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Short communication

Rapid GC–MS analysis of methamphetamine and its metabolites in urine—application of a short narrow-bore capillary column to GC–MS☆

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Abstract

A rapid analysis of methamphetamine and its metabolites in urine was performed by gas chromatography–mass spectrometry (GC–MS) using a short narrow-bore capillary column (NBC) (5 m × 0.1 mm I.D.). For detection, selected ion monitoring (SIM) was performed for the characteristic ions of each of the compounds. The analytes were independently detected within 2 min. Linearity was demonstrated over a range from 25–2500 ng/ml. As an application of this study, a urine sample from a drug-abuse suspect was analyzed. The analytes from the actual sample were detected with reasonable reproducibility. The results indicate the possibility of rapid analysis using a conventional GC–MS with a short NBC at a relatively low inlet pressure.

Keywords: GC–MS; Short narrow-bore capillary column; Amphetamines

1. Introduction

The precise and rapid demonstration of drugs in the urine of an illegal drug abuser is needed in the field of forensic toxicology. As there are various kinds of illegal drugs, several drug examinations need to be performed even for just one case. Furthermore, we frequently run into a large number of cases over a very short period. However, both staff and analytical instruments are in limited supply. For these reasons, rapid analytical methods utilizing accurate and simple procedures are required.

With that in mind, many attempts at drug analysis have been carried out using gas chromatography–mass spectrometry (GC–MS). The concept of fast GC or fast GC–MS has appeared within the past few years [1–5]. Selected ion monitoring (SIM) in GC–MS has been performed using a narrow-bore capillary column (NBC) (10 m × 0.1 mm I.D.) and this has successfully achieved the detection of polychlorinated biphenyls with a high sensitivity and selectivity within a short period [2]. For the screening of multiple pesticides in foods, SIM was used by utilizing an analytical column (10 m × 0.53 mm I.D.) coupled with a restricted capillary column (3 m × 0.15 mm I.D.) at a low inlet pressure [3]. As a fast GC–MS system, GC–MS–MS was used with an analytical column connected to a short narrow-bore guard column [4]. In one of the attempts, the combination of a short narrow-bore capillary column (5 m × 0.1 mm I.D.) with a fast temperature programming system enabled the analysis of various pesticides [5]. Ultimately, many methods developed for fast GC or fast GC–MS require special systems or new systems.

In this study, we attempted to use conventional GC–MS with a short NBC (5 m × 0.1 mm I.D.) without any new systems or special systems, for the rapid analysis of amphetamines in urine.

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For this rapid urinary analysis, the method of extractive derivatization described in our previous report [6] was used.

2. Experimental

2.1. Materials

2.1.1. Reagents

Methamphetamine (MAMP) hydrochloride was purchased from Dainippon Sumitomo Pharma Co. Ltd. (Osaka, Japan). Amphetamine (AMP) sulfate was obtained as official standards for criminal drug testing from the National Institute of Health Sciences in the Ministry of Health, Labour and Welfare of Japan. 4-hydroxymethamphetamine (4-HMAMP) was prepared as described in our previous report [7]. Phentermine (α, α-dimethylphenethylamine) hydrochloride as an internal standard (IS) and heptafluoro-*n*-butyryl (HFB) chloride were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Diatomaceous Earth (granular) was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The other common reagents used in this study were of an analytical grade.

2.1.2. Preparation of standard solutions and internal standard solution

MAMP hydrochloride, AMP sulfate, 4-HMAMP base and phentermine hydrochloride were dissolved in 0.1 M HCl to obtain 1 mg/ml solutions as stock solutions. Calibration standard solutions at three different concentrations (1, 5, 10 μg/ml) were prepared by the appropriate dilution of the stock solutions using 0.1 M HCl. Three calibration standard solutions were used to create five calibration points (25, 50, 100, 500, 2500 ng/ml). Phentermine stock solution was diluted in 0.1 M HCl to prepare internal standard (IS) solution at 10 μg/ml solution. All the solutions were stored at 4 °C until use.

2.1.3. Urine for standard samples and selectivity test

The urine used in this experiment was provided by two male and three female volunteers who had not taken any drugs within the previous one-month period. The urine demonstrated no interfering peaks when MAMP, AMP, 4-HMAMP and IS were detected using the method described below.

