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Determination of methamphetamine and related compounds by capillary electrophoresis with UV and laser-induced fluorescence detection

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Abstract

Methods for the simultaneous determination of methamphetamine (MP) and its related compounds (MPs) using capillary electrophoresis (CE) with UV absorbance and laser-induced fluorescence (LIF) detection are described. In UV detection, MPs were applied to CE without any derivatization procedure and detected at 210 nm for a rapid and simple analysis. Capillary zone electrophoresis (CZE) and electrokinetic capillary electrophoresis (MEKC) were used. MP, amphetamine (AP), 1-phenylethylamine (1-PA as an I.S.), 2-phenylethylamine (2-PA), 4-hydroxymethamphetamine (4-HMP) and 4-hydroxyamphetamine (4-HAP) were separated within 15 min by both CZE and MEKC. Detection limits of MPs were in the range 48–72 fmol/injection for CZE and 85–191 fmol/injection for MEKC. MEKC was successfully applied to the determination of MPs in urine. For a highly sensitive analysis, LIF detection was also examined using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) as a fluorescent derivatization reagent. By the method, in which MPs derivatives were separated within 45 min by MEKC, 22–40 amol/injection of primary amines (AP, 4-HAP and 2-PA) and 690 amol/injection of MP and 300 amol/injection of 4-HMP were detected. The concentration of MP and AP in 50 μl urine from MP addicts were successfully determined. A comparison of the characteristics for both UV and LIF detections was also discussed. © 1998 Elsevier Science B.V.

Keywords: Methamphetamines; Amphetamines; Phenylethylamines

1. Introduction

Methamphetamine (MP) and amphetamine (AP) are well known central nervous system stimulants that bring about wakefulness and anorexia. The escalation of abuse of these drugs in Japan is causing serious social problems from the point of view of drug dependence and an increase in crime. A reliable method for identification and quantitation of MP and its metabolites is thus very important for forensic as well as clinical and pharmaceutical studies. Many methods have been developed for this purpose: gas chromatography (GC) [1–3], GC–mass spectrometry (GC–MS) [4–6], polarization fluoroimmunometry [7] and high-performance liquid chromatography (HPLC) [8–11]. Each method has its own advantages and disadvantages regarding sensitivity, precision and simplicity. Mainly, urine was employed as the sample in the methods described above. However, the sample type for the analysis of MP and its metabolites is diversely growing: determination of MP in nails [12] and hair [5,12] to elucidate the

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history of drug abuse, and also sweat [13] have been reported. Therefore, the suitable method should be selected by considering the sensitivity and selectivity required, and the time for analysis according to the sample type.

Capillary electrophoresis (CE) has been reported as a powerful tool for a wide range of analytes, including many applications to the analyses of drugs in recent years. CE is especially advantageous for the simultaneous determination of a number of analytes with high resolution and speed [14]. In the present paper, we have investigated the potential of CE to determine MP and its related compounds (MPs). Both UV and laser-induced fluorescence (LIF) detection were examined and a comparison of the results is made regarding the separation, sensitivity, precision and simplicity. The applicability of these methods to practical samples was also studied by using urine samples.

2. Experimental

2.1. Reagents and materials

1-Phenylethylamine (1-PA) and 2-phenylethylamine (2-PA) were purchased from Aldrich (Milwaukee, WI, USA). Methamphetamine hydrochloride (MP) was obtained from Dainippon Pharmacy (Osaka, Japan). Amphetamine sulfate (AP), 4-hydroxyamphetamine (4-HAP) and 4-hydroxymethamphetamine (4-HMP) were synthesized in our laboratory. These compounds were dissolved in water to give 10^{-2} *M* solutions and then diluted with water to the appropriate concentrations prior to use. 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), used as a fluorescent derivatization reagent, was obtained from Tokyo Chemical Industries (Tokyo, Japan) and dissolved in EtOH. Water was deionized and passed through a Pure line WL21P water purification system (Yarnato kagaku, Tokyo, Japan). All other reagents and solvents were of analytical reagent grade.

Urine samples from healthy volunteers were obtained in our laboratory and those from MP addicts were kindly provided for this investigation by the Forensic Science Laboratory of Nagasaki Prefectural Police Headquarters.

