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Research Article

Determination of cocaine and its metabolites using cation-selective exhaustive injection and sweeping-MEKC

We have employed a high-sensitivity on-line preconcentration method, cation-selective exhaustive injection (CSEI) and sweeping MEKC, for the analysis of cocaine, benzoylecgonine, norcocaine, and cocaethylene. We monitored the effects of several of the CSEI-sweeping-MEKC parameters – including the pH, the concentrations of SDS and organic modifier, the injection length of the high-conductivity buffer, and the injection time of the sample – to optimize the separation process. The optimal BGE was 100 mM phosphoric acid (pH 1.8) containing 75 mM SDS with 10% 2-propanol and 10% tetrahydrofuran as the organic modifier. In addition, electrokinetic injection of the sample at 15 kV for 900 s provided both high separation efficiency and enhanced sweeping sensitivity. The sensitivity enhancements for cocaine, norcocaine, and cocaethylene ranged from 2.06×10^4 to 3.96×10^4; for benzoylecgonine it was 1.75×10^3; the coefficients of determination exceeded 0.9958. The LODs, based on an S/N ratio of 3:1, of sweeping-MEKC ranged from 33.5 to 52.8 ng/mL; in contrast, when using CSEI-sweeping-MEKC the sensitivity increased to range from 29.7 to 236 pg/mL. Under the optimal conditions, we analyzed cocaine in a human urine sample prepared using off-line SPE to minimize the influence of the matrix. The recovery of the SPE efficiency was satisfactory (*ca.* 74.9–87.6%). Our experimental results suggest that, under the optimal conditions, the CSEI-sweeping-MEKC method can be used to determine cocaine and its metabolites with high sensitivity in human urine.

Keywords:
Cocaine and its metabolites / CSEI-sweeping-MEKC / On-line preconcentration / Sweeping-MEKC
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1 Introduction

Cocaine, which is found in the leaves of *Erythroxylon coca*, a South American shrub, has been utilized widely in medicine as a local anesthetic agent [1]. Recently, it has become one of the most widespread abused drugs, increasingly so during the last two decades [2]. For illicit use, cocaine is often taken in its hydrochloride form, through nasal insufflations or intravenous injection, or as its free base, through smoking [1]. Because of its potent stimulant effect on the central nervous system, cocaine is a personal health safety risk that can result in serious societal problems; authorities generally attempt to admonish or manage its use. Therefore, it is becoming increasingly important to establish detection methods to determine the presence of cocaine, and other illicit drugs, in the human body.

Cocaine is metabolized spontaneously to benzoylecgonine, the major metabolite, and to norcocaine and ecgonine methyl ester [3]. When cocaine and ethanol are co-administered, cocaethylene is formed, and its traces can be detected in urine after 24 h [1]. On account of the short half-life of cocaine, its metabolites in urine appear to be appropriate analytical targets for monitoring cocaine abuse [4]. Immunoassays are often employed for screening large numbers of samples, but they often possess insufficient sensitivity when attempting to confirm the presence of cocaine or its metabolites [5]. Thus, immunoassays are employed generally as the first screening line to judge whether specific drugs are present or not; positive screening tests are then confirmed using GC/MS [3, 6–9]. Nevertheless, GC/MS is not suitable for analyzing polar cocaine and its metabolites; it requires a derivatization step, possibly a complicated and time-consuming process, to be performed prior to analysis [10].

HPLC is a powerful technique for analyzing polar, nonvolatile, and thermally labile compounds. Although HPLC with UV detection has been employed to determine

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Abbreviations: CSEI, cation-selective exhaustive injection; **HCB,** high-conductivity buffer

cocaine and its metabolites with satisfactory resolution [11, 12], the LODs have been too high. Cocaine and its metabolites have also been determined successfully using LC/MS coupled with ESI or atmospheric pressure chemical ionization tandem mass spectrometry (LC/MS/MS) to increase the accuracy of their quantitative analyses and to raise the sensitivity of analyses of biological fluids [13–19]. As an analytical tool, CE has several advantageous features, including rapid analyses, good separation efficiencies, and minimal sample requirements. CE-based techniques employing MEKC coupled with UV detection allow the separation of cocaine and it common impurities, including benzoylecgonine and norcocaine [20]. Most of these methods have been applied to the analysis of biological samples such as urine, blood, and hair. Urine is most commonly used because it is easy to obtain and provides direct evidence of short-term illicit drug use. Pretreatment of biological fluids is usually performed using SPE [9, 12, 17] to limit interference during subsequent detection.