2.2. Sample preparation

Five microlitres of 10 μg/ml IS solution and 0.5 ml of 0.04 M borate buffer (pH 9) were added to 0.2 ml of a male volunteer's urine, among five urine samples. The urine pH measured was 5.6. After being adjusted to pH 12.6 with several drops of 1 M NaOH, the urinary sample was applied to a column (3 ml Glass Tube, 5.5 cm × 0.8 cm I.D., Supelco, Bellefonte, PA, USA) filled with 1 g of diatomaceous earth. After penetration for 15 min, the analytes were eluted as their HFB derivatives with 3 ml of *n*-hexane containing 10% propyl acetate and 1% HFB chloride. The derivatives were condensed to about 20 μl under a nitrogen stream at room temperature. The condensed solution was diluted with 50 μl of ethyl acetate for GC–MS analysis. It is recommended that sample preparation is carried out in a fume hood because of the toxic nature of HFB chloride.

2.3. Sample preparation for calibration curves

For the running of calibration curves, calibration standard solutions for five calibration points with 5 μl of 10 μg/ml IS solution were appropriately added to 0.2 ml of urine and treated in the same manner as that described above. Standard calibration curves were established by three replicates per concentration and constructed using peak area ratios of standard to IS.

2.4. Intra-assay and inter-assay

In order to indicate the reliability of intra- and inter-assays, precision and accuracy were evaluated over the linear dynamic range of the method at 25, 100 and 1000 ng/ml standard concentrations. Each of the three authentic standard solutions and 5 μl of 10 μg/ml IS solution were added to 0.2 ml of an old male volunteer's urine and treated in the same manner as that described above. Intra-assay precision was evaluated by analyzing five replicates per concentration prepared in a day and inter-assay precision by analyzing two replicates prepared everyday for five days. Accuracy was determined by comparing mean intra-assay measured concentrations of three analyses to target, and expressed as percentage of target concentration.

2.5. GC/MS conditions

GC–MS analysis was performed by a 5973 Network mass spectrometer coupled with a 6890 series gas chromatograph (GC) equipped with a 7683 series autosampler (Agilent Technologies, Palo Alto, CA, USA). The whole system was controlled by Chemistation software (version G1701CJ). As for column, an EQUITY^{TM}-5 fused silica capillary column, 5 m × 0.1 mm I.D., with a film thickness of 0.10 μm (Supelco, Bellefonte, PA, USA) was used. The temperature of the GC oven was initially set at 100 °C for 0.5 min and then increased to 325 °C by programming at 50 °C/min. The carrier gas; high-purity helium (99.999%) was controlled at a constant flow mode. The flow rate was 0.9 ml/min. The inlet pressure was initially set at 310 kPa for 0.5 min, with a final pressure of 510 kPa. The temperatures of the injection port and the interface were 250 °C and 280 °C, respectively. Sample injection was performed in the split mode (split ratio = 1:10) with a 1 μl injection volume. The ionizing energy was 70 eV. For detection, SIM was performed by monitoring seven selected ions indicated in Table 1. The dwell

Table 1
Selected ions for SIM

Compound	Monitoring ions (*m/z*)		
AMP	91	<u>118</u>	240
MAMP	118	210	<u>254</u>
4-HMAMP	210	<u>254</u>	330
IS	91	132	<u>254</u>

The underlined numbers were used for quantification.

time for each selected ion was 10 ms. The scanning cycle speed for seven ions detected in SIM mode was in fact 5.56 cycle/s.

3. Results

The HFB derivatives of AMP, MAMP, 4-HMAMP and IS appeared within 2 min as seen in Fig. 1, and the variability of their retention times was less than 0.3% as a coefficient of variation, as shown in Table 2. The respective peak widths at the target ions are also indicated in Table 2. Their peak widths at the base means that each of the peaks was composed of more than 8 points as the scanning cycle speed was 5.56 cycle/s. Then, 8 scans across a peak were sufficient for peak integration.

Calibration curves of each analyte to IS were respectively linear over a range of 25 to 2500 ng/ml; $y = 0.00135x + 0.01404$ ($r^2 = 0.9999$) for AMP, $y = 0.00587x - 0.15274$ ($r^2 = 0.9993$) for MAMP, $y = 0.00555x - 0.0142$ ($r^2 = 1$) for 4-HMAMP. The reliability of this method was indicated as precise and accurate, estimated by the measurements of five replicates at three concentrations: 25, 100 and 1000 ng/ml (see Table 3). The peaks at a concentration of 25 ng/ml (the lowest level) were detected as a signal-to-noise ratio of more than 14 for AMP, more than 39 for MAMP and more than 93 for 4-HMAMP in SIM mode.