2.2. Apparatus.

The following CE instruments and conditions were used in all the analyses. For UV detection: CAPI-3100A (Otsuka Electronics, Osaka, Japan); siphoning injection (20 s with 20 mm height differential which corresponded to 9 nl injection for CZE and 17 nl injection for MEKC, these volumes were calculated according to the manual by using the data of peak area and electrophoretic velocity obtained with theophylline as a standard sample to be measured in each separation mode); detection at 210 nm; separation voltage, 10 kV for CZE and 15 kV for MEKC; fused-silica capillary (50 cm×75 μm I.D.), effective length of 37.5 cm; temperature, 25°C. The CZE was performed using 50 m*M* CH_3COONa–HCl buffer (pH 3.0) as a running solution. A running solution for MEKC was 50 m*M* $Na_2B_4O_7$ solution containing 15 m*M* SDS (pH 9.3). Both running solutions were filtered through 0.45 μm filters (Millipore, Yonezawa, Japan) before use. For LIF detection: Beckman P/ACE 5510 (Palo Alto, CA, USA) with a laser module [air-cooled argon ion laser (4 mW) at 488 nm]; pressure injection (1 s with 0.5 p.s.i, pressure which corresponded to 6 nl injection; 1 p.s.i.=6894.76 Pa); detection of fluorescence at 520 nm filtered by a band pass filter (520 nm); separation voltage, 15 kV; fused-silica capillary (57 cm×75 μm I.D.), effective length of 50 cm; temperature, 25°C. The running solution used was the same as that for the MEKC with UV detection except for that 10 m*M* SDS was employed. The capillaries for both the systems were rinsed with the corresponding running solutions for 5 min before each run.

2.3. Fluorescent derivatization procedures

To 100 μl of a sample in 50 m*M* $Na_2B_4O_7$–NaH_2PO_4 buffer (pH 6.7) was added 100 μl of 20 m*M* NBD-F in EtOH. The mixture was mixed well and heated at 80°C for 10 min. After cooling to room temperature, 200 μl of 0.15 *M* HCl in EtOH was added, and then the mixture was applied to the CE system.

2.4. Sample preparation

The assay procedure for urine with UV detection

was as follows: to 5.0 ml urine or diluted urine in a test tube, 100 μl of I.S. solution (5.0×10^{-4} *M*) was added followed by the addition of 1.0 ml of concentrated HCl. The mixture was heated at 80°C for 1 h, chilled in tap water, and neutralized with 1.5 ml of 28% aqueous ammonia. To it were added 3.5 ml of 500 m*M* H_3BO_3–NaOH solution (pH 10.5) containing 4.5 *M* NaCl and 2.0 ml of chloroform–2-propanol (3:1, v/v), the mixture was shaken vigorously for 1 min, and then centrifuged at 1500 g for 10 min. This extraction was repeated again and the organic layers obtained were combined (ca. 4 ml), washed with 2.5 ml of water, and centrifuged at 1500 g for 10 min. After the addition of 400 μl of acetic acid, the organic layer was evaporated and dissolved in 200 μl of water. This solution filtered through 0.45 μm filters was applied to the CE system.

The following procedure for urine was used in the CE–LIF detection system: to 50 μl urine or diluted urine were added 10 μl of I.S. solution (2.1×10^{-4} *M*) and 20 μl of concentrated HCl. After heating at 80°C for 1 h, the mixture was chilled in tap water, and to it were added 30 μl of 28% aqueous ammonia and 2.0 ml of 100 m*M* Na_2CO_3–$NaHCO_3$ buffer (pH 9.0). The mixture was then applied to a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA). After washing the cartridge with 5.0 ml of water, elution was carried out with 4.0 ml of MeOH. After the addition of 400 μl of acetic acid, the eluate was evaporated. To the residue of the eluate, 100 μl of 50 m*M* $Na_2B_4O_7$–NaH_2PO_4 buffer (pH 6.7) was added and the resultant solution was subjected to the fluorescent derivatization with NBD-F.

3. Results and discussion

3.1. CE–UV detection

CE separation conditions were first optimized for CZE and MEKC. In CZE, separation of MPs was examined using 50 m*M* CH_3COONa–HCl buffer at various pHs (2.0–5.0) as running solutions; the longest and constant migration time was observed over the range of pH 2.5–3.5. The concentration of CH_3COONa in the buffer slightly affected the separation; the migration time for each peak increased with an increase in the concentration range 10–100 m*M*. However, the increase in the concentration of CH_3COONa resulted in a peak broadening. 50 m*M* CH_3COONa–HCl buffer at pH 3.0 was selected in this experiment. The use of the voltage at higher than 10 kV increased the baseline noise level and thus 10 kV was chosen. Under the conditions, MPs were separated from each other within 13 min (Fig. 1A). The effect of pH on the separation of MPs by MEKC was tested with the following solutions containing 10 m*M* SDS; 50 m*M* $Na_2B_4O_7$–NaH_2PO_4 (pH 8.5–9.0), $Na_2B_4O_7$ (pH 9.3) and $Na_2B_4O_7$–NaOH (pH 9.5). Since almost the same results were obtained, 50 m*M* $Na_2B_4O_7$ (pH 9.3) solution was selected. Concentrations of $Na_2B_4O_7$ above 75 m*M* made the width of each peak to be broad; 50 m*M* was chosen. The migration time for each peak increased with an increase in SDS concentration from 10 to 20 m*M*; 15 m*M* was employed for further experiments resulting in rapid separation. The voltage was tentatively set at 15 kV by considering the current not to exceed 150 μA recommended to obtain a better resolution in this CE equipment. As shown in Fig. 1B, MPs were well separated within 15 min.

