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# Rapid analysis of 3,4-methylenedioxymethamphetamine: a comparison of nonaqueous capillary electrophoresis/fluorescence detection with GC/MS

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**Abstract**

Because of the increasing use of 3,4-methylenedioxymethamphetamine (3,4-MDMA), a rapid and sensitive analytical technique is required for its detection and determination. Using nonaqueous capillary electrophoresis/fluorescence spectroscopy (NACE/FS) detection, it is possible to determine this drug at the level 0.5 ppm without any pre-treatment in less than 5 min. After liquid–liquid extraction, the sample can be condensed and a detection limit of 3,4-MDMA in urine of 50 ppb ($S/N = 3$) can be achieved. The precision of the method was evaluated by measuring the repeatability and intermediate precision of migration time and the corrected peak height by comparison with a 3,4-MDMA-$D_5$ internal standard. With the conventional GC/MS method, it is necessary to derivatize the 3,4-MDMA before injection and the GC migration time also is in excess of 20 min. Therefore, NACE/FS represents a good complementary method to GC/MS for use in forensic analysis. 

*Keywords:* 3,4-Methylenedioxymethamphetamine (3,4-MDMA); Nonaqueous capillary electrophoresis/fluorescence spectroscopy (NACE/FS); Gas chromatography/mass spectrometry (GC/MS)

## 1. Introduction

3,4-Methylenedioxymethamphetamine (3,4-MDMA) is a strong central nervous system stimulant, which is classified as an illicit drug, and is currently a source of serious social problems in many Asian countries. Because of the rapid growing abuse of this, and similar substances, a simple, economic, fast and consistent method for their determination is necessary, not only for clinical research, but for forensic analysis as well. So far, GC/MS remains the official prescribed method. Only data obtained by GC/MS can be considered as a scientific proof and accepted in the court in Taiwan. However, it is necessary to derivatize these MDMAs prior to their injection into the GC system [1–4]. The GC migration time also is longer (20 min) when a 25 m GC column is used. All of these operation processes are time consuming. Because it is the necessary to analyze hundreds of samples, a rapid and complementary method would be highly desirable. Capillary electrophoresis (CE) represents a rapidly growing separation technique that might well meet this need, since it is now a well-established and widely used analytical method in many fields, such as bioscience, pharmaceutical, environmental studying, food science and forensic research [5–7]. Given its high separation efficiency, short analysis time, the use of higher electric field strength without untoward effects of Joule heating, and improved detection limits, nonaqueous capillary electrophoresis (NACE) has rapidly grown in popularity over the past few years [8–13]. Recently, we described a NACE method which is coupled with low-temperature fluorescence detection for the separation and on-line determination of 3,4-MDMA in human urine at 77 K [14,15]. In this study, we describe optimum conditions for the rapid analytical determination of 3,4-MDMA in suspect urine samples. Without any pre-treatment, and by spiking an internal standard (IS) of 3,4-MDMA-$D_5$ to urine samples, the qualitative and

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quantitative analysis of 3,4-MDMA could be achieved by comparing the migration time (less than 5 min) and the peak height of the IS. This method, which was found to be accurate, sensitive and rapid, can be considered to be a reliable complementary method to GC/MS for use in forensic analysis.

## 2. Materials and methods

### 2.1. Chemicals

3,4-MDMA and 3,4-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). Sodium cholate (SC) and methanol (99.8%) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Formamide and ammonium acetate ($CH_3COONH_4$) was purchased from Acros and Riedel-de Haen (RdH Laborchemikalien GmbH&Co. KG), respectively. All of the suspect urine samples were generously donated by Command of the Army Force of Military Police, Forensic Science Center, Taiwan.

