available. However, these tests provide for only a cursory and qualitative examination and are not legally acceptable as scientific proof.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) is a very popular and powerful tool that is routinely used in the analysis of biomolecules [12–20]. Recently, the analysis of low-mass ($m/z < 500$) molecules using this method has been reported [21–28]. A major problem associated with MALDI quantitation is irreproducible signal intensities, which are basically due to the fluctuation of a pulsed-laser source and heterogeneity of the surface on which the analyte-matrix is formed, although some efforts have been made to overcome such problems [29–34]. One of these methods is the use of a mixture of the surfactant of cetrimonium bromide (CTAB), a surfactant and the conventional matrix of α-cyano-4-hydroxycinnamic acid (CHCA) to prepare the MALDI samples [21]. During the MALDI process, the presence of CTAB could suppress the matrix-related ion background. As a result, the low-mass range can be obtained. Otherwise, the use of porous silicon [26] or the use of ionic liquid matrixes [35] can also provide desirable results in the low-mass range. In this study, we report on the rapid drug-screening and quantitation of MDMA in urine based on the linear relationship between the concentrations and the ratios of intensity MDMA/intensity MDMA-D_5. Actual urine samples from 10 suspects were also examined by MALDI-TOFMS and the results are reported herein.

1. Experimental

1.1. Materials

The MDMA and MDMA-D_5 were obtained from Radian International (Catalog No. M-013, 1 mg/1 mL methanol; M-011, 0.1 mg/1 mL methanol). α-Cyano-4-hydroxycinnamic acid, cetrimonium bromide (CTAB), acetonitrile, methanol and trifluoroacetic acid were purchased from Aldrich (St. Louis, MO, USA) and Acros (New Jersey, USA), respectively.

1.2. Sample preparation

The blank urine sample was collected from an adult male volunteer. All of the suspect urine samples were generously donated by Command of the Army Force of Military Police, Forensic Science Center, Taiwan. In the initial steps of the MALDI experiment, a CHCA-solution was prepared (10 mg/mL in a mixed solution; water/acetonitrile = 50/50,

v/v, 1% trifluoroacetic acid) for use. Various concentrations of MDMA-D_5 standards were added to the urine samples and aliquots were then sonicated, centrifuged and filtered through a 0.45 μm filter. After this, a 1.0 μL aliquot of urine was mixed with 1.0 μL of CHCA-solution and loaded on the MALDI substrate for drying. A simple liquid–liquid extraction step is recommended if the concentration levels of MDMA in urine are low. For this, 1.0 mL of urine sample was made alkaline by the addition of excess K_2CO_3. The free bases were then extracted into 2 mL of a hexane/CH_2Cl_2 (3:1, v/v) solution by mixing for 1 min. After centrifugation, the upper layer was collected and this organic phase was then evaporated to dryness. The residue was dissolved in 10 μL of water for the subsequent experiments. For the GC/MS experiments, 1.0 mL of urine sample was also made alkaline by the addition of excess K_2CO_3. After the same liquid–liquid extraction procedures, 0.1 mL of acetic anhydride was added to derivatize the MDMA. The residue was dissolved in 100 μL of ethyl acetate for use in the GC/MS experiments.

1.3. MALDI-TOFMS apparatus

The linear type of time-of-flight mass spectrometer (TOFMS) used in these experiments was a modified Wiley-McLaren design (R.M. Jordan Co., Grass Valley, CA, USA); the flight distance was 1.1 m and the mass resolution was ~300. The instrument was equipped with a 4-in. turbo (flight tube) and a 6-in. diffusion (ionization region) pump system to maintain the vacuum below ~5×10^{-7} Torr during the experiments. All mass spectra were obtained using 355-nm radiation from a GCR-170 Nd:YAG laser system (Spectra-physics, Mountain View, CA, USA). The voltages used for the repeller and extraction grid were 10 and 6 kV, respectively. A stainless steel target was used as the MALDI substrate and the samples were deposited directly on it. The instrument was equipped with a video camera, for displaying the sample image on a monitor, thus permitting the laser to be focused on a specific spot within the area of the target. The ions formed by MALDI were produced in a field-free region, and then directly migrated toward the detector. A 25 mm triple microchannel plate (MCP) was used for ion detection. Data were recorded using a LeCroy 9350A digital oscilloscope (500 MHz) and processed by a personal computer. A time-of-flight range of 0–20 μs was generally used as the mass acquisition period, which corresponds to a m/z range of 0–627. All spectra were obtained as 200-shot averages. Mass calibration was conducted using fragments produced from a CHCA standard. This has described previously [36] and is abbreviated herein.

