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On-line stacking and sweeping capillary electrophoresis for detecting heroin metabolites in human urine

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ABSTRACT

Heroin metabolites including morphine, codeine, and 6-acetylmorphine were determined by cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography (CSEI–sweep-MEKC). Liquid–liquid extraction was used for urine pretreatment. An uncoated fused silica capillary (Ld = 30 cm, 50 μm ID) was filled with phosphate buffer (50 mM, pH 2.5) containing 30% methanol, then high conductivity buffer (100 mM phosphate, 41.3 kPa for 18 s) was followed. Samples were injected electrokinetically (20 kV, 300 s). The sweeping and separation were performed at −25 kV using phosphate buffer (20 mM, pH 2.5) and 80 mM sodium dodecyl sulfate. The baseline separation was done within 10 min. During method validation, the calibration curves were linear over a range of 50–500 ng/mL ($r \geqq 0.994$). The RSD and RE values in intra-day and inter-day assays were all below 20%, which showed good precision and accuracy. Their detection limits were 10 ng/mL (S/N = 3). The optimized method was applied to determine real urine samples from addicts. These samples were confirmed by liquid chromatography/mass spectrometry.

1. Introduction

Heroin (H, diacetylmorphine) is an illegal, highly addictive drug [1]. Because H abusers do not know the actual strength of the drug or its true contents, they are at risk of overdose or death. This drug is rapidly metabolized to 6-acetylmorphine (6-AM) by enzymatic hydrolysis, and its plasma half-life has been estimated about 2–8 min. Morphine (M) results from further hydrolysis of 6-AM, which has a plasma half-life of 10–40 min. M may be further metabolized to codeine (C) in liver and intestine (see Fig. 1) [2,3]. Due to labile nature of H, we have to monitor its metabolites for confirmation of H addiction. 6-AM is regarded as the best marker for H use because it is the unique H metabolite, and there is no known natural source [4].

In the past, opiates have been detected in biological samples by radioimmunoassay (RIA) or enzyme immunoassay (EIA), thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC). RIA and EIA are rapid and widely used to detect opiates in biological fluids. These methods still have some drawbacks such as cross-reaction of opiate metabolites [2]. TLC is rapid, simple but lacks sensitivity and high resolution. GC often requires pre-column derivatizations and time-consuming. Analysis of common opiates and H metabolites in urine by HPLC has been demonstrated [5].

Capillary electrophoresis (CE) provides many advantages of high-resolution power, low reagents consumption, small sample volume, and automation. It has attracted much attention. However, conventional absorbance detectors are not sensitive enough because the optical light-path and sample volume are small. Among laser induced fluorescence, mass spectrometry, electrochemical, and amperometric detectors, they are expensive and/or not easily adaptable with CE [6]. Some CE-UV methods were used for the analysis of clandestine H preparations and H metabolites in human urine [2,7]. Lurie et al. used dynamically coated capillaries for the determination of H and other controlled drugs [8,9]. Poly(ethylene oxide)-coated CE was also applied for the determination of H, C, methadone (ME) in urine [3].

On-line concentration techniques have been intensively used to improve concentration sensitivity. Quirino and Terabe reported a million-fold sensitivity enhancement by using cation-selective

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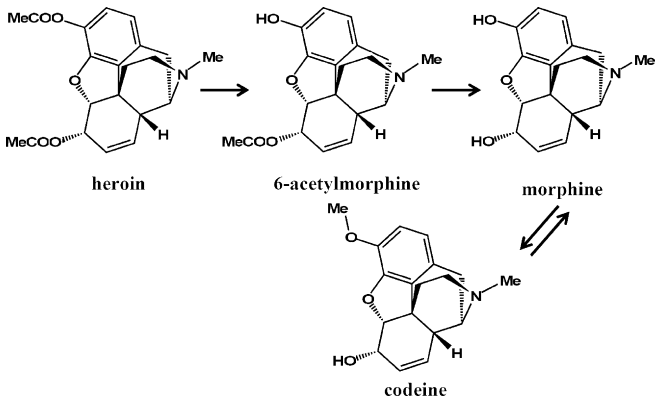

Fig. 1. Metabolic pathway of heroin.

exhaustive injection and sweeping micellar electrokinetic chromatography (CSEI–sweeping-MEKC) [10]. Some studies have shown their applications such as lysergic acid diethylamide, ephedra-alkaloid, herbicides, and environmental aromatic amines [11–15]. We have also applied this combined stacking method for the determination of some forensic drugs in urine or hair [16–19]. The aim of this study is to establish a simple, fast and sensitive method for the simultaneous determination of H metabolites in urine. Liquid–liquid extraction was used for sample pretreatment, and CSEI–sweep-MEKC method has been applied for on-line stacking and separation. Optimization of parameters and validation of this method has been studied.