Table 2
Retention time and peak width of the compound

Compound	Retention time[a] (min) (% CV)	Peak width at the base (s)
AMP	1.008 (0.2)	2.76
MAMP	1.241 (0.2)	2.40
4-HMAMP	1.726 (0.1)	1.56
IS	1.027 (0.3)	2.76

CV: coeficient of variation ($n = 5$ each).
[a] The retention time was measured at the peak top of the target ion for quantification (see Table 1).

The sample preparation time was on average 20 min. The GC–MS analysis time per sample was 10 min, including 4 min for the GC oven to cool, injector needle cleaning and conditioning time. The total analysis time was therefore 30 min per sample.

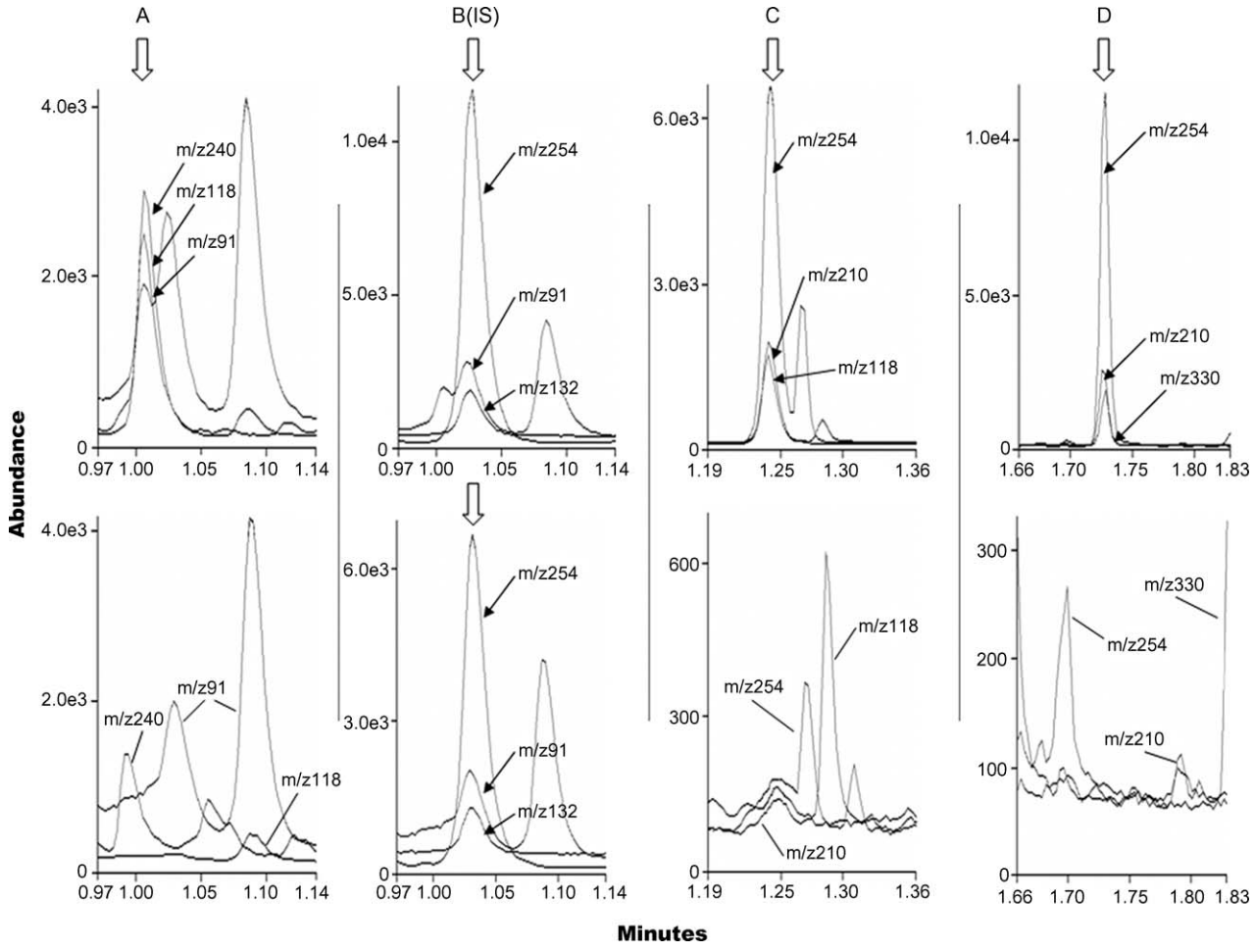

Fig. 1. SIM chromatograms obtained from a male volunteer's urine spiked with (A) amphetamine, (B) phentermine (IS), (C) methamphetamine, (D) 4-hydroxymethamphetamine (100 ng/ml each) and IS (250 ng/ml). As a blank sample, SIM chromatograms of a female volunteer's urine only spiked with IS (250 ng/ml) to confirm IS peak on a chromatogram were indicated under them. Peaks (C) and (D) on chromatogram of *m/z* 254, and peak (A) on chromatogram of *m/z* 118 were not interfered with any peaks although there were some small peaks around the target peaks. However, AMP peak on chromatogram of *m/z* 240 was disturbed at low concentration of AMP.