1.4. GC/MS apparatus

A gas chromatograph (GC 6890 Hewlett-Packard, Avondale, PA, USA) equipped with a mass spectrometer (Hewlett-Packard 5973 mass selective detector) was used for detection.

The experimental conditions were similar to those described previously [37] and are abbreviated herein.

2. Results and discussion

Fig. 1A shows the selected ion current ($m/z = 58$, 100 and 162) profile for MDMA-derivative extracted from a suspect urine sample, the internal standard used was methoxyphenamine hydrochloride. The inset, at the left in Fig. 1A, shows the fragmenting mechanism for the derivatized MDMA; the other inset, at the right, shows the mass fragmentation spectrum of the derivative. The production of specific fragments permitted its characterization. However,

the GC retention time was relatively long (20 min, a 25 m GC column was used) compared to the results obtained by the MALDI-TOFMS method, only a decade microsecond (as shown in Fig. 1B). The mass spectrum (Fig. 1B) was obtained from a 20 ppm of MDMA standard, which was mixed with CHCA and CTAB at a concentration ratio of 1000–1. CHCA is a common matrix that is frequently used in MALDI-TOFMS when used in conjunction with a UV pulsed laser (particularly a N_2 laser, wavelength: 337 nm). CTAB is a surfactant, and is used as a suppressant to reduce the production of CHCA fragments during the laser desorption ionization process. It is clear that, when the mixture of the matrix of CTAB/CHCA was present, the spectrum contained only two strong peaks corresponding to matrix-related ions, the flight
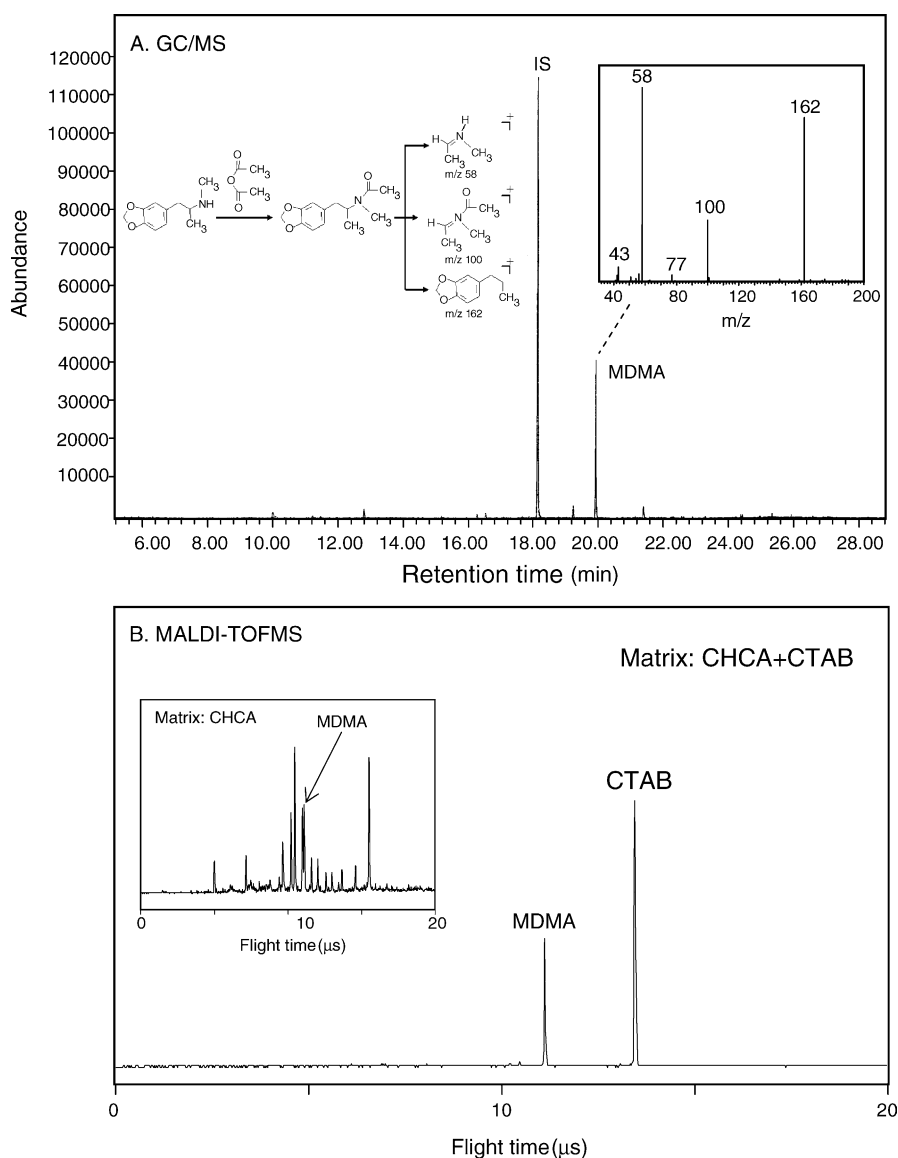