2. Materials and methods

2.1. Materials

All chemicals used were analytical grade. H, M, C, and methamphetamine (MA) were obtained from Taiwan National Bureau of Controlled Drugs, Executive Yuan, Taiwan. 6-AM, ME (Cerilliant Corporation, Round Rock, TX, USA), cimetidine (300 ng/mL, internal standard, IS), ketamine (K) (Sigma–Aldrich, St. Louis, MO, USA), NaH_2PO_4, sodium hydroxide, hydrochloric acid, methanol, dichloromethane, isopropanol and sodium dodecyl sulfate (SDS) (Merck, Darmstadt, Germany) were commercial products. Milli-Q Water (Millipore, Bedford, MA, USA) was used for the preparation of buffer and other aqueous solutions.

2.2. Sample preparation

Stock solutions of analytes (H, M, C) were prepared in methanol (1 mg/mL). 6-AM was obtained in acetonitrile (1 mg/mL). They were diluted by urine as reference standards. The blank urine

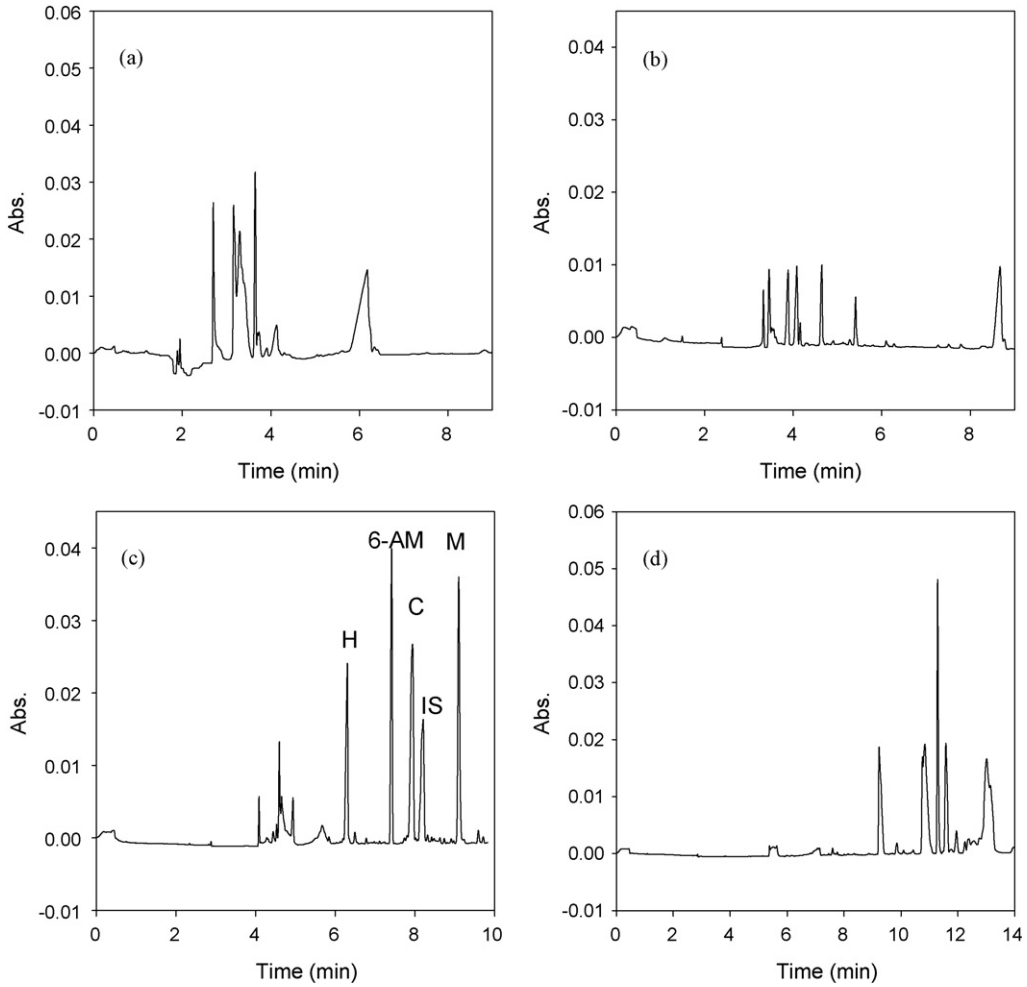

Fig. 2. Effect of methanol level (a) none, (b) 20%, (c) 30% and (d) 40% in buffer on separation of heroin and its metabolites in urine. Conditions: separation buffer, 50 mM phosphate (pH 2.5); HCB, 100 mM phosphate, 41.3 kPa for 18 s; sweeping buffer, 20 mM phosphate (pH 2.5) and SDS 80 mM; applied voltage, −25 kV (detector at anode side); uncoated fused-silica capillary, 30 cm (effective length) ×50 μm ID; sample size, electrokinetic injection 20 kV, for 300 s; wavelength, 214 nm. Sample concentrations: 250 ng/mL (H, 6-AM, C and M), 300 ng/mL (IS). Peaks: H, heroin; 6-AM, 6-acetylmorphine; C, codeine; M, morphine; IS, cimetidine.

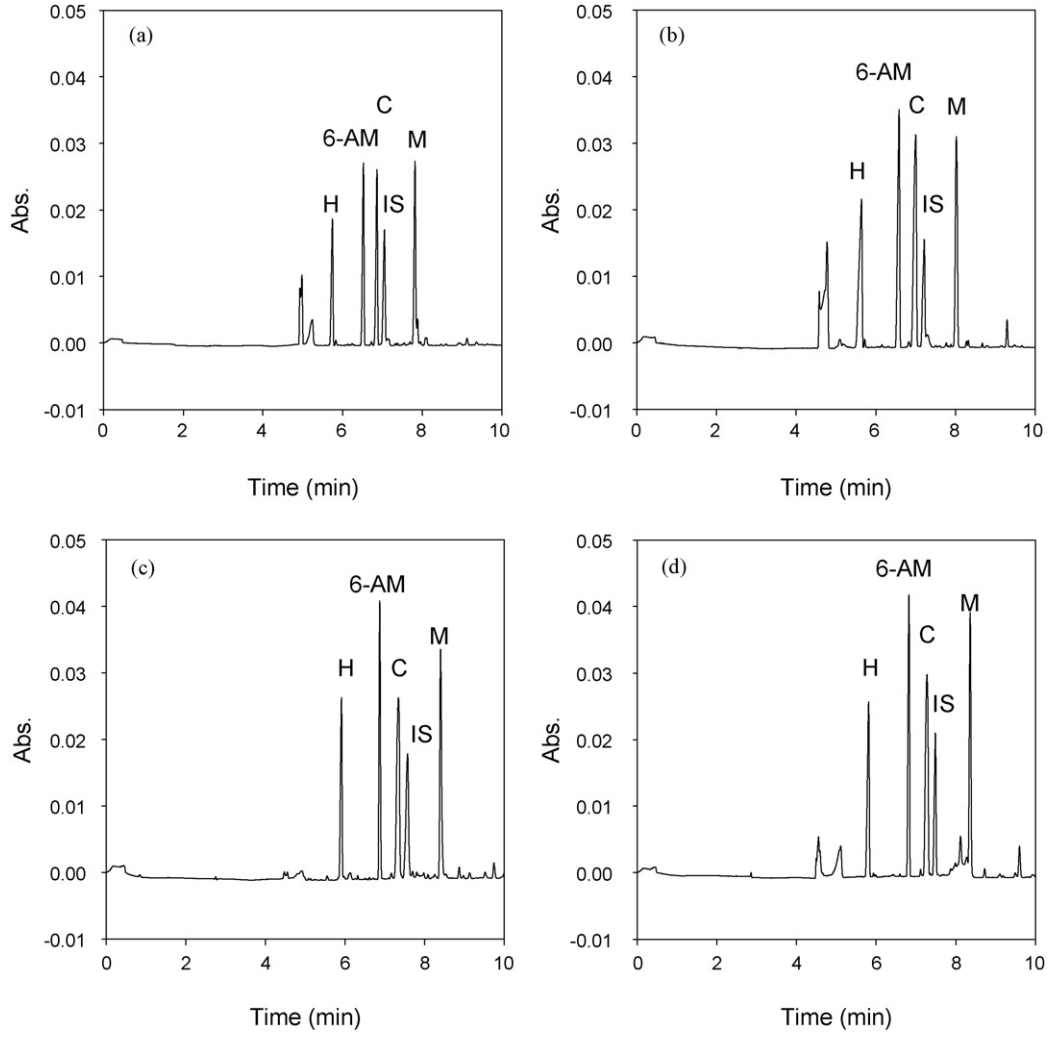

Fig. 3. Effect of injection time (a) 180 s, (b) 240 s, (c) 300 s, and (d) 360 s on separation of heroin and its metabolites in urine. See Fig. 2 for other conditions.