### 2.2. CE apparatus and method

The CE set-up is laboratory-made and is similar to that described previously [14,15]. Briefly, a high-voltage power supply (Model RR30-2R, 0–30 KV, 0–2 mA, reversible, Gamma, FL, USA) was used to drive the electrophoresis. A 75 μm i.d. fused silica capillary (J&W Scientific, California) was used for the separation. Prior to injection, the separation buffer was filtered through a 0.45 μm syringe filter and then degassed for 5 min. The sample was hydrodynamically injected by raising the reservoir by 10 cm relative to the exit reservoir for 3 s (∼1.5 nl), depending on the conditions used. The excitation source was selected by a monochromator (ARC, Acton Research Corporation; Model SP-150, 1200 grooves/mm grating) connected to an Xe lamp (Muller Elektronik. Optik, SVX/LAX 1450, 500 W) which was used to provide the output power of over 6.1 W. CE-separated analytes are probed with the light. The fluorescence is collected at a right angle to the light source and dispersed by a second monochromator (ARC Model SP-300i, 2400 and 300 grooves/mm grating) and detected by a photomultiplier tube (ARC Model P2-R928, for 190–900 nm). Electropherograms were collected at a speed of 200 ms/point with a data acquisition system (ARCs SpectraSense NCL package), which was connected to a personal computer. The internal standard was 3,4-MDMA-$d_5$ (50 ppm).

### 2.3. GC/MS apparatus and methods

A gas chromatograph (GC 5890 Hewlett–Packard, Avondale, PA, USA) equipped with a mass spectrometer

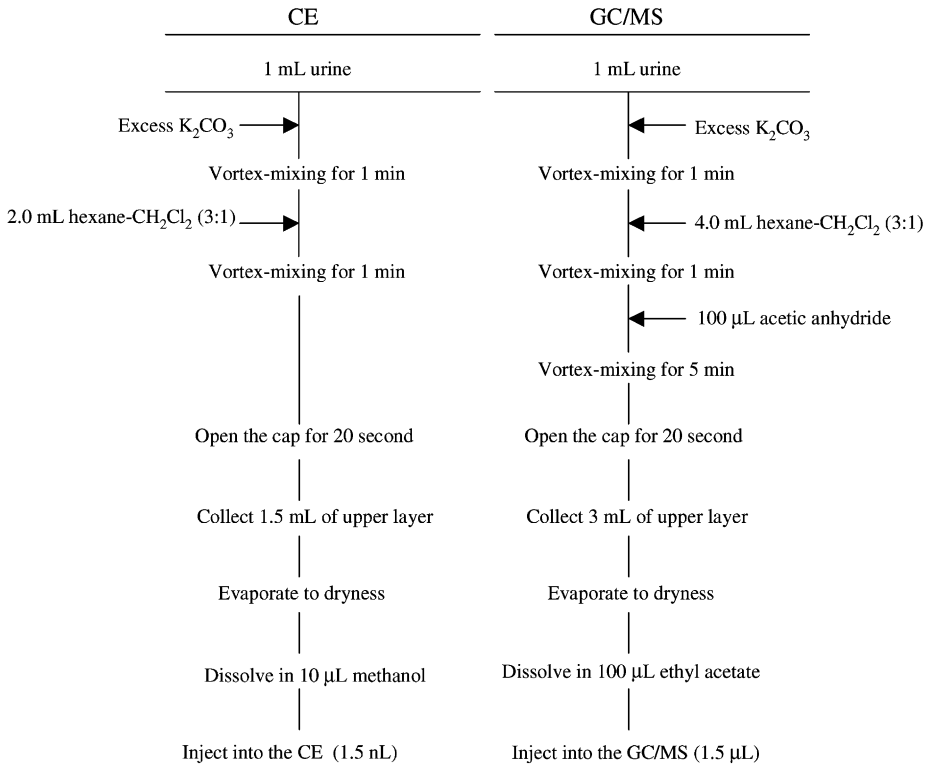


Fig. 1. Flow chart of the sample preparation procedures for CE and GC/MS.