Fig. 1. (A) Selected ion current profile for a MDMA derivative extracted from a urine sample of a suspect and measured using GC/MS. Internal standard: methoxyphenamine hydrochloride. Insets: left, fragmentation mechanism for the derivatized MDMA; right, mass fragmentation spectrum of the derivative. (B) MALDI-TOF mass spectrum obtained from a 20 ppm MDMA standard, which was mixed with CHCA and CTAB, the concentration ratio of CTAB/CHCA was 1–1000. Inset, when the analyte (MDMA) was mixed with CHCA (without CTAB), a complicated spectrum was obtained.

times of 11.15 and 13.49 μs correspond to $[MDMA + H]^+$ and $[CTAB - Br]^+$ ions (m/z 194.24 and 284.53), respectively. For comparison, the inset shows the result obtained from a non-CTAB matrix under exactly the same conditions. As can be seen from the mass spectrum, numerous strong peaks corresponding to matrix-related ions of CHCA are produced (arrow, $[MDMA + H]^+$). These observed ion peaks are in general agreement with previous literature reports [38,39], and were used to construct the mass calibration equation. It also appears that, at an appropriate ratio of CTAB to CHCA, the addition of CTAB to CHCA is effective in suppressing CHCA-related matrix ion signals. The inset shows that when the analyte (MDMA) was mixed with CHCA (without CTAB), a complicated spectrum was obtained. However, we unexpectedly found that the use of CTAB is not actually necessary if the analyte is a urine sample, since a number of compounds present in the urine absorb UV light and can serve

as reagents for matrix suppression. For this reason, CTAB was not used in the subsequent experiments. The quantitation of MDMA in urine was achieved by adding the MDMA-D$_5$ internal standard because we found that the intensity ratio of MDMA/MDMA-D$_5$ can be maintained in the form of a linear relationship during the MALDI experiments.