Although CE had many benefits when applied to detecting abused drugs, the short optical path length for UV detection usually results in poor sensitivity. Quirino and Terabe [21] were the first to develop on-line preconcentration techniques to increase the sensitivity of CE analyses. Because these methods are simple to use, provide high sensitivity, and do not require additional equipment, they have found widespread use. An appropriate preconcentration technique can be selected based on a particular analyte's properties. If sweeping-MEKC concentration is employed, the detection limit can be improved by *ca.* 10–1000-fold [22, 23]; if, however, sweeping-MEKC is coupled with selective electrokinetic injection, the sensitivity can be further enhanced by up to 10^6-fold [24]. Among the many on-line preconcentration methods available, one of the best preconcentration efficiencies is provided by the cation-selective exhaustive injection (CSEI)-sweeping-MEKC technique – used for the detection of cationic analytes – performed in conjunction with selective electrokinetic injection, followed by the addition of surfactants to perform the sweeping process. The CSEI-sweeping-MEKC technique has been applied, for example, to the analysis of amphetamine and its derivatives [25] and to methamphetamine, ketamine, morphine, and codeine in hair [26]. These studies have indicated the practicality of the method in terms of the linearity of the quantitative data, the reproducibility of the measurements, and the applicability to real samples.

In this study, we used CSEI-sweeping-MEKC to enhance the sensitivity of the determination of cocaine, benzoylecgonine, norcocaine, and cocaethylene. We optimized several separation parameters, including the pH, the concentrations of SDS and organic modifier, the injection length of the high-conductivity buffer (HCB), and the injection time of the sample. We also compared the sensitivity enhancements using CSEI-sweeping-MEKC with sweeping-MEKC and normal MEKC. In addition, we have applied this method to the analysis of these materials in human urine samples.

2 Materials and methods

2.1 Chemicals and reagents

All reagents and chemicals were of analytical grade. Cocaine (1.0 mg/mL in acetonitrile), benzoylecgonine (1.0 mg/mL in methanol), norcocaine hydrochloride (1.0 mg/mL in acetonitrile), and cocaethylene (1.0 mg/mL in acetonitrile) were obtained from Cerilliant Corporation (Round Rock, TX, USA); their chemical structures are shown in Fig. 1. Methanol was purchased from Echo Chemical (Miaoli, Taiwan, ROC). Hydrochloric acid (HCl), sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), 2-propanol (IPA), and THF were purchased from Sigma–Aldrich (St. Louis, MO, USA). SDS and phosphoric acid were purchased from J. T. Baker (Phillipsburg, NJ). Dichloromethane (CH_2Cl_2) was obtained from TEDIA (Fairfield, Ohio, USA). Water was purified through a Milli-Q water system (Millipore, Milford, MA, USA). The blank urine samples were obtained through donation.

2.2 Apparatus

A Beckman P/ACE MDQ CE system (Beckman, Fullerton, CA, USA) was used to effect the separations. A diode-array detector was employed for detection. Separations were performed in a 60-cm (effective length: 50 cm) × 50-μm ID fused-silica capillary tube (Polymicro Technologies, Phoenix, AZ, USA). The capillary tube was assembled in the cartridge format. A personal computer using 32 Karat software controlled the P/ACE instrument and allowed data analysis. Prior to use, the separation capillary was preconditioned sequentially with methanol (10 min), 1 M HCl (10 min), deionized water (5 min), 1 M NaOH (10 min), and then deionized water again (5 min). Under optimal conditions, the non-micellar BGE consisted of 100 mM phosphoric acid (pH 1.8) containing 10% IPA and 10% THF. The HCB solution was 150 mM phosphoric acid

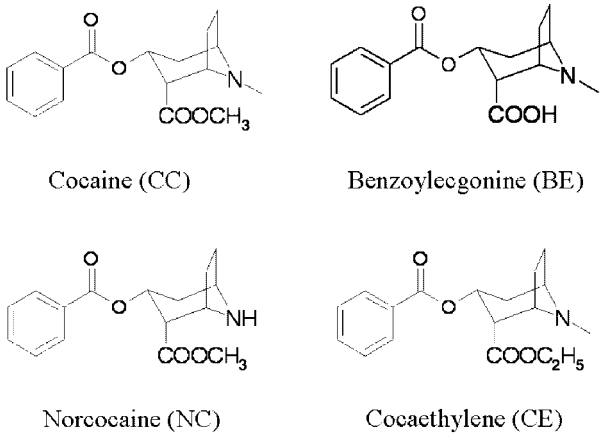

Figure 1. Molecular structures of cocaine and its metabolites.