(Hewlett–Packard 5973 mass selective detector) was used. A capillary column (30 m × 0.25 μm i.d.) with an HP-1 (100% dimethylpolysiloxane) bonded stationary phase film 0.33 μm thickness (Agilent Technologies, USA) was used. The inlet temperature was maintained at 230 °C. The column oven was held at 80 °C for 5 min, then programmed from 80–270 °C at 10 °C/min and, finally, held for 5 min. Helium carrier gas was used at a constant flow-rate of 1 ml/min. Data were collected using the Hewlett-Packard Chem-Station software. The mass conditions were as follows: SIM mode; ionization energy, 35 eV; ion source temperature, 280 °C. The mass selective detector was operated in the SIM mode, at a scan rate of 1.25 scans/s. The internal standard was methoxyphenamine hydrochloride, 5 ppm.

### 2.4. Liquid–liquid extraction procedure

One milliliter 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 MeOH for the subsequently CE separation. For the GCMS experiments, 1 ml of urine sample was also made alkaline by the addition of excess $K_2CO_3$. After the same process of liquid–liquid extraction, 0.1 ml acetic anhydride was added, to derivatize the 3,4-MDMA. Using the same subsequent procedures as described above, the residue was dissolved in 100 μl of ethyl acetate for use in the GCMS experiments. Details of procedures are summarized in Fig. 1.

## 3. Results and discussion

### 3.1. Separation of 3,4-MDMA standards by nonaqueous CE buffer

Fig. 2 shows the excitation and fluorescence spectra of 3,4-MDMA (spectra a and c, solid lines) and 3,4-MDMA-$D_5$ (spectra b and d, dashed lines) in the methanol matrix; the concentration for each was 100 ppm. The fluorescence spectra are nearly the same with a maximum at 317 nm; whereas the fluorescence excitation spectra provide different maxima at 289 and 291 nm (monochromator resolution = 0.3 nm), respectively. Fig. 3 exhibits a typical fluorescence CE chromatogram of the 3,4-MDMA and 3,4-MDMA-$D_5$ standards by NACE separation ($\lambda_{ex} = 285$ nm, $\lambda_{em} = 320$ nm). In the presence of a formamide–methanol solution (3:7, v/v) containing 100 mM SC and 20 mM of ammonium acetate, the separation was completed in less than 5 min (applied voltage, 15 kV; current, ~47 μA; capillary, 35 cm/30 cm to detector). A separation process in less than 3 min can be achieved, when the applied voltage is up to 20 kV, but the currents became too high (~70 μA) to maintain a stable migration time. The ratio of the height of the two peaks of

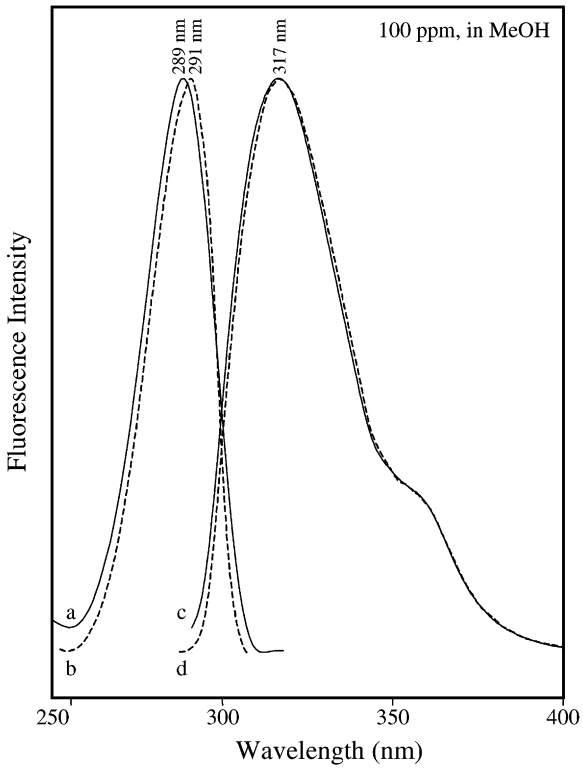


Fig. 2. Typical excitation and fluorescence spectra of 3,4-MDMA (spectra a and c, solid lines) and 3,4-MDMA-$D_5$ (spectra b and d, dash lines) in the methanol matrix; concentration of each was 100 ppm.