Table 3
Precision and accuracy were evaluated over the linear dynamic range of the method at 25, 100, 1000 ng/ml standards concentrations

Target concentration (ng/ml)		Precision		Accuracy (% Target, $n=5$)
		Intra-assay (% CV, $n=5$)	Inter-assay (% CV, $n=10$)	
AMP	25	9.1	17.8	45
	100	1.8	9.8	92
	1000	0.1	3.0	102
MAMP	25	5.0	6.3	101
	100	1.2	1.5	99
	1000	0.4	2.0	100
4-HMAMP	25	3.3	10.0	129
	100	3.8	2.3	97
	1000	4.3	1.9	99

Each of the three authentic standard solutions and 5 μl of 10 μg/ml IS solution were added to 0.2 ml of a male volunteer's urine. Intra-assay precision was evaluated by analyzing five replicates per concentration prepared in a day, inter-assay precision by analyzing two replicates prepared everyday for five days. Accuracy was determined comparing mean intra-assay measured concentrations of three analyses to target, and expressed as percentage of target concentration.

4. Application

4.1. Case

A 30-year-old man was arrested on suspicion of MAMP consumption. For confirmation of his crime, his urine, which had been collected by an urologist on the basis of a warrant, was subjected to drug testing. Amphetamines were detected by Triage® and a conventional drug test. We analyzed his urine by the current method to assess its utility.

Under interrogation, the suspect initially insisted that he had not taken any MAMP, but he finally made a statement confessing that he had swallowed one capsule containing about 20 mg of MAMP hydrochloride two days before his urine sample was collected.

4.2. Analysis

Peaks corresponding to AMP, MAMP and 4-HMAMP were detected, as indicated in Fig. 2. The relative abundance ratios of the characteristic ions at the peak (C) corresponding to MAMP were identical to MAMP derivative. The peak (D) was also identified as identical to the mass spectrum of 4-HMAMP derivative. Although the peak (A) at *m/z* 91 overlapped with the IS peak (B), the relative abundance ratios at peak (A) were identical to AMP derivative.

In an effort to estimate the concentrations, the urine was diluted 20 times with distilled water, and five replicates were prepared. The estimated concentrations (mean ± SD) were 3.31 ± 0.12 μg/ml for AMP, 20.5 ± 0.2 μg/ml for MAMP and 3.76 ± 0.35 μg/ml for 4-HMAMP.