(pH 1.8). The micellar BGE comprised 75 mM SDS in 100 mM phosphoric acid (pH 1.8) containing 10% IPA and 10% THF. Between runs, the capillary was flushed sequentially with methanol (3 min), water (7 min), and non-micellar BGE (5 min) in the CSEI-sweeping-MEKC mode or with micellar BGE (5 min) in the sweeping-MEKC and MEKC modes.

2.3 Preparing standards and urine samples

Stock standard solutions (1 mg/mL) of cocaine, benzoylecgonine, norcocaine, and cocaethylene were prepared in methanol or acetonitrile and refrigerated at 4°C. Prior to analysis in the CSEI-sweeping-MEKC mode, the stock solution was diluted to 10 μg/mL. The working standard was then diluted with pure water to the desired concentration. For analyses in the sweeping-MEKC mode, the sample was diluted with non-micellar BGE buffer to provide the same conductivity as the background solution. In the MEKC mode, the sample was also diluted with pure water. The drug-free urine samples were frozen at −20°C; when required for assaying, they were thawed and warmed to room temperature.

2.4 Method procedures

The column employed was a bare fused-silica capillary conditioned initially using a low-pH electrolyte. The EOF was suppressed by the low pH (pH 1.8). In the MEKC procedure, samples were pressure-injected at 3.45 kPa for 3 s. The detection wavelength was set at 230 nm. The separation proceeded with the micellar BGE and a negative applied potential (−20 kV). In the sweeping-MEKC procedure, samples were pressure-injected at 3.45 kPa for 120 s. The capillary was filled with micellar BGE and a negative voltage (−20 kV) was applied. When the anionic micelles entered the sample zone, sweeping and separation were achieved through MEKC. In the CSEI-sweeping-MEKC procedure, the capillary was conditioned with non-micellar BGE and then the HCB solution was injected hydrodynamically, followed by a sample of low conductivity (pure water) injected electrokinetically. Finally, the inlet and outlet of the capillary were placed in micellar BGE and a negative voltage (−20 kV) was applied to effect separation.

2.5 SPE

Oasis MCX column-type cartridges were obtained from Waters (Milford, MA, USA) for SPE. The cartridges (3 cc/60 mg) were first conditioned with methanol (2 mL) and H_2O (2 mL). The loading sample was a drug-free urine sample (2 mL) spiked with cocaine, norcocaine, and cocaethylene (each 10 μg/mL, 4 μL) and benzoylecgonine (10 μg/mL, 20 μL). The column was washed sequentially with 0.1 M HCl (2 mL) and methanol (3 mL). The elution solution was a mixture of CH_2Cl_2, IPA, and NH_4OH (78:20:2, v/v); the eluted solution was dried under a stream of nitrogen gas while heating at 40°C. For assaying, the residue was dissolved in pure water (4 mL).

2.6 Calibration curves

In the sweeping-MEKC mode, calibration curves were obtained after preparing individual standards at concentrations of 0.2, 0.5, 1, 2, and 5 μg/mL. In the CSEI-sweeping-MEKC mode, calibration curves were obtained after preparing cocaine, norcocaine, and cocaethylene standards at concentrations of 0.2, 0.5, 1, 3, 5, and 10 ng/mL and benzoylecgonine standards at 1, 2.5, 5, 15, 25, and 50 ng/mL in pure water. For each concentration, three replicate measurements were performed to assure the precision of the data.

3 Results and discussion

3.1 Optimizing conditions for separation using CSEI-sweeping-MEKC

To obtain the optimal separation and enhancement efficiencies, we considered the effects of several parameters of the CSEI-sweeping-MEKC method, including the pH, the proportion of organic modifier, the concentrations of SDS, the injection length of the HCB, and the injection time of the sample. Our attempts to optimize each of these conditions are described in detail below.

3.1.1 Optimizing the pH

We examined the influence of buffers at pH 1.8, 2.8, 3.8, and 4.8 while maintaining constant the concentrations of the buffer, SDS, and the proportion of organic modifier. Decreasing the pH to 1.8 enhanced the intensities of the analytes' peaks. The pH change correlated closely with the charge of the analytes and the magnitude of the EOF. The values of pK_a of cocaine, norcocaine, and cocaethylene are all greater than 8.0; for benzoylecgonine, the values are 3.1 and 10.1 [12]. Thus, when the pH is less than 3.1, the four analytes will all be positively charged; if the pH exceeds 3.1, benzoylecgonine will have neutral charge (because of charge equilibrium), whereas the three other analytes will retain their positive charges. The greater the degree of analyte protonation, the larger the number of sample ions injected electrokinetically into the CSEI-sweeping-MEKC system. Therefore, at values of pH greater than 3.1, benzoylecgonine was introduced into the capillary in the lowest amounts because its carboxylate ion was not protonated. At pH 1.8, each analyte had its greatest degree of positive charge and, therefore, its signal had the highest intensity. Hence, for subsequent experiments, we chose a buffer having a pH of 1.8.