3,4-MDMA-$D_5$ and 3,4-MDMA was 10:9. This is the basic relationship used for the calculation of 3,4-MDMA for all of the actual urine samples. Other types of surfactants were also tested for the separation, including SDS (sodium dodecyl sulfate) and DOSS (sodium dioctylsulfosuccinate), but SC provided the best separation of these isotopic isomers. We also checked the efficiency of different organic solvents by using a single organic solvent, e.g. formamide or methanol, instead of a mixed solvent, and found that the separation was degraded. By a simple variation of organic solvents a significant change in selectivity can be achieved. Fig. 4 shows the CE chromatograms of one urine sample from a normal person (Frame A) and two suspect urine samples (Frames B and C) for comparison before and after the spiking standards of 3,4-MDMA-$D_5$. In Frame A, electropherograms a and b show normal human fresh urine (without any pretreatment) and this same urine spiked with 100 ppm of 3,4-MDMA-$D_5$ standard. In this case, the UV excitation and fluorescence emission wavelength were $280 \pm 8$ nm and $320 \pm 2$ nm, respectively. It is clear that in a typical human urine sample, only a few native fluorescent compounds are present which illuminate in the wavelength range of $320 \pm 2$ nm. Using this fluorescence detection, the electropherogram was much simple than UV detection due to the UV-absorption of

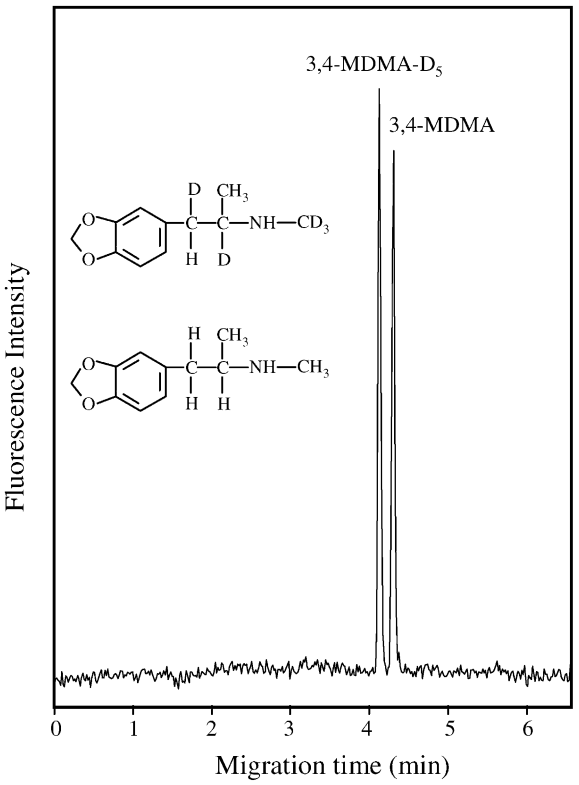


Fig. 3. Electropherograms of 3,4-MDMA-$D_5$ and 3,4-MDMA standards with fluorescence detection by nonaqueous capillary electrophoresis. The sample concentrations were 100 ppm. CE conditions: capillary, 35 cm (30 cm to detector); pumping and detection wavelength, $\lambda_{ex} = 285$ nm/$\lambda_{em} = 320$ nm; running buffer, 100 mM SC (Sodium cholate), 20 mM ammonium acetate ($CH_3COONH_4$) in the formamide-methanol solution (30:70, v/v); applied voltage 15 kV; current, 47 μA.