was obtained from the pooled urine of two healthy volunteers in our laboratory. The real urine samples of H and its metabolites were taken from addicts who showed up to private local hospitals (Kaohsiung, Taiwan). All samples were stored in frig (−70 °C) until analysis.

2.3. CE system

A Beckman P/ACE System MDQ (Fullerton, CA, USA) equipped with a UV detector and a liquid-cooling device was used. An uncoated fused-silica capillary (50 μm ID and 30 cm effective length) was used (Polymicro Technologies, Phoenix, AZ, USA) and detected at 214 nm. Any new capillary was preconditioned with water, methanol, water, 1 M HCl, water, 1 M NaOH and water, each for 5 min. Before the first run, a 5-min rinse with running buffer was performed. Before daily use, the capillary was washed with water, 0.1 M HCl, water, 0.1 M NaOH, and water, each for 5 min. The capillary was flushed between consecutive analyses to ensure its repeatability, with methanol (5 min), water (2 min), 0.1 M HCl (5 min), water (2 min), and running buffer (5 min).

Table 1
Regression analysis for the determinations of heroin and its metabolites in urine.

Concentration range (50–500 ng/mL)	Regression equation	Coefficient of correlation (r)
Intra-day ($n = 3$)[a]		
H	$Y = (0.0068 \pm 0.0001)X + (0.1335 \pm 0.0113)$	0.994
6-AM	$Y = (0.0065 \pm 0.0001)X + (0.0956 \pm 0.0110)$	0.997
C	$Y = (0.0099 \pm 0.0001)X + (0.0560 \pm 0.0273)$	0.999
M	$Y = (0.0067 \pm 0.0002)X + (0.0587 \pm 0.0181)$	0.995
Inter-day ($n = 5$)[b]		
H	$Y = (0.0069 \pm 0.0002)X + (0.1333 \pm 0.0216)$	0.996
6-AM	$Y = (0.0078 \pm 0.0003)X + (0.0563 \pm 0.0350)$	0.996
C	$Y = (0.0099 \pm 0.0001)X + (0.0926 \pm 0.0508)$	0.997
M	$Y = (0.0067 \pm 0.0002)X + (0.0859 \pm 0.0176)$	0.994

[a] The regression equations of intra-day analysis were calculated from the assay values of prepared standards on a single day ($n = 3$).
[b] The regression equations of inter-day analysis were calculated from the assay values of prepared standards on five different days ($n = 5$).

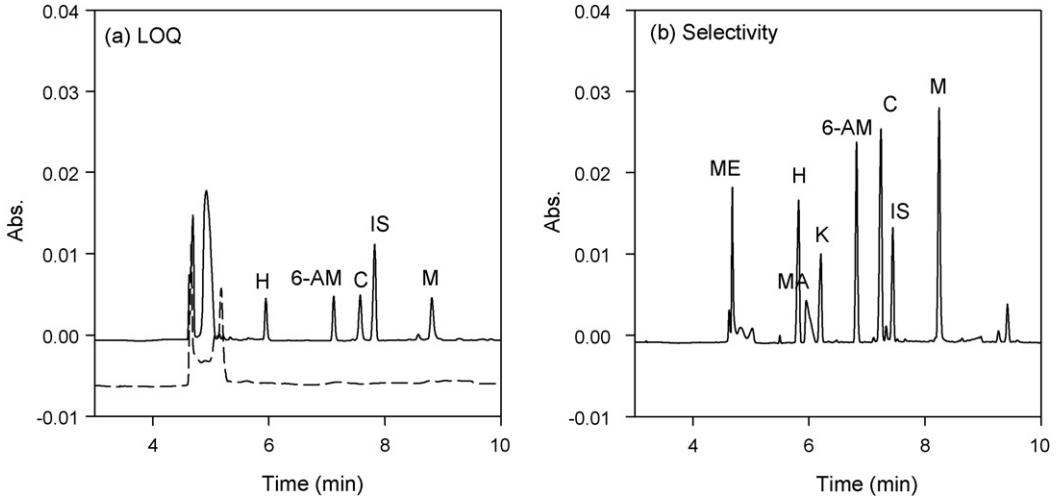

Fig. 4. Electropherogram of (a) LOQ and (b) selectivity test. See Fig. 2 for conditions, and peaks: MA, methamphetamine; ME, methadone; K, ketamine.