3.1.2 Optimizing the organic modifier

The organic solvent was the most important factor influencing the separation efficiency of the analytes. Although the addition of methanol and acetonitrile did not improve the baseline separation, adding IPA or THF did advance the separation efficiency. In the absence of IPA or THF in the buffer, the four analytes migrated together and could not be identified individually. Because the addition of IPA alone (0–30%) did not effect complete separation, we considered the use of co-solvent system. When the IPA concentration was 10% in the absence of THF in the buffer, norcocaine and cocaine migrated together. Increasing the content of THF to 10% resulted in baseline separation and adequate resolution of norcocaine and cocaine; exceeding 10% THF led to long analysis times. Thus, the optimum separation conditions were achieved when adding 10% IPA and 10% THF to the buffer.

3.1.3 Optimizing the SDS concentration

We employed SDS in the concentration range 50–125 mM to evaluate the second on-line concentration sweeping step. The use of 75 mM SDS as the sweeping buffer provided the greatest peak enhancement without losing the separation efficiency. A lower SDS concentration provided good resolution, but the sweeping effect was worse. A higher concentration of SDS did not increase the peak height – in fact, it broadened the peak, worsening the resolution. The negatively charged SDS from the micellar BGE entered the capillary under an applied reverse polarity of −20 kV and then interacted with the cationic sample to perform the sweeping concentration. Because the EOF was suppressed at pH 1.8, the negatively charged micelles migrated toward the anode. Therefore, an increase in the number of negative micelles would accelerate the migration of the cationic sample. We observed that the analysis time shortened when the SDS concentration increased. Comparing the separation efficiency and the peak enhancement factor, we concluded that 75 mM SDS was optimal as the micellar BGE.

3.1.4 Optimizing the HCB injection length

The HCB injection length is the most important factor affecting the stacking efficiency. Pressure injection for 0.5, 1.0, 2.0, and 3.0 min at 20.7 kPa provided sample lengths of 9.1, 18.1, 36.3, and 54.4 cm, respectively. The peak heights were greater when the HCB lengths were longer (Fig. 2). At a longer HCB length, the cations stacked into a narrower zone, thereby enhancing the efficiency; nevertheless, in terms of the resolution of cocaine and norcocaine, an injection HCB length of 36.3 cm provided optimal improved sensitivity and separation efficiency. A few papers have suggested that inserting a plug of water after injecting the HCB zone can improve the sample stacking as a result of the different electric field strengths accelerating the cations into the capillary [23, 25]. In our hands, however, this process did not have any obvious effect and, therefore, we did not inject water in our subsequent experiments.