numerous organic compounds in the urine sample. This approach also provides a simple way to observe non-natural compounds in urine, such as MDMAs. Under exactly the same experimental conditions, two suspect urine samples were randomly chosen as the test samples, as shown in Frame B and C. The electropherograms c and e show that the urine samples of suspects I and II before spiking. The peaks marked with asterisks (∗) indicated that 3,4-MDMA was detected at 4.2 min by comparing the migration times with the spiked 3,4-MDMA standards. Electropherograms d and f show these urines spiked with 100 ppm of 3,4-MDMA-$D_5$ standards. Due to the biological matrix effect in the urine samples, the separation efficiency was not good as the standards (Fig. 3). Some of the commercial computer software could help us to calculate the reasonable area. For convenience, we use the peak heights for the calculation. By comparing the peak heights, the concentration of 3,4-MDMA in these urine samples were calculated to be 66.8 (suspect I) and 30.8 ppm (suspect II), respectively. All of the

Table 1
Concentrations of 3,4-MDMA in a series of suspect urine samples by NACE/FS detection

| No. | Concentration (ppm) |
|---|---|
| I | 66.8 |
| II | 30.8 |
| III | 12.4 |
| IV | 31.0 |
| V | 46.8 |
| VI | 11.3 |
| VII | 17.2 |
| VIII | 14.7 |
| IX | 20.0 |
| X | 52.9 |

results are shown in Table 1. Indeed, there are many methods for taking drugs, including by ingesting a pill or drinking a solution of a drug. Any of these procedures would resolute positive reaction and would result in a concentration of 3,4-MDMA in the urine, in the range of 1–90 ppm. In Taiwan, if the concentration of amphetamines or its related metabolite exceeds 0.5 ppm, then the suspect is judged guilty. Other types of abuse drugs such as cocaine, opium have more strict limitations which are set at 0.3 ppm. The limitation for cannabis is 0.05 ppm. The method proposed here provides sufficient sensitivity to permit the identification of such low levels of MDMAs.

### 3.2. Method sensitivity and precision

3,4-MDMA is naturally fluorescent, so that the derivatization is unnecessary. A calibration curve was constructed for 3,4-MDMA at concentrations between 5 and 100 ppm in MeOH (5, 10, 25, 50, 100 ppm). Fig. 5 shows that a linear relationship exists in the 5–100 ppm range of 3,4-MDMA standards. A 3:1 signal-to-noise ratio was found for <0.5 ppm of 3,4-MDMA. The linearity of the method for 3,4-MDMA was fairly good in the range of concentrations from 100 to 5 ppm, being described by the equation ($y = 0.4776x + 0.1887$, $r^2 = 0.9977$). As shown in the inset, under the conditions of liquid–liquid extraction, the sample can be condensed and the detection limit of 3,4-MDMA in urine was ∼50 ppb. The intra-day and day-to-day precision are described in Table 2. Peak areas were characterized by RSDs of 7–8% for concentration of 50 ppm. In day-to-day repeatability tests ($n = 6$) R.S.D.s were ∼9%.

### 3.3. Comparison with GCMS

The results obtained by NACE/FS were compared with GCMS. It has been reported that 3,4-MDMA only provided an imine fragment ($m/z = 58$), which was a major peak in the electron impact mass spectrum of MDMAs. Our data are