2.4. Liquid–liquid extraction

The spiked urine sample (including IS solution) was prepared to 300 μL. Subsequently, 200 μL of the borate buffer (0.1 M) was added. The resulting mixtures were extracted using 1 mL of a solution of dichloromethane and isopropanol (85:15, v/v). The aqueous solutions were separated from the organic phase. 700 μL of organic phase was collected, added with 10 μL of 0.1 M HCl/methanol solution, and was evaporated by a centrifugal vaporizer (EYELA CVE-200D, Japan). The residue was reconstituted with 100 μL of H_2O before CE analysis [3].

2.5. CSEI–sweep-MEKC

The capillary was first filled with phosphate buffer (50 mM, pH 2.5) containing 30% (v/v) methanol, followed by the injection of a high conductivity buffer (HCB) (100 mM phosphate, 41.3 kPa for 18 s). With the application of 20 kV with normal polarity, the cationic analytes entered the capillary with high velocities and were then slowed down in HCB. The electrokinetic injection was performed for 300 s. The voltage was then switched to the reverse polarity (−25 kV), thus permitting the entry of micelles from the cathodic vial into the capillary to sweep the stacked analytes to a narrower band. Finally, the separation was performed using surfactant mediated CE in the reversed-migration mode. Phosphate buffer (20 mM, pH 2.5) and 80 mM SDS were used as the sweeping buffer at both ends of the capillary [16–19]. All operations and electropherograms were computer-controlled using Beckman 32 Karat software (Fullerton).

3. Results and discussion

3.1. Optimization of CSEI–sweep-MEKC

The CSEI–sweep-MEKC technique [10,16–19] was optimized with several parameters. First, different concentrations (30–60 mM) and pH values (2.0, 2.5, 3.0 and 3.5) of phosphate buffer, and amounts of methanol (0–40%) were tested to optimize the separation buffer. From the results (data not shown), all analytes could be separated and stacked well when phosphate buffer were greater than 40 mM and at pH 2.0–3.5.The results indicated these factors did not affect stacked samples dramatically. However, methanol showed the greatest effect on separation. Using organic modifier could change partition coefficient of the focused analytes between the fast moving micellar phase and slow moving aqueous phase. Without methanol, all peaks migrated together and could not be distinguished. Baseline separation of analytes could be achieved when methanol level was 30%. Up to 40%, it was noted that a higher concentration of the organic solvent may damage the micelle structure (shown in Fig. 2). Finally, phosphate buffer (50 mM, pH 2.5) containing 30% methanol was selected as separation buffer.

We tested different HCB concentrations (60, 80, 100 and 120 mM), and found all analytes could be separated under these conditions. The HCB concentration (60–120 mM) showed less effect on separation (data not shown). We selected 100 mM as the HCB concentration. The effect of using a HCB (100 mM phosphate) with different plug lengths (none, 20.7 kPa, 41.3 kPa, 62.0 kPa, each for

Table 2
Precision and accuracy for the determinations of heroin and its metabolites in an intra-day and inter-day analysis.

	Concentration know (ng/mL)	Concentration found (ng/mL)	RSD (%)	RE (%)
Intra-day (n = 3)				
H	100	85.1 ± 4.4	5.1	−15.0
	250	246.4 ± 23.8	9.7	−1.5
	450	424.3 ± 17.9	4.2	−5.7
6-AM	100	97.8 ± 5.6	5.7	−2.3
	250	257.3 ± 22.5	8.8	2.9
	450	413.4 ± 11.1	2.7	−8.1
C	100	82.5 ± 3.2	3.9	−17.5
	250	270.4 ± 13.6	5.0	8.2
	450	452.0 ± 10.1	2.2	0.5
M	100	110.2 ± 14.4	11.3	10.2
	250	257.0 ± 20.9	8.1	2.8
	450	477.7 ± 14.1	3.0	6.2
Inter-day (n = 5)				
H	100	81.7 ± 7.6	9.3	−18.4
	250	282.6 ± 16.8	5.9	13.1
	450	426.6 ± 20.7	4.9	−5.2
6-AM	100	96.8 ± 8.3	8.6	−3.2
	250	269.8 ± 12.2	4.5	7.9
	450	428.5 ± 29.4	6.9	−4.8
C	100	90.2 ± 5.3	5.9	−9.8
	250	277.2 ± 29.0	10.5	10.9
	450	452.3 ± 35.1	7.8	0.5
M	100	106.8 ± 8.8	8.3	6.8
	250	295.7 ± 10.2	3.5	18.3
	450	465.5 ± 34.7	7.5	3.5

Table 3
Absolute recoveries of heroin and its metabolites in urine.