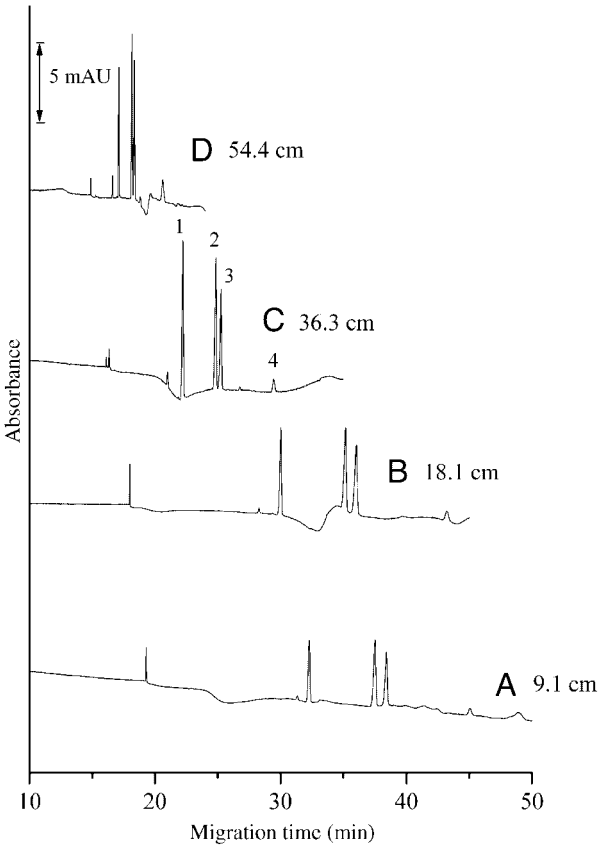

Figure 2. Effect of injection length of HCB on CSEI-sweeping-MEKC analysis. (A) 9.1 cm; (B) 18.1 cm; (C) 36.3 cm; (D) 54.4 cm. Conditions: non-micellar BGE, 100 mM phosphoric acid (pH 1.8) containing 10% IPA and 10% THF; HCB, 150 mM phosphoric acid (pH 1.8); micellar BGE, 75 mM SDS in 100 mM phosphoric acid containing 10% IPA and 10% THF; electrokinetic injection, 15 kV for 900 s; separation voltage, −20 kV; detection at 230 nm; effective capillary length, 50 cm. Peak identification: (1) CE, 10 ng/mL; (2) NC, 10 ng/mL; (3) CC, 10 ng/mL; (4) BE, 50 ng/mL.

3.1.5 Optimizing the sample injection time

Increasing the length of time of the electrokinetic injection generally increases the intensity of the signals of analytes. In this study, we varied the sample injection time from 300 to 1200 s at 15 kV. Figure 3 reveals that the intensities of the signals for cocaine, norcocaine, and cocaethylene increased up to an injection time of 900 s; beyond that time, the intensities did not increase significantly and the resolution worsened. For benzoylecgonine, the intensity of the signal increased until the injection time reached 1200 s. When increasingly more of the cationic sample enters the capillary, a greater sweeping power is required to provide satisfactory resolution and enhancement. Nevertheless, taking into account the simultaneous detection of the four analytes,

we found that a sampling time of 900 s at 15 kV provided the optimal resolution and maximum peak enhancement.

3.2 Comparing MEKC, sweeping-MEKC, and CSEI-sweeping-MEKC

The chromatographs shown in Fig. 4, obtained using the conventional MEKC, sweeping-MEKC, and CSEI-sweeping-MEKC methods, indicate the dramatically amplified efficiency of the latter method. The detailed data in Table 1 indicate that the sensitivity of the CSEI-sweeping-MEKC method relative to the conventional MEKC was improved by a factor of between 1.75×10^3 and 3.96×10^4; relative to sweeping-MEKC, the improvement factor was between 81 and 1.74×10^3. In the CSEI-sweeping-MEKC method, the non-micellar BGE (100 mM phosphoric acid, pH 1.8, containing 10% IPA and 10% THF) filled the capillary initially. Next, a 36.3-cm-long HCB (150 mM phosphoric acid, pH 1.8) was injected. The analytes were then introduced into the capillary through electrokinetic injection, followed by injection of a buffer containing SDS and organic solvent as sweeping and separation agents, respectively. The enhancement factors obtained – up to 3.96×10^4-fold – for the peak heights when using the CSEI-sweeping-MEKC method indicate that it is a powerful tool for improving the detectability of these analytes.

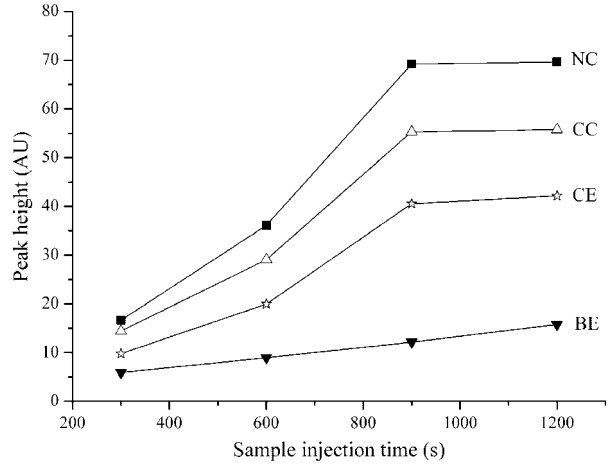

Figure 3. Effect of electrokinetic injection time on CSEI-sweeping-MEKC analysis. HCB length, 36.3 cm; other conditions were the same as those employed to obtain Fig. 2.

3.3 Method validations

Under the optimal conditions, we performed a quantitative analysis (see Table 2) to obtain a set of calibration curves. For the CSEI-sweeping-MEKC method, the linear range was 0.2–10 ng/mL for cocaine, norcocaine, and cocaethylene and 1–50 ng/mL for benzoylecgonine. When using the

Table 1. Sensitivity enhancements for cocaine and its metabolites

Compound	SE_{height} [a]	SE_{height} [b]
Cocaethylene	20 600	1030
Norcocaine	39 600	1390
Cocaine	30 000	1740
Benzoylecgonine	1750	81

a) Peak height of CSEI-sweeping-MEKC/peak height of MEKC × dilution factor.

b) Peak height of CSEI-sweeping-MEKC/peak height of sweeping-MEKC × dilution factor.