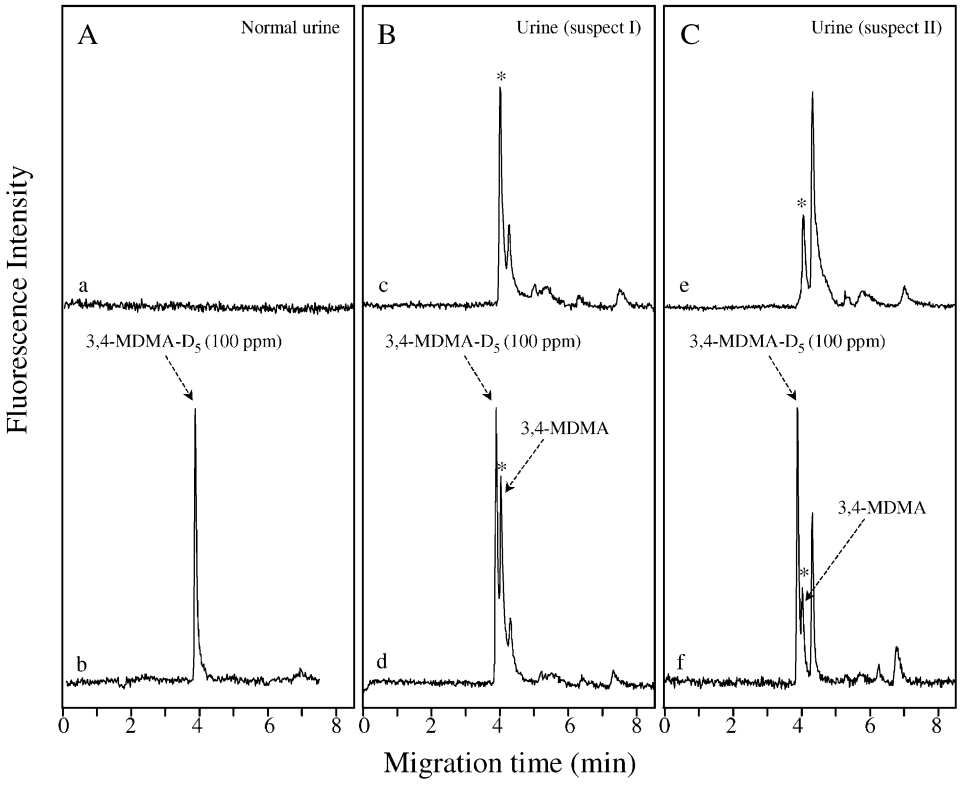


Fig. 4. CE chromatograms of urine samples from a normal subject (Frame A) and suspect I and II (Frame B and C). Chromatogram (a), normal urine sample; (b), a normal urine sample spiked with 100 ppm of 3,4-MDMA-$D_5$ standard; c and e, suspect I and II urine samples; d and f, suspect I and II urine samples spiked with 100 ppm of 3,4-MDMA-$D_5$. CE conditions are as stated in Fig. 3.

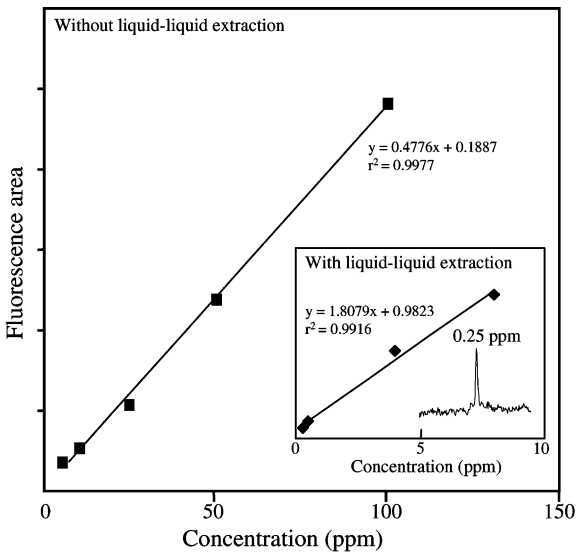


Fig. 5. Calibration graph of 3,4-MDMA at concentrations between 5 and 100 ppm in MeOH. Inset, calibration graph of 3,4-MDMA at concentration between 0.25 and 8 ppm after liquid–liquid extraction.

in agreement with this. Thus, optimum derivatization of 3,4-MDMA was performed via the reaction with acetic anhydride. Fig. 6A shows the selected ion current ($m/z = 58$, 100 and 162) profile for 3,4-MDMA extracted from the urine sample of suspect III and Fig. 6B shows the fragmenting mechanism for the derivatized 3,4-MDMA. The identification carried out by spiking extracts with an internal standard (methoxyphenamine hydrochloride). After the derivatization and liquid-liquid extraction procedures, the total operating time was ~1 h for one sample and the detection limit was 0.1 ppm.