Calibration curves were prepared by using known concentrations of MPs. Table 1 summarizes the linear regressions with the detection limits obtained by CZE and MEKC. In case of CZE, a slight improvement of the sensitivity was observed in comparison with MEKC.

3.2. Determination of MPs in urine with CE–UV detection

In a preliminary experiment, urine sample were applied to both CZE and MEKC. Since the peaks for 4-HAP and 4-HMP were overlapped with unknown peaks derived from urine sample in CZE, MEKC was thus used for further investigations. The extraction step was carried out as described in the literature [10] with minor modifications. The number of repeated extractions with 2.0 ml of chloroform–2-propanol (3:1, v/v) affected the recoveries of MPs (Fig. 2). Although the recoveries of 1-PA, 2-PA, AP and MP reached constant for more than 2 times of extraction, those of 4-HAP and 4-HMP gradually increased; the number of extraction was tentatively set at twice to facilitate the extraction procedure.

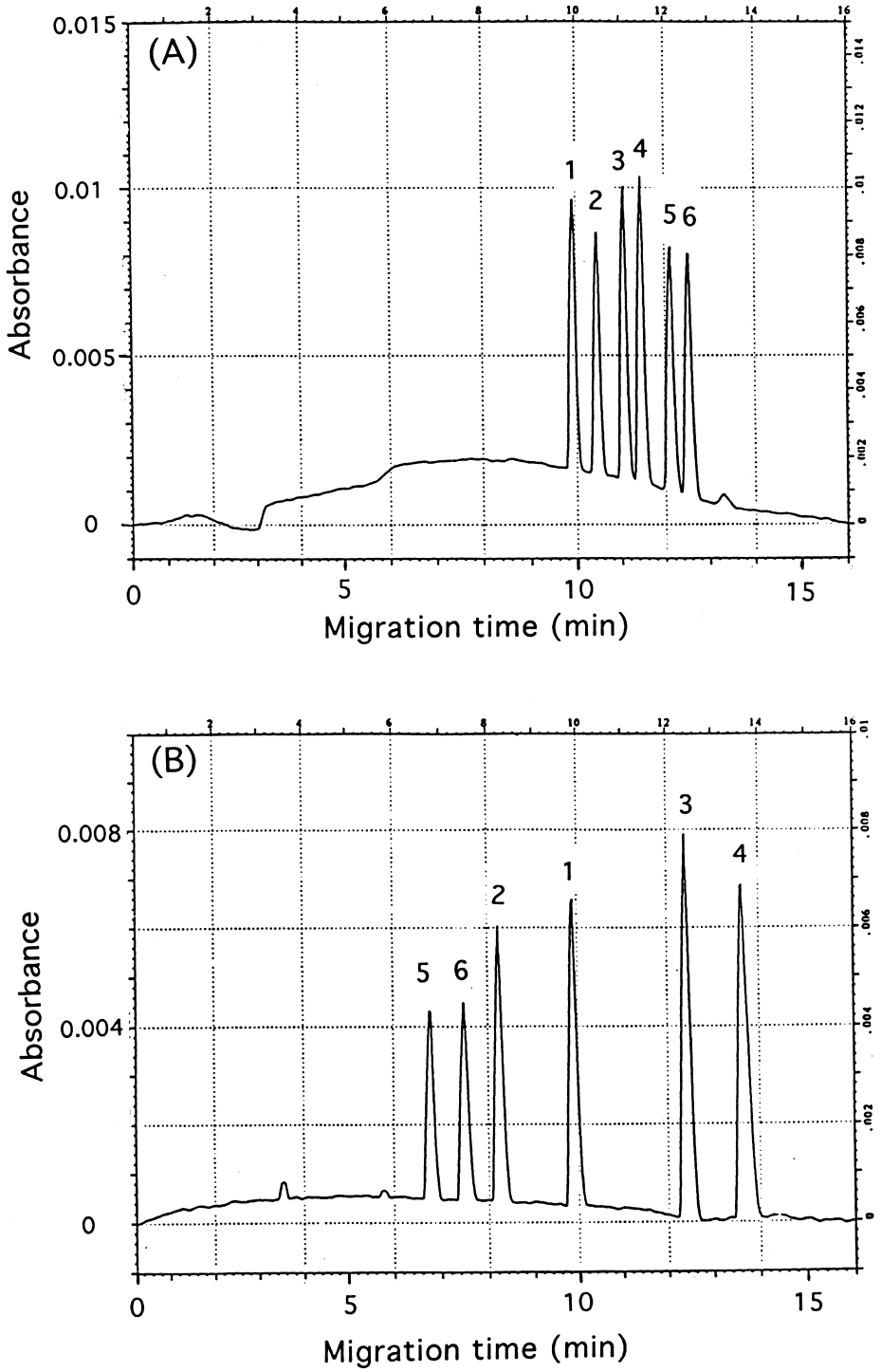

Fig. 1. Electropherograms of MPs with UV detection by (A) CZE, and (B) MEKC. Peak: 1=2-PA; 2=1-PA; 3=AP; 4=MP; 5=4-HAP; 6=4-HMP. Sample injected: 17 pmol each for CZE; 10 pmol each for MEKC. CE conditions: capillary, 50 cm (37.5 cm to detector)×75 μm I.D.; detection, UV at 210 nm; temperature, 25°C; running solution, (A) 50 m*M* CH_3COONa–HCl (pH 3.0), (B) 50 m*M* $Na_2B_4O_7$ containing 15 m*M* SDS (pH 9.3); voltage, (A) 10 kV, (B) 15 kV.

Table 1
Calibration curves and detection limits for MPs with UV detection by CZE and MEKC

Separation	Compound	Range (pmol/injection)	Equation[a]	r	Detection limit[b] (fmol/injection)
CZE	MP	0.09–9.0	$y=3596x-95$	1.000	68
	AP	0.09–9.0	$y=3390x-64$	1.000	59
	4-HMP	0.09–9.0	$y=3012x+157$	1.000	50
	4-HAP	0.09–9.0	$y=2849x+164$	1.000	48
	2-PA	0.09–9.0	$y=2765x-80$	1.000	72
	1-PA	0.09–9.0	$y=2994x-62$	1.000	72
MEKC	MP	0.17–85	$y=1612x-318$	1.000	87
	AP	0.17–85	$y=1445x+117$	1.000	85
	4-HMP	0.17–85	$y=613x+426$	1.000	170
	4-HAP	0.17–85	$y=555x+398$	1.000	191
	2-PA	0.17–85	$y=1086x+367$	1.000	107
	1-PA	0.17–85	$y=871x+473$	1.000	120