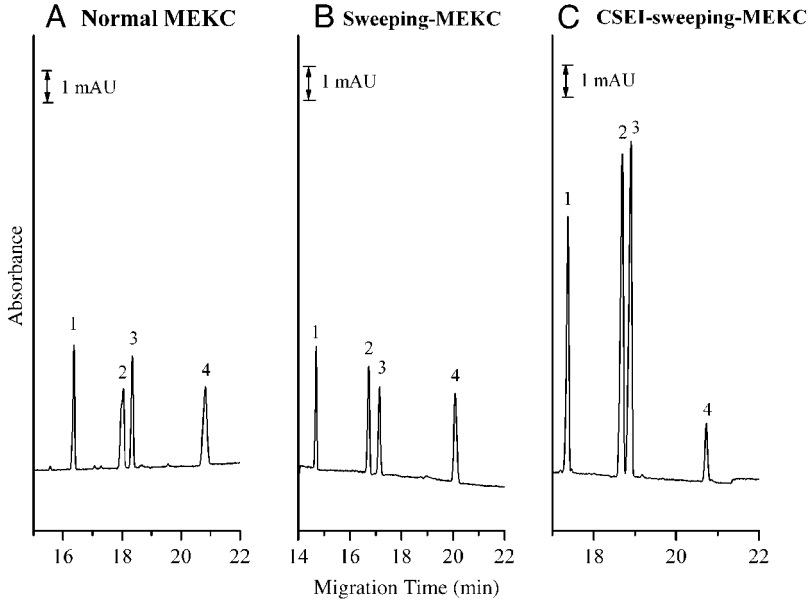

Figure 4. Comparison of normal MEKC, sweeping-MEKC, and CSEI-sweeping-MEKC analyses. (A) Normal MEKC conditions: separation buffer; 75 mM SDS in 100 mM phosphoric acid (pH 1.8) containing 10% IPA and 10% THF; sample concentration, 100 μg/mL in water; injection length, 1.5 mm. (B) Sweeping-MEKC conditions: separation buffer, 75 mM SDS in 100 mM phosphoric acid (pH 1.8) containing 10% IPA and 10% THF; sample concentration, 5 μg/mL in buffer (without SDS); injection length, 60 mm. (C) CSEI-sweeping-MEKC conditions: HCB length, 36.3 cm; other conditions were the same as those employed to obtain Fig. 2.

Table 2. Ranges of linearity, calibration curves, coefficients of determination (r^2), LODs, and values of RSD for cocaethylene, norcocaine, cocaine, and benzoylecgonine using both sweeping-MEKC and CSEI-sweeping-MEKC analytical methods

	Cocaethylene	Norcocaine	Cocaine	Benzoylecgonine
Sweeping-MEKC				
Range of linearity (ng/mL)	200–5000	200–5000	200–5000	200–5000
Calibration curve	$y = 568.6x + 58.10$	$y = 829.6x + 79.30$	$y = 709.4x + 46.30$	$y = 1113x + 111.8$
Coefficient of determination	0.9989	0.9992	0.9994	0.9992
LOD ($S/N = 3$; ng/mL)	52.8	39.7	50.3	33.5
RSD (%; $n = 5$)				
Migration time (min)	1.76	2.09	2.14	2.43
Peak area	6.85	6.42	8.57	4.66
Peak height	7.47	6.52	6.55	2.18
CSEI-sweeping-MEKC				
Range of linearity (pg/mL)	200–10 000	200–10 000	200–10 000	1000–50 000
Calibration curve	$y = 2892x - 417.7$	$y = 4323x - 590.1$	$y = 3165x - 444.2$	$y = 164.0x + 48.46$
Coefficient of determination	0.9989	0.9994	0.9980	0.9958
LOD ($S/N = 3$; pg/mL)	42.2	29.7	36.7	236
RSD (%; $n = 3$)				
Migration time (min)	3.37	3.96	4.06	4.71
Peak area	10.7	8.38	11.1	4.06
Peak height	15.9	11.9	15.9	9.98

sweeping-MEKC method, linearity was confined to the range 0.2–5 μg/mL. The coefficients of determination (r^2) were all greater than 0.9958, suggesting that our developed method has good linearity. The LODs ($S/N = 3$) for the four analytes ranged from 29.7 to 236 pg/mL. These values reveal that the CSEI-sweeping-MEKC method is much more sensitive than the sweeping-MEKC method, which exhibited LODs of 33.5–52.8 ng/mL. The quantitative precision – in terms of the repeatability, *i.e.* the RSDs, of the migration time, peak area, and peak height – of the sweeping-MEKC method was better than that of the CSEI-sweeping-MEKC method. This behavior might have resulted from electrokinetic injection being influenced by the sample matrix, causing diversity between one injection and another. In total, these results clearly indicate the superior linearity and higher sensitivity provided by the CSEI-sweeping-MEKC method, and its acceptable relative repeatability when analyzing these four compounds.