Analyte	H	6-AM	C	M
Concentration (ng/mL)	500	500	500	500
Recovery (%) [a]	86.2 ± 4.1	94.7 ± 3.7	83.3 ± 2.7	75.8 ± 6.7

[a] Mean ± SD of triplicate analysis.

Table 4
Relative recoveries of heroin and its metabolites in urine.

Analytes	Concentration spiked (ng/mL)	Concentration found (ng/mL)	Recovery[a] (%)
H	120	115.3 ± 3.1	96.1 ± 3.1
	280	244.1 ± 9.4	87.1 ± 4.1
	480	438.8 ± 12.7	91.4 ± 3.2
6-AM	120	121.2 ± 1.7	101.0 ± 1.7
	280	271.8 ± 1.0	97.1 ± 0.4
	480	463.2 ± 9.7	96.5 ± 2.5
C	120	111.5 ± 2.0	92.9 ± 2.0
	280	251.2 ± 3.3	89.7 ± 1.4
	480	452.9 ± 7.3	94.6 ± 2.0
M	120	118.0 ± 1.1	98.4 ± 1.2
	280	247.5 ± 12.7	88.4 ± 5.6
	480	452.4 ± 1.4	94.3 ± 0.3

[a] Mean ± SD of triplicate analysis.

18 s) was tested. We found that it was necessary to use HCB plug for sample stacking (data not shown). When the plug length increased, the peaks became sharper. We selected the hydrodynamic injection of 41.3 kPa for 18 s as the best length of HCB. We tried different SDS concentrations (60–120 mM) in different phosphate buffers (10–40 mM, pH 2.5). SDS did not show significant differences. However, the analytes were not separated from the interferences in urine when 30 and 40 mM phosphate buffers were used in sweeping buffers. Finally, 20 mM phosphate and 80 mM SDS were selected.

Electrokinetic injection was operated between 180, 240, 300 and 360 s at 20 kV, the sensitivity increased while the increasing of injection time (Fig. 3). The peak heights showed little increasing above 300 s injection. We evaluated both time and peak height, and selected 300 s as the sample injection time. The effect of separation voltage (−20, −25, and −30 kV) was examined. Considering separation speed and joule heat from different voltages, −25 kV was chosen.

3.2. Method validation

The linearity of this method was tested within the range of 50–500 ng/mL using IS (300 ng/mL). The analytes can be quantified at level below that generally accepted as the cut-off level for heroin metabolites (300 ng/mL). Linear regression equations were calculated between analyte-to-IS peak area ratio and analyte concentration by using the least-square method. The matrix-matched calibrations were performed on intra-day ($n = 3$) and on five consecutive days ($n = 5$). As shown in Table 1, the coefficients of correlation (r) for the four analytes were greater than 0.994. The limits of detection (LOD, S/N = 3, electrokinetic injection 20 kV, 300 s) of the four compounds were 10 ng/mL, and the limits of quantitation (LOQ, S/N = 10) were 50 ng/mL as shown in Fig. 4(a). The precision (relative standard deviation, RSD) and accuracy (relative error, RE) of the proposed methods were studied, in terms of peak-area ratios for three replicate injections at three different concentrations. The results were shown in Table 2. All of the RSD and RE were all less than 11.3% and 18.4%, respectively. Absolute recoveries were calculated from the ratio of peak area of each analyte extracted from the urine, and the peak area of each standard without extraction. These recoveries were greater than 75% ($n = 3$), and shown in Table 3. Relative recoveries were calculated from the matrix-matched calibrations. Those were greater than 87% ($n = 3$), and shown in Table 4. The results indicated good precision.

Table 6
Comparisons of detection limits of heroin, 6-acetylmorphine, morphine, codeine in CZE and this method.