[a] y=peak area ($\times 10^7$ AU min); x=concentration (pmol/injection).
[b] Concentration giving a $S/N=3$.

Salts dissolved in the organic phase after the extraction step disturbed the separation of MPs by broadening the peak width on CE separation; washing the organic phase with more than 2.0 ml of water improved this phenomenon and therefore the washing step with 2.5 ml of water was introduced in the procedure. Fig. 3 shows typical electropherograms obtained for normal human urine and that spiked with MPs under the conditions established.

Working curves were prepared by using urine spiked with known concentrations of MPs. A peak area providing better precision than peak height was measured for preparing the working curves. The peak area ratios of each compounds to I.S. were linear from 0.85 to 60 pmol/injection (2.0×10^{-9}–1.4×10^{-7} mol/ml urine) for MP ($r=0.998$), AP ($r=0.999$) and 2-PA (0.999), from 8.5 to 60 pmol/injection (2.0×10^{-8}–1.4×10^{-7} mol/ml urine) for 4-HMP ($r=0.997$) and from 26 to 60 pmol/injection (6.1×10^{-8}–1.4×10^{-7} mol/ml urine) for 4-HAP ($r=0.992$). The detection limits calculated by peak height ratio were from 350 to 580 fmol/injection (8.2×10^{-10}–1.4×10^{-9} mol/ml urine) for MP, AP and 2-PA, 7.5 pmol/injection (1.8×10^{-8} mol/ml urine) for 4-HMP and 9.2 pmol/injection (2.2×10^{-8} mol/ml urine) for 4-HAP at a signal-to-noise ratio (S/N) of 3. Recovery and precision test were carried out by adding MPs (73.7–81.8 nmol) to 5.0 ml of urine. The mean recoveries ($n=5$) were from 81.8 to 92.5% for MP, AP and 2-PA, 22.7% for 4-HMP and 13.6% for 4-HAP. The precision expressed as R.S.D. for MPs were from 2.3 to 5.1% (within-day, $n=5$) and from 2.7 to 9.0% (between-day, $n=3$). The increase in the number of repeated extractions with chloroform–2-propanol (3:1, v/v) should improve the recoveries and the detection limits for 4-HMP and 4-HAP.

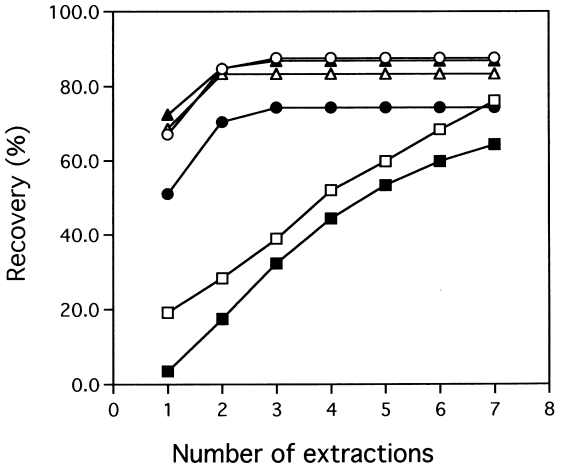

Fig. 2. Effect of the number of repeated extractions on the recoveries of MPs. Sample (spiked concentration, nmol/ml): ○=1-PA (19.6); ●=2-PA (15.1); △=AP (14.5); ▲=MP (15.8); □=4-HAP (15.9); ■=4-HMP (16.1).