3.4 Separating and determining cocaine and its metabolites in urine samples

In this study, we combined SPE with CSEI-sweeping-MEKC to determine the levels of cocaine and its metabolites in urine samples. Biological samples have complexity that makes them difficult subjects for analysis. To avoid the large matrix effect that results from using real samples, we chose SPE rather than liquid–liquid extraction to remove interfering impurities. Figure 5 indicates that after we pretreated blank urine samples through SPE, we could not detect any of the four analytes. When we spiked the four analytes into the blank urine samples prior to performing the SPE process, we detected cocaine and its metabolites at the same migration times obtained for the standard samples; thus, it appears that through SPE treatment we can determine the presence of these four analytes in urine samples without other impurities interfering with the CSEI process. Under the optimized SPE conditions, we obtained recoveries of 74.9% for cocaine, 77.5% for norcocaine, 75.5% for cocaethylene, and 87.6% for benzoylecgonine; the reproducibilities (RSD; $n = 5$) of the extractions were 14.8, 17.0, 13.0, and 12.7%, respectively; the LOQs of these analytes in urine samples were 0.61, 0.43, 0.52, and 6.99 ng/mL, respectively. Thus, pretreating urine samples through SPE provided satisfactory recoveries.

4 Concluding remarks

This study demonstrates that CSEI-sweeping-MEKC is easy to operate, requires a short analysis time, and can be used efficiently in conjunction with on-line sample preconcentration to determine the presence of cocaine and its metabolites. Under the optimized separation parameters, the enrichment factors for cocaine, norcocaine, cocaethylene, and benzoylecgonine when using CSEI-sweeping-MEKC (relative to MEKC) ranged from 1.75×10^3 to 3.96×10^4. The LODs for the four compounds in pure water ranged from 29.7 to 236 pg/mL. We successfully applied the CSEI-sweeping-MEKC method, in conjunction with SPE, to the analysis of these four compounds in human urine. This developed analytical method functioned with acceptable reproducibilities. Therefore, this technique

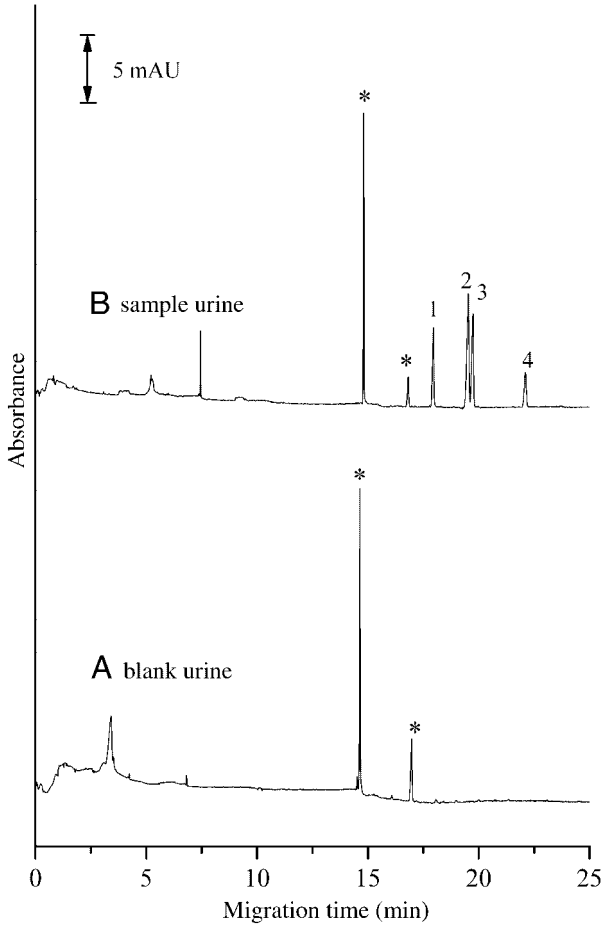

Figure 5. CSEI-sweeping-MEKC electropherograms of SPE-extracted (A) blank and (B) spiked urine samples. *: Unknown. Analyses were performed according to the optimized conditions of the CSEI-sweeping-MEKC method; sample concentrations: (1) CE, 10 ng/mL; (2) NC, 10 ng/mL; (3) CC, 10 ng/mL; (4) BE, 50 ng/mL.

should prove useful for detecting, with high sensitivity, the amounts of cocaine and its metabolites in biological fluid samples.