Analyte	CZE (ng/mL)	CSEI–sweep-MEKC (ng/mL)	Sensitivity enhancement
H	500	10	50×
6-AM	500	10	50×
C	1000	10	100×
M	1000	10	100×

Because this quantification range of 50–500 ng/mL was limited, most real samples must be diluted to the range. We tested diluted urine if affect the quantitative applicability of the method when urine matrix was changed. The stock solutions were suitably diluted by 10- and 100-fold diluted urine. They were prepared within the range of 50–500 ng/mL. The results were shown in Table 5. Comparing the three slopes of no dilution urine, 10-fold diluted urine, and 100-fold diluted urine, those RSDs were within 0.9–5.5%. There was little difference no matter whether the urine was diluted or not. About selectivity test, we found the analytes could separate from ME, MA and K in Fig. 4(b). Comparing with the LOD of capillary zone electrophoresis mode (S/N = 3, injecting 6.89 kPa for 5 s, running buffer 50 mM phosphate (pH 2.5) containing 30% methanol), the sensitivity could be improved about 50- to 100-fold (shown in Table 6).

3.3. Analysis of urine samples from addicts

Real urine samples were tested positive for opiates using immunoassay system. The electropherograms of the two real samples were shown in Fig. 5(a) and (b). The H metabolites could be identified with no interference. The concentrations (Table 7) were detected by CE, and confirmed by LC/MS. The results showed good

Table 5
Regression analysis for the determinations of heroin and its metabolites in urine with additional dilution.

Concentration range (50–500 ng/mL)	Regression equation	Coefficient of correlation (r)
Intra-day (10× dilution)[a]		
H	$Y = (0.0067 \pm 0.0001)X + (0.0679 \pm 0.0217)$	0.991
6-AM	$Y = (0.0074 \pm 0.0002)X + (0.0128 \pm 0.0408)$	0.997
C	$Y = (0.0097 \pm 0.0002)X + (0.1846 \pm 0.0081)$	0.997
M	$Y = (0.0069 \pm 0.0001)X + (0.0902 \pm 0.0386)$	0.996
Intra-day (100× dilution)[a]		
H	$Y = (0.0067 \pm 0.0003)X + (0.1198 \pm 0.0329)$	0.996
6-AM	$Y = (0.0072 \pm 0.0003)X + (0.0739 \pm 0.0352)$	0.996
C	$Y = (0.0095 \pm 0.0002)X + (0.1895 \pm 0.0154)$	0.995
M	$Y = (0.0064 \pm 0.0000)X + (0.0840 \pm 0.0226)$	0.995

[a] The regression equations of intra-day analysis were calculated from the assay values of prepared standards on a single day ($n = 3$).

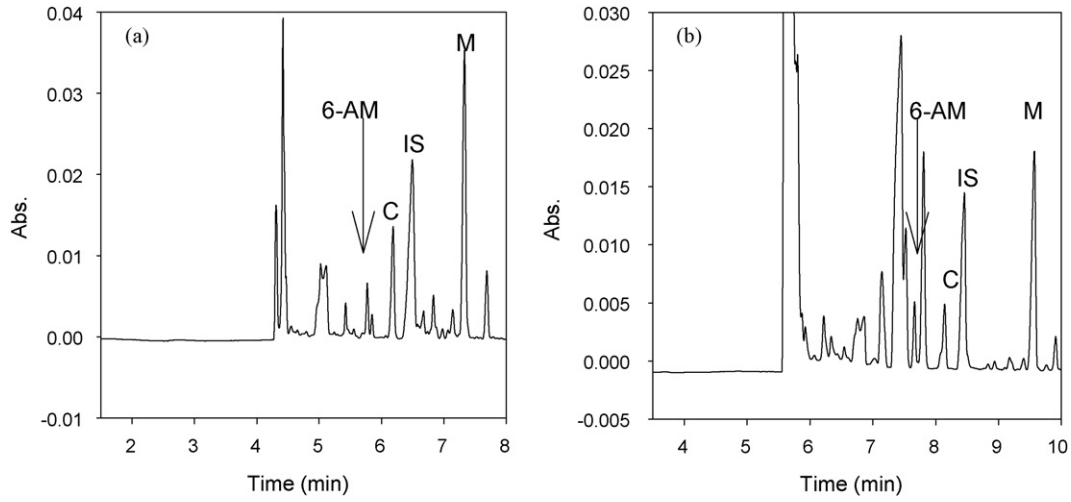

Fig. 5. Electropherograms of two abusers' urine (a and b). See Fig. 2 for conditions.

Table 7
Data of urine samples from nine opiate addicts tested by CE method.