3.3. CE–LIF detection

It was reported that the excitation and emission

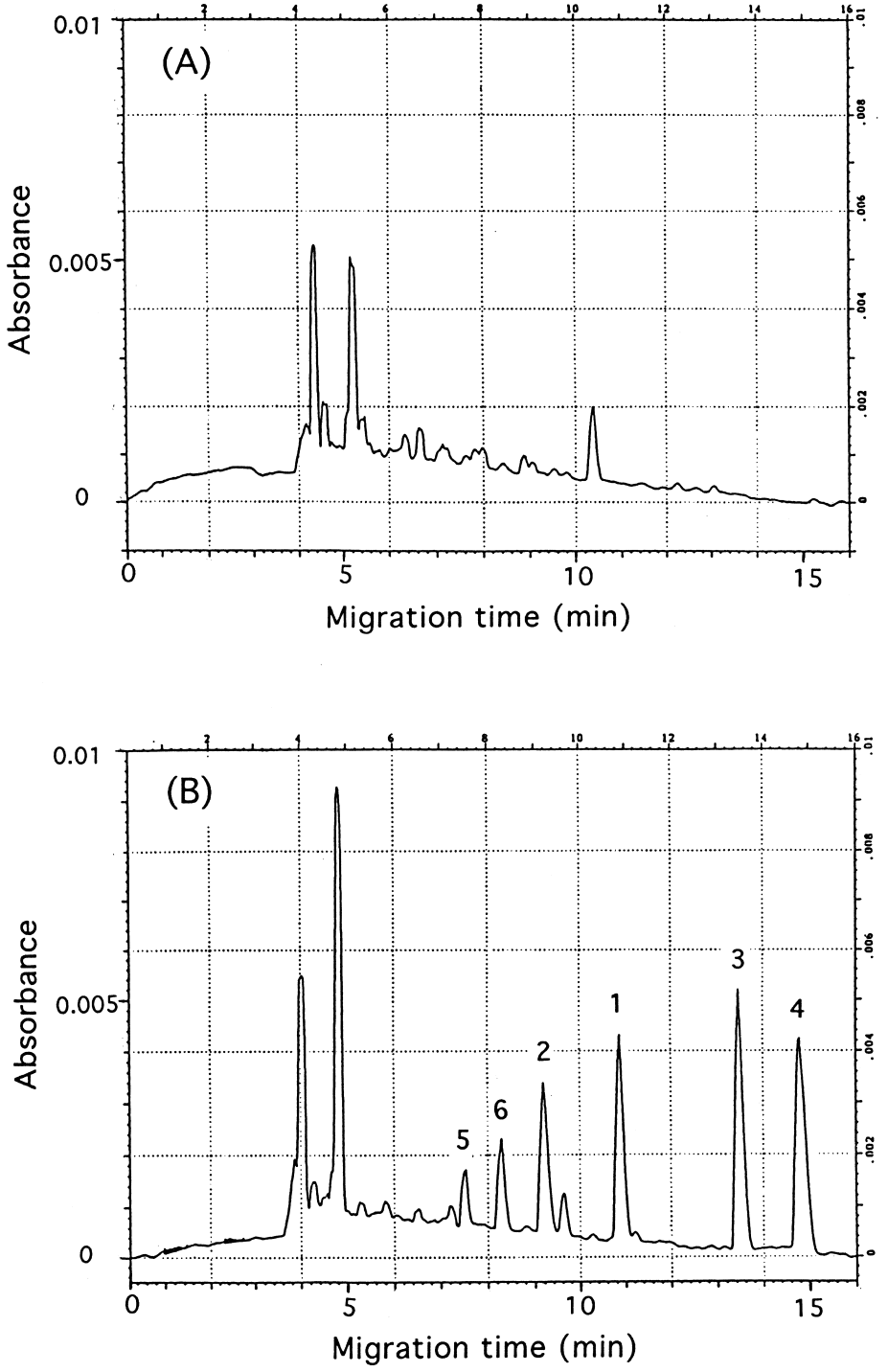

Fig. 3. Electropherograms of (A) a normal urine sample, and (B) a urine sample spiked with MPs by UV detection. Peak (spiked concentration, nmol/ml): 1=2-PA (15.1); 2=1-PA (19.6); 3=AP (14.5); 4=MP (15.8); 5=4-HAP (15.9); 6=4-HMP (16.1). CE conditions: capillary, 50 cm (37.5 cm to detector)×75 μm I.D.; detection, UV at 210 nm; temperature, 25°C; running solution, 50 m*M* $Na_2B_4O_7$ containing 15 m*M* SDS (pH 9.3); voltage, 15 kV.