Using the MALDI-TOFMS method, Fig. 2 shows the mass spectra of a blank urine sample (spectrum a), the same urine sample after spiking with 50 ppm of MDMA standard (spectrum b) and after spiking with MDMA/MDMA-D$_5$ (20/10 ppm, spectrum c), respectively. As can be seen from spectrum a, a number of unidentified peaks appear. Among these peaks, the peaks at 3.830 and 4.987 μs of flight time correspond to Na^+ and K^+, respectively, which are the most common metal ions in a typical urine sample. The flight time of $[MDMA + H]^+$ was calculated at a position of 11.15 μs (arrow mark), but none of the characterized peak was detected. In other words, the position of 11.15 μs flight time (under the experimental conditions described above) would not appear in any contingency peak from a normal human urine sample, if the MDMA had not been dosed. A high resolution mass spectrometer would provide better mass resolution. After spiking with the MDMA standard (50 ppm), a peak appeared at the position of 11.15 μs of flight time, as shown in spectrum b (arrow mark). The peak was identified by comparison with the mass calibration equation. For the quantitation, additional MDMA-D$_5$ standard was added in conjunction with the MDMA standard, as shown in spectrum c. The relationship between the sample concentrations and the intensity ratios of MDMA/MDMA-D$_5$ was plotted, as shown in Fig. 3. The linearity of this method for MDMA was fairly good in the range of concentrations from 100.0 to 0.1 ppm, being described by the equation
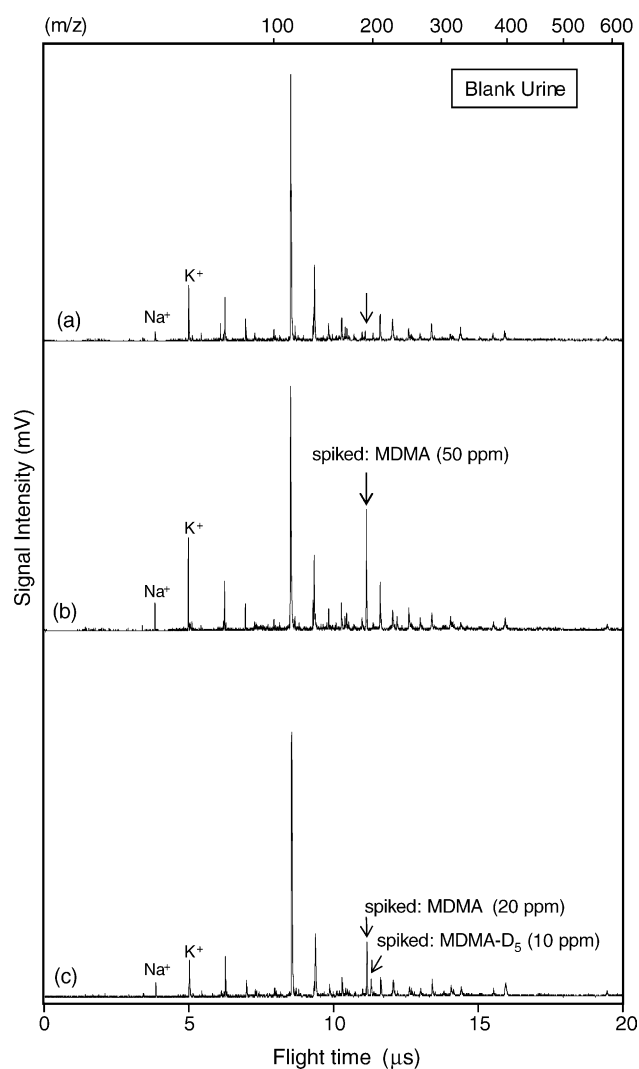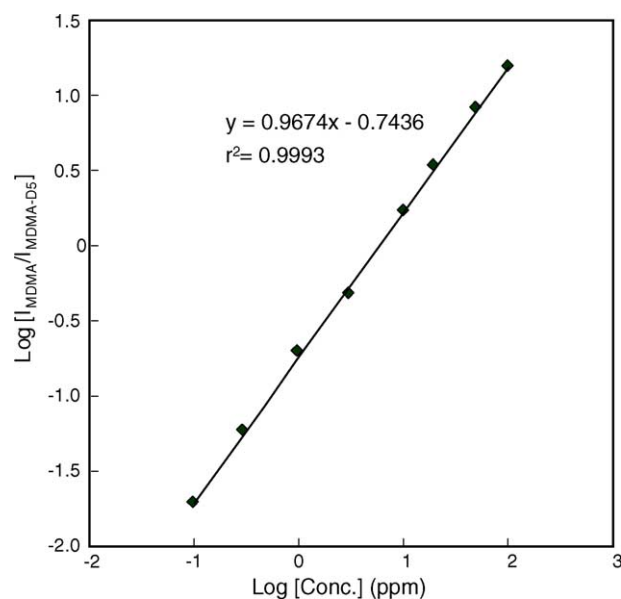

Fig. 2. MALDI-TOF mass spectra obtained from a blank urine sample (spectrum a), the same urine sample after spiking with 50 ppm of MDMA standard (spectrum b) and after spiking with MDMA/MDMA-D$_5$ (20/10 ppm, spectrum c), respectively.

Fig. 3. Logarithmic concentration diagram for a calibration graph of MDMA at concentrations between 0.1 and 100 ppm.

Table 1
Recovery data

Concentration of spiked standards (ppm)	MALDI-TOFMS (ppm)	Recovery (%)
0.5	0.47	94
1.0	1.04	104
5.0	4.83	96
10.0	10.47	104
30.0	28.25	94

Various concentrations of MDMA standards were spiked to a blank urine sample.