Table 2
Precious of retention times and peak areas (R.S.D.%)

| | Intra-day ($n = 6$) | | Day-to-day ($n = 6$) | |
|---|---|---|---|---|
| | Time | Area | Time | Area |
| 3,4-MDMA 50 (μg/ml) | 1.48 | 7.47 | 3.86 | 9.88 |
| 3,4-MDMA-$D_5$ 50 (μg/ml) | 1.56 | 8.71 | 3.54 | 9.12 |

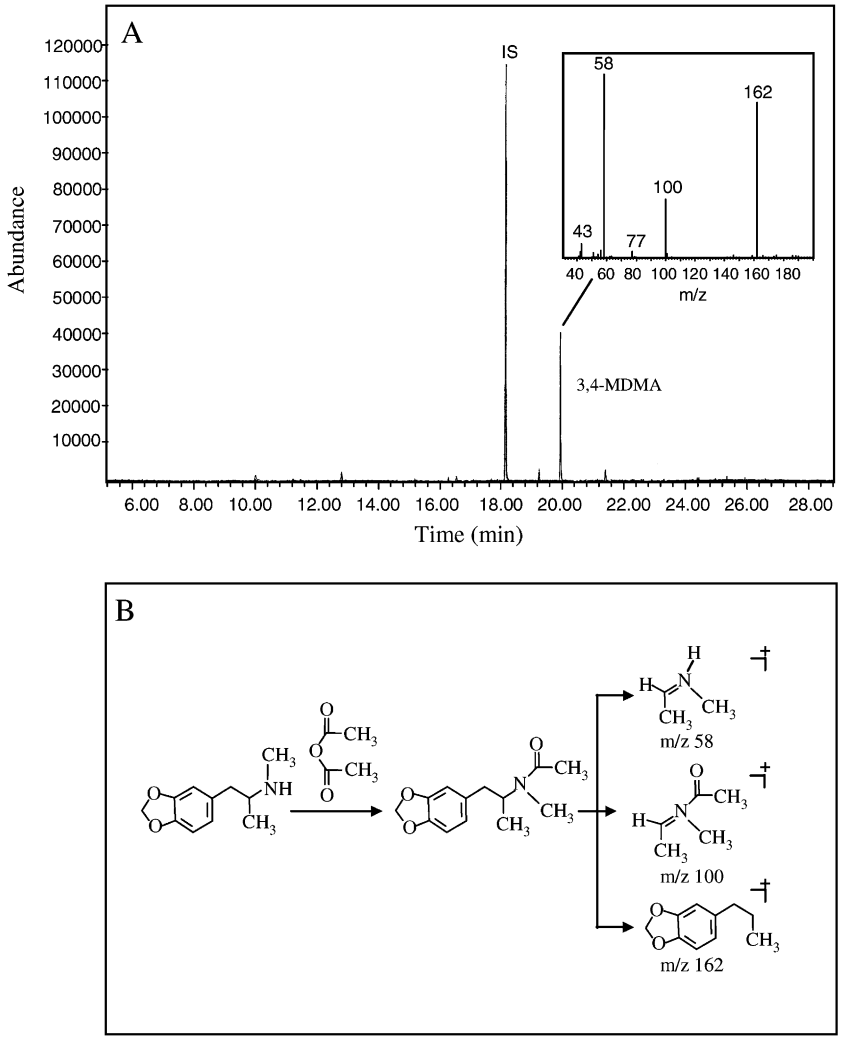


Fig. 6. Selected ion current profile for the 3,4-MDMA derivative extracted from a urine sample of suspect III and measured using GC/MS. Internal standard: methoxyphenamine hydrochloride.

## 4. Conclusions

We have demonstrated that a NACE/FS method can be successfully used for the separation and identification of 3,4-MDMA in urine samples of suspects. The method we proposed here can provide the results in less than 5 min with no need for pre-treatment resulting in a 0.5-ppm detection limit of 3,4-MDMA (using the liquid–liquid extraction developed in this study, the detection limit can further improved to ~50 ppb); whereas GCMS requires derivatization and extraction for success.

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