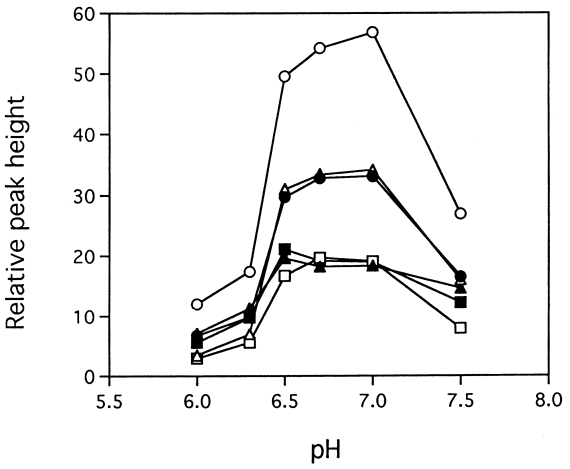

Fig. 4. Effect of pH of the buffer used for the derivatization of MPs on peak heights. Sample: ○=1-PA; ●=2-PA; △=AP; ▲=MP; □=4-HAP; ■=4-HMP. Sample injected: 720 fmol each.

wavelength of NBD-derivative of norephedrine are 470 and 533 nm, respectively [15]. This excitation wavelength was considered to be well suited for LIF detection with argon ion laser excitation (488 nm). The conditions for fluorescence derivatization was first investigated using NBD-F. The effect of NBD-F concentration was examined over the range of 1–60 m*M* and almost maximum and constant peak height for each derivative was obtained at NBD-F concentrations higher than 10 m*M*; 20 m*M* was chosen. MPs were more reactive with NBD-F at pH 6.5–7.0 (Fig. 4); 50 m*M* $Na_2B_4O_7$–NaH_2PO_4 buffer at pH 6.7 was employed for further experiments.

The effect of temperature on the derivatization reaction was examined at 80, 60°C and room temperature: the maximum fluorescence intensity for each derivative was observed at 80°C, in which each fluorescence intensity reached maximum after the reaction for 10 min; derivatization was carried out at 80°C for 10 min.

The effect of pH on the separation of MPs derivatives with NBD-F by MEKC was tested using 50 m*M* $Na_2B_4O_7$–NaH_2PO_4 (pH 8.0–9.2), $Na_2B_4O_7$ (pH 9.3) and $Na_2B_4O_7$–NaOH (pH 9.4) solutions as running solutions, all of these contained 10 m*M* SDS; the migration time for each peak kept constant at pH 8.0–9.0 and then increased with an increase in pH. $Na_2B_4O_7$ (pH 9.3) solution was used tentatively. The migration time for each peak increased with an increase in SDS concentration from 5 to 25 m*M*: 10 m*M* was employed as optimum.

Calibration curves were prepared by using known concentrations of MPs. The linear regressions with detection limits obtained by CE–LIF detection are listed in Table 2. MPs could be detected at amol levels, but MP and 4-HMP as secondary amines showed relatively lower sensitivities as detection limits. The latter compounds gave corresponding single peak by using MEKC–UV detection. Furthermore, the fluorescence spectra for MP and 4-HMP after the derivatization with NBD-F observed by using fluorescence spectrophotometer (650-10S, Hitachi, Tokyo, Japan) were almost the same as those for AP, 1-PA and 2-PA. These results suggest that the derivatization reaction of MP and 4-HMP with NBD-F proceed completely but the derivatives showed relatively weak fluorescence by unknown reasons. Detailed study concerning this phenomenon will be discussed elsewhere. The electropherogram of MPs derivatives is presented in Fig. 5. Under the conditions, MPs were separated within 45 min.

Table 2
Calibration curves and detection limits for MPs derivatives with NBD-F by LIF detection

Compound	Range (fmol/injection)	Equation[a]	*r*	Detection limit[b] (amol/injection)
MP	1.50–150	$y=0.045x-0.171$	0.999	690
AP	0.15–150	$y=0.567x+0.187$	0.999	40
4-HMP	1.50–150	$y=0.073x+0.132$	0.999	300
4-HAP	0.15–150	$y=0.495x+0.074$	0.999	34
2-PA	0.15–150	$y=0.575x+0.203$	0.999	31
1-PA	0.15–150	$y=0.675x+0.220$	0.999	22

[a] y=relative fluorescence intensity (arbitrary unit); x=concentration (fmol/injection).
[b] Concentration giving a $S/N=3$.