NO.	CE	
	Drug found	Concentration[c] (ng/mL)
S1[a]	6-AM	$(1.7 \pm 0.3) \times 10^2$
	C	$(2.7 \pm 0.2) \times 10^2$
	M	$(1.3 \pm 0.0) \times 10^3$
S2[a]	6-AM	$(2.9 \pm 0.0) \times 10^2$
	C	$(1.7 \pm 0.0) \times 10^2$
	M	$(2.2 \pm 0.0) \times 10^3$
S3	C	$<5.0 \times 10^1$
S4[b]	ND	ND
S5	C	$<5.0 \times 10^1$
	M	$(2.1 \pm 0.0) \times 10^2$
S6[b]	ND	ND
S7[a]	6-AM	$(1.7 \pm 0.0) \times 10^3$
	C	$(1.8 \pm 0.0) \times 10^2$
	M	$(4.1 \pm 0.3) \times 10^2$
S8	6-AM	$<5.0 \times 10^1$
	C	$<5.0 \times 10^1$
	M	$(5.7 \pm 0.1) \times 10^1$
S9[a]	6-AM	$(9.1 \pm 0.1) \times 10^2$
	C	$(7.6 \pm 0.0) \times 10^2$
	M	$(1.8 \pm 0.0) \times 10^3$

[a] Urine with additional 10-fold dilution was analyzed.
[b] Not found analytes in this study.
[c] Mean ± SD ($n = 3$).

agreement between them. Among them, five addicts were tested containing 6-AM, and validated as H users.

4. Conclusions

This CSEI–sweeping-MEKC method combined two on-line preconcentration techniques—stacking and sweeping, and provided the advantages of speed, accuracy, and precision. This method was successfully applied to the analysis of H metabolites in addicts'urine. CE-UV is probably not first choice as confirmation assay. Rather it is the other way round that it may be a screening method and GC–MS is then employed as confirmation method.

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References

[1] E.J. Rook, A.D.R. Huitema, W.V.D. Brink, J.M.V. Ree, J.H. Beijnen, Curr. Clin. Pharmacol. 1 (2006) 109.
[2] A. Alnajjar, B. McCord, J. Pharm. Biomed. Anal. 33 (2003) 463.
[3] N.D. Pietro, G. Placanica, I. Fiorini, J. Anal. Toxicol. 30 (2006) 679.
[4] A.C. Spanbauer, S. Casseday, D. Davoudzadeh, K.L. Preston, M.A. Huestis, J. Anal. Toxicol. 25 (2001) 515.
[5] R.B. Taylor, A.S. Low, J. Chromatogr. B 663 (1995) 225.
[6] T. Odake, T. Kitamori, T. Sawada, Analysis 26 (1998) 41.
[7] M. Macro, G. Manetto, C. Mori, C. Papi, J. Chromatogr. A 924 (2001) 499.
[8] I.S. Lurie, P.A. Hays, A.E. Garcia, S. Panicker, J. Chromatogr. A 1034 (2004) 227.
[9] I.S. Lurie, P.A. Hays, K. Parker, Electrophoresis 25 (2004) 1580.
[10] J.P. Quirino, S. Terabe, Anal. Chem. 72 (2000) 1023.
[11] C. Fang, S.H. Chou, C.H. Lin, J.T. Liu, Electrophoresis 24 (2003) 1031.
[12] C. Fang, C.H. Lin, J.T. Liu, J. Chromatogr. B 775 (2002) 37.
[13] H.Y. Chiang, S.J. Sheu, Electrophoresis 25 (2004) 670.
[14] O. Nunez, J.B. Kima, J. Chromatogr. A 961 (2002) 65.
[15] J.P. Quirino, Y. Iwai, K. Otsuka, S. Terabe, Electrophoresis 21 (2000) 2899.
[16] Y.H. Lin, J.H. Li, W.K. Ko, S.M. Wu, J. Chromatogr. A 1130 (2006) 281.
[17] H.L. Cheng, Y.J. Jong, J.H. Li, W.K. Ko, S.M. Wu, Electrophoresis 27 (2006) 4711.
[18] Y.H. Lin, M.R. Lee, R.J. Lee, W.K. Ko, S.M. Wu, J. Chromatogr. A 1145 (2007) 234.
[19] Y.H. Lin, J.F. Chiang, M.R. Lee, R.J. Lee, W.K. Ko, S.M. Wu, Electrophoresis 29 (2008) 2340.