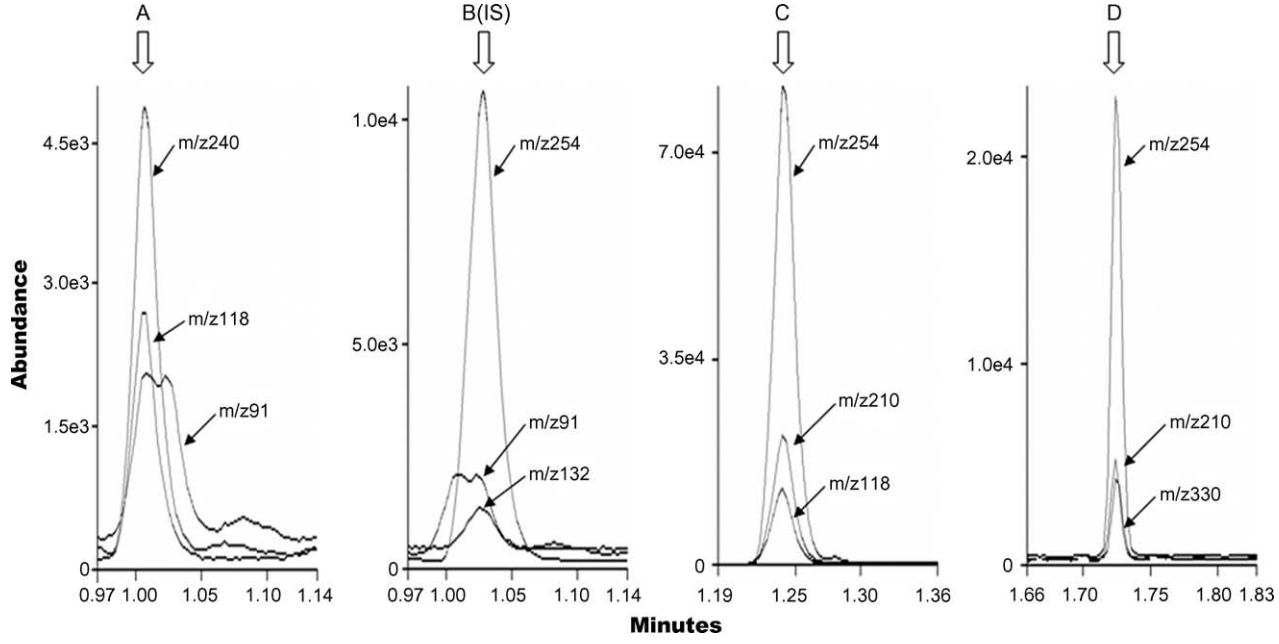

Fig. 2. SIM chromatograms obtained from the urine of a 30-year-old male suspect in an actual case. The corresponding peaks to AMP, MAMP and 4-HMAMP are indicated as (A), (C) and (D). Peak (B) is the IS. The estimated concentrations (mean ± SD); 3.31 ± 0.12 μg/ml for AMP, 20.5 ± 0.2 μg/ml for MAMP and 3.76 ± 0.35 μg/ml for 4-HMAMP.

5. Discussion

In this study, we used a 5 m NBC in order to obtain a short analytical time. In comparison with our previous method that used a regular sized capillary column (30 m × 0.25 mm I.D., film thickness, 0.25 μm) [6], this method gave dramatically shorter retention times for AMP, MAMP and 4-HMAMP. With a view to reducing the analytical time, Mastovska and Lehotay [1] made some suggestions such as shortening the capillary column, rapidly altering the column temperature, selecting a different stationary phase, and so on. Meanwhile, Covaci and Schepens demonstrated the effect of using a narrow-bore column (0.1 mm I.D.) to achieve a fast GC–MS performance [2]. Although the NBC is used for the fast GC–MS under a condition of high inlet pressure, it is difficult to control the pressure using a conventional GC–MS apparatus. In order to apply a conventional instrument to the fast GC–MS, we used a 5 m NBC.

In this study, we observed the effect of a 5 m NBC on the fast GC–MS. However, the results obtained by SIM performance seem to be insufficient for the purposes of forensic toxicology. The peak widths were too narrow to acquire intact mass spectra under the GC–MS conditions, as already described [1]. Instead of obtaining mass spectra, we attempted to identify the peaks with the relative ratios of the peak areas of three ions selected for each of the compounds.

For the sample injection of GC–MS, we need to select the split mode because of the very small sample loading capacity of an NBC. The sensitivity of this method was satisfactory for detecting every objective at a low level with a urinary concentration of 25 ng/ml, as relatively high intensive peaks were obtained. Furthermore, to enable fast analysis, we improved, in part, the method of extractive derivatization which we had previously developed and reported earlier [6]. That is to say, we cut the volume of hexane solution through a column by less than half to decrease condensation time and added propyl acetate to the solution. Proply acetate plays two roles. It aids the dissolution of compounds poorly soluble in hexane, and delays evaporation the eluted solution entirely at the end of condensation under a nitrogen stream. Although the preparation takes 20 min per sample, analysis of samples in parallel with sample preparation can reduce the overall time taken when analysing a lot of samples. Through the combination of the sample preparation and the GC–MS analysis with a 5 m NBC, the entire procedure of testing for amphetamines abuse can now be successfully performed in just 30 min.

As for precision and accuracy test, phentermine as IS is more reproducible for quantification of MAMP than those of AMP and 4-HMAMP. Coefficient of variation for each analyte at the lowest concentration is so variable that quantification analysis around the concentration is unavailable. However, phentermine as IS becomes a beneficial substitute except for around the concentration if it is hard to obtain deuterated analogs for AMP, MAMP and 4-HMAMP.

6. Conclusion

Using a 5 m narrow-bore capillary column after extractive derivatization successfully reduced the time needed for urinary amphetamines analysis by GC–MS. This method of analysis will be useful in a busy forensic toxicological laboratory.

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