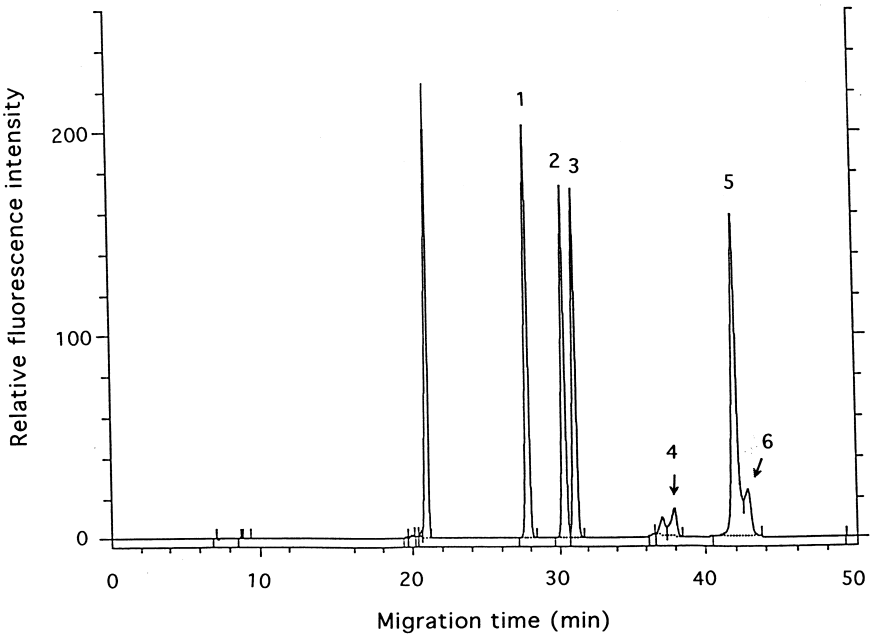

Fig. 5. Electropherograms of MPs derivatives with NBD-F by LIF detection. Peak: 1=1-PA; 2=2-PA; 3=AP; 4=MP; 5=4-HAP; 6=4-HMP. Sample injected: 150 fmol each. CE conditions: capillary, 57 cm (50 cm to detector)×75 μm I.D.; detection, fluorescence at 520 nm; temperature, 25°C; running solution, 50 m*M* $Na_2B_4O_7$ containing 10 m*M* SDS (pH 9.3); voltage, 15 kV.

3.4. Application of CE–LIF detection to the determination of MPs in urine

To test the applicability of the CE–LIF detection of MPs to biological samples, the determination of MPs in urine was attempted. 50 μl of urine or diluted urine was used for the following investigation. When using the liquid–liquid extraction as described above for CE–UV detection, unknown substances or water exist in the residue after the evaporation of organic phase disturbed the reaction of MPs with NBD-F to give relatively lower peak heights. Therefore, solid-base extraction with Sep-Pak C_{18} cartridge was employed. The effect of buffer's pH on the recoveries of MPs was examined using 100 m*M* $Na_2B_4O_7$–NaH_2PO_4 (pH 7.0–9.0) and Na_2CO_3–$NaHCO_3$ (pH 9.0–11.0) buffer: the latter buffer at pH 9.0 showed the maximum recoveries and thus was used. The effect of the concentration of Na_2CO_3–$NaHCO_3$ was also investigated over the range 25–125 m*M*; 75–125 m*M* gave the maximum recoveries for each derivative and 100 m*M* was chosen as optimum. MPs retained on the cartridge were completely eluted with 4.0 ml of MeOH.

In LIF detection of MPs, the sample solution applied to CE system contains 75% of EtOH. In such a solution, measurements of peak height were found to be slightly better than peak area for more precise results, and thus was used for preparing working curves. The peak height ratios of each MPs to I.S. were linear from 1.03 to 103 fmol/injection (1.38×10^{-9}–1.38×10^{-7} mol/ml urine) for AP ($r=0.999$) and 2-PA ($r=1.000$), from 20.6 to 206 fmol/injection (2.76×10^{-8}–2.76×10^{-7} mol/ml urine) for MP ($r=0.987$). Unfortunately, 4-HMP and 4-HAP could not be determined under the conditions examined because the peaks of those overlapped with fluorescent peaks in case of urine sample. The detection limits obtained were 4.37 fmol/injection (5.87×10^{-9} mol/ml urine) for MP, 517 amol/injection (6.86×10^{-10} mol/ml urine) for AP and 322 amol/injection (4.2×10^{-10} mol/ml urine) for 2-PA at a S/N of 3. Recovery and precision studies were carried out by adding 0.37 nmol for AP, 0.80 nmol for MP and 0.38 nmol for 2-PA to 50 μl of urine.

The recoveries obtained were 74% for AP, 86% for MP and 61% for 2-PA, and the precision for AP, MP and 2-PA were from 4.5 to 6.3% (within-day, $n=5$) and from 3.1 to 4.7% (between-day, $n=3$) as R.S.D.s. Typical electropherograms obtained with normal human urine and that spiked with MPs are demonstrated in Fig. 6. A slight delay of the migration time for each MPs was observed in urine sample.