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The authors have declared no conflict of interest.

5 References

[1] Baselt, R. C., *Disposition of Toxic Drugs and Chemicals in Man*, 4th Edn., Chemical Toxicology Institute, Foster City, CA 1995, pp. 186–188.

[2] Johansen, S. S., Bhatia, H. M., *J. Chromatogr. B* 2007, *852*, 338–344.

[3] Cognard, E., Rudaz, S., Bouchonnet, S., Staub, C., *J. Chromatogr. B* 2005, *826*, 17–25.

[4] Brunetto, M. R., Cayama, Y. D., García, L. G., Gallignani, M., Obando, M. A., *J. Pharmaceut. Biomed. Anal.* 2005, *37*, 115–120.

[5] Caslavska, J., Allemann, D., Thormann, W., *J. Chromatogr. A* 1999, *838*, 197–211.

[6] Cardenas, S., Gallego, M., Valcarcel, M., *Rapid Commun. Mass Spectrom.* 1996, *10*, 631–636.

[7] Matta Chasin, A. A. da., Mídio, A. F., *Forensic Sci. Int.* 2000, *109*, 1–13.

[8] Kintz, P., Mangin, P., *Forensic Sci. Int.* 1995, *73*, 93–100.

[9] Cristoni, S., Basso, E., Gerthoux, P., Mocarelli, P. *et al.*, *Rapid Commun. Mass Spectrom.* 2007, *21*, 2515–2523.

[10] Gottardo, R., Fanigliulo, A., Bortolotti, F., Paoli, G. D. *et al.*, *J. Chromatogr. A* 2007, *1159*, 190–197.

[11] Virag, L., Mets, B., Jamdar, S., *J. Chromatogr. B* 1996, *681*, 263–269.

[12] Foulon, C., Menent, M.-C., Manuel, N., Pham-Huy, C. *et al.*, *Chromatographia* 1999, *50*, 721–727.

[13] Jeanville, P. M., Estapé, E. S., de Jeanville, I. T.-N., Martí, A., *J. Anal. Toxicol.* 2001, *25*, 69–75.

[14] Jeanville, P. M., Estapé, E. S., Needham, S. R., Cole, M. J., *J. Am. Soc. Mass Spectrom.* 2000, *11*, 257–263.

[15] Needham, S. R., Jeanville, P. M., Brown, P. R., Estapé, E. S., *J. Chromatogr. B* 2000, *748*, 77–87.

[16] Jeanville, P. M., Estapé, E. S., de Jeanville, I. T.-N., *Int. J. Mass Spectrom.* 2003, *227*, 247–258.

[17] Pichini, S., Pacifici, R., Pellegrini, M., Marchei, E. *et al.*, *J. Chromatogr. B* 2003, *794*, 281–292.

[18] Srinivasan, K., Wang, P., Eley, A. T., White, C. A., Bartlett, M. G., *J. Chromatogr. B* 2000, *745*, 287–303.

[19] Xia, Y., Wang, P., Bartlett, M. G., Solomon, H. M., Busch, K. L., *Anal. Chem.* 2000, *72*, 764–771.

[20] Hilhorst, M. J., Van Hout, M. W. J., Somsen, G. W., Franke, J. P., De Jong, G. J., *J. Capillary Electrophor.* 1998, *5*, 159–164.

[21] Quirino, J. P., Terabe, S., *Science* 1998, *282*, 465–468.

[22] Quirino, J. P., Terabe, S., *Anal. Chem.* 1999, *72*, 1638–1644.

[23] Jen, H.-P., Tsai, Y.-C, Su., H.-L., Hsieh, Y.-Z., *J. Chromatogr. A* 2006, *1111*, 159–165.

[24] Quirino, J. P., Terabe, S., *Anal. Chem.* 2000, *72*, 1023–1030.

[25] Meng, P., Fang, N., Wang, M., Liu, H., Chen, D. D. Y., *Electrophoresis* 2006, *27*, 3210–3217.

[26] Lin, Y.-H, Lee, M.-R, Lee, R.-J, Ko, W.-K, Wu, S.-M., *J. Chromatogr. A* 2007, *1145*, 234–240.