($y = 0.9674x - 0.7436$, $r^2 = 0.9993$). Based on this equation, a series of suspect urine samples were examined. Herein, all of the urine samples were used directly without any derivatization procedures after spiking with MDMA-D$_5$ (1 or 10 ppm depending on various conditions). Table 1 shows the recoveries obtained when the MALDI-TOFMS method was used. Various concentrations of MDMA standards (0.5, 1.0, 5.0, 10.0 and 30.0 ppm, respectively) were spiked to a blank urine sample. As a result, the recoveries were 94, 104, 96.6, 104.7 and 94.2% (average: $98.7 \pm 5.3\%$). Fig. 4 shows the mass spectrum of suspect urine sample (e). As can be seen from the mass spectrum, a number of peaks appear. Although each of the urine samples provides a different mass pattern (data not shown), we assigned the peaks with flight times of 11.15 and 11.29 µs to [MDMA + H]$^+$ and spiked [MDMA − D$_5$ + H]$^+$, respectively. We also assigned the peak with flight time of 10.53 µs to [MDA + H]$^+$, the major metabolite of MDMA, based on the flight time and spiking method. The quantitation of MDA is also possible, if the MDA-D$_5$ standard was used. By comparing the intensity ratios of MDMA (in the suspect urine samples) and MDMA-D$_5$ (spiked to the suspect urine samples), the concentration levels of MDMA in these urine samples were calculated and the results are shown in Table 2. Indeed, there are many methods for taking drugs, including ingesting a pill or drinking a solution of a drug. Any of these procedures would lead to urine samples con-
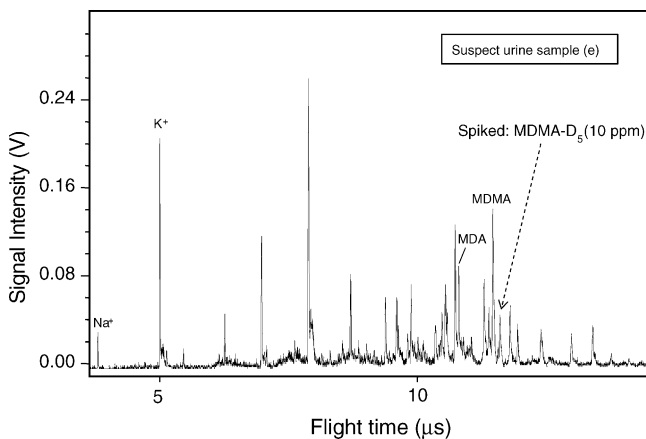

Fig. 4. MALDI-TOF mass spectra obtained from a urine sample of suspect (e). The urine sample was spiked with 10 ppm of MDMA-D$_5$ standard.

Table 2
Comparison of the results (concentration: ppm) obtained by MALDI-TOFMS (in this study) and GC/MS data obtained from the (Forensic Science Center, Command of the Army Force of Military Police, Department of Defense, Taipei, Taiwan) for the analysis of 3,4-MDMA in a series of suspect urine samples

No. of urine sample	MALDI-TOFMS	GC/MS
a	18.6	Not detected
b	11.7	12.0
c	1.9	32.9
d	6.3	19.8
e[a]	19.2	24.0
f	4.7	15.3
g	8.3	9.0
h	8.1	6.7
i	4.8	5.3
j	23.5	Not detected

Concentration levels in ppm.
[a] The mass spectrum of urine sample (e) is shown in Fig. 4.

taining MDMA, which would result in a positive reaction as shown in this study. In Taiwan, if the concentration of amphetamines or related metabolites exceeds 0.5 ppm, then the suspect is judged guilty. The method proposed here provides sufficient sensitivity to permit the identification of such low levels of MDMAs. In the Table 2, the data (right column) obtained from a GC/MS are also present. No significant bias is shown between the two methods by *t*-test at 95% confidence level. Thus, we conclude that MALDI-TOF mass spectrometry, which is clearly accurate, sensitive and rapid, can be considered for use in drug-screening and is sufficiently reliable to serve as a complementary method to GC/MS for use in forensic analysis.

3. Conclusions

We demonstrate here that a MALDI-TOFMS method can be rapidly and successfully used for the identification and quantitation of MDMA in urine samples of suspects. The quantification of MDMA was achieved using MDMA-D$_5$ standards since the ratios of intensity MDMA/intensity MDMA-D$_5$ and the concentrations can be maintained as a linear relationship during the MALDI experiments. The method proposed here can be used in the rapid drug-screening of MDMA in urine samples with no need for pre-treatment and resulting in a 0.1 ppm detection limit, whereas GC/MS requires derivatization and extraction for success.

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