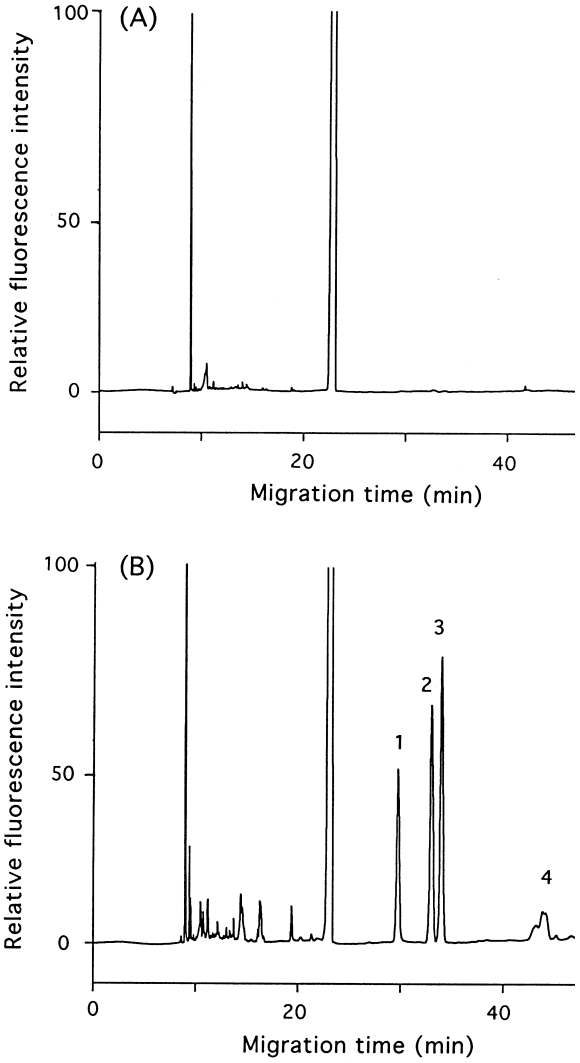

Fig. 6. Electropherograms of (A) a normal urine sample, and (B) a urine sample spiked with MPs by LIF detection. Peak (spiked concentration, nmol/ml): 1=2-PA (23.1); 2=1-PA (30.0); 3=AP (22.2); 4=MP (24.1). CE conditions: capillary, 57 cm (50 cm to detector)×75 μm I.D.; detection, fluorescence at 520 nm; temperature, 25°C; running solution, 50 m*M* $Na_2B_4O_7$ containing 10 m*M* SDS (pH 9.3); voltage, 15 kV.

Table 3
Concentrations of MPs in urine from MP addicts

Age	Gender	Concentration in urine (nmol/ml)		
		MP	AP	2-PA
44	M	916.1	81.9	N.D.[a]
31	F	229.0	10.7	N.D.
37	M	103.7	36.0	N.D.
32	M	675.0	202.9	N.D.
44	F	91.6	21.0	N.D.

[a] N.D.: not detectable.

Contents of AP, MP and 2-PA in urine from MP addicts were tried to be determined by the proposed method. The results of the assay are listed in Table 3. 2-PA was not detected in the urine samples tested.

4. Conclusions

In CE–UV detection, both the CZE and MEKC have made MPs separable within 15 min. The sensitivity of MPs using CZE (48–72 fmol/injection) was slightly better than that of CZE (85–191 fmol/injection), but the applicability to urine sample in MEKC was found to be superior to that in CZE regarding the separation. MEKC determination of MP and its related compounds spiked in urine could be performed in a short separation time without any pre-derivatization procedure. Determination of AP in biological fluids with CE–UV detection has also been reported by Hyötyläinen et al., in which AP was separated within 16 min from caffeine and morphine analogues by MEKC [16]. The sensitivity for AP of our method was several fold higher than that of the reported method. The proposed MEKC-UV method is simple, rapid and reliable, and might be applicable to the routine analysis of MP and its related compounds for forensic and legal purposes by increasing the recoveries of 4-HMP and 4-HMP from urine.

In CE–LIF detection, a highly sensitive analysis was attempted to be developed using NBD-F as a fluorescent derivatization reagent. NBD-F derivatives of MPs could be separated within 45 min by MEKC. Although the secondary amines such as MP and 4-HMP showed relatively weak fluorescence, amol

levels of detection for all MPs was achieved. The sensitivities for primary amines such as AP, 4-HAP and 2-PA obtained with LIF detection were more than 1000 times higher than those with UV detection. When the method was applied to urine sample, in which 4-HMP and 4-HAP could not be determined by the interference due to unknown components in urine, amol levels of AP and 2-PA and lower fmol levels of MP spiked in urine were determined. The method was successfully applied to the determination of MP and AP in 50 μl of urine or diluted urine from MP addicts. In the presented study, the usefulness of the method has been demonstrated for only the urine sample. However, the proposed method with high sensitivity might be advantageous for MPs including primary amines where only a small amount of sample (such as sweat and hair) is obtainable.

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