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Separations

FAST AND HIGH-PERFORMANCE SCREENING OF NARCOTIC DRUGS ON A MICROFLUIDIC DEVICE BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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This work presents a new approach for fast and sensitive ultraviolet detection of 12 kinds of narcotic drugs on a microfluidic device by micellar electrokinetic capillary chromatography. Under optimal sampling and separation conditions the baseline separation of 12 drugs with resolution values ranging from 1.06 to 4.04 and separation efficiency up to 5.14×10^5 plates m^{-1} was achieved within 200 s. The widest linear range for detection of these analytes was 1.0 to 1500.0 μg mL^{-1}. The correlation coefficients are higher than 0.9994. This system can successfully be applied to analyze narcotic drugs in human urine with the aid of liquid-liquid extraction of the samples. This method allows minimum detectable concentrations of these drugs down to 33 ng mL^{-1} at a signal-to-noise ratio of 3. This rapid method with high resolution and sensitivity, and little solvent consumption possessed potential application in screening of narcotic drugs in forensic analysis.

Keywords: Micellar electrokinetic capillary electrophoresis; Microfluidic device; Narcotic drugs; Ultraviolet detection

INTRODUCTION

Narcotics are prescription drugs that relieve severe pain in medical therapy. However, as powerfully addictive drugs, the abuse of narcotics is harmful because narcotics are strong stimulants to the central nervous system. Therefore, the determination of the use of these drugs is required for forensic toxicology and for the

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monitoring of drug addicts during therapy (Rendle 2005; Brettell, Butler, and Almirall 2009). A number of analytical methods have been described using gas chromatography (Leis et al. 2000; Lewis, Johnson, and Hattrup 2005) and liquid chromatography (Ghazi-Khansari et al. 2006; Salomonsson, Bondesson, and Hedeland 2008). Although these methods have adequate sensitivity to detect low concentrations in samples, large sample volumes and high-cost equipment are involved. Methods based on immunoassay can be used for fast detection, but they need specific antibodies and antibody immobilization under specific conditions (Yang et al. 2004; Stubbs, Lee, and Hunt 2005). Raman spectroscopy is also favored for the application of determining narcotics, but the Raman effect is an inherent limitation and thus hinders the detection of low-concentration analytes (Ryder 2005). Recently, capillary electrophoresis (CE) has been widely applied for the analysis of drugs and metabolites due to the high resolving power and relative small amounts of related reagents (Jong et al. 2009; Lin et al. 2008; Tagliaro et al. 2010). But, the fussy pretreatment of capillaries and long-time separation limit the application in practice.

Micellar electrokinetic capillary chromatography (MECC) is an attractive approach by adding a surfactant to the electrophoresis buffer for the analysis of narcotic drugs. For example, based on the hydrophilic ionic liquid and sodium dodecyl sulfate (SDS), the first CE microchip was reported for MECC separation and electrochemiluminescent detection of 4 narcotic drugs (Du and Wang 2008). This was done by combining the advantages of a microfluidic device, such as low cost, small consumption of reagents and samples, short separation time, and easy miniaturization (Chan, Zohar, and Lee 2009; Dang et al. 2006; Kraly et al. 2009; Lenshof and Laurell 2010; Mark et al. 2010; Wang et al. 2008). Our previous work proposed a quartz capillary/poly(methyl methacrylate) (PMMA) microfluidic device coupled

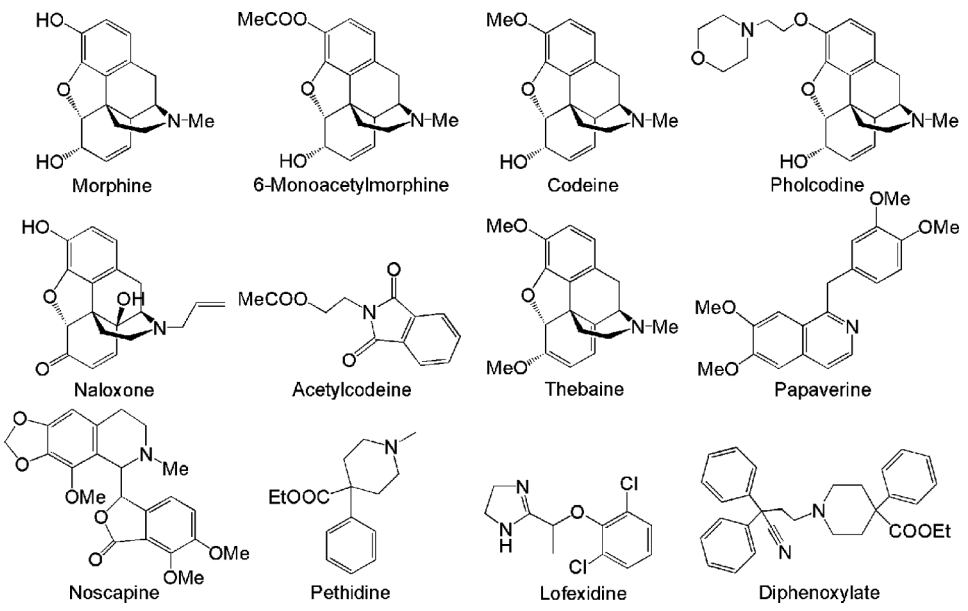

Figure 1. Chemical structures of 12 narcotic drugs.

with ultraviolet (UV) detection for separation of eight illicit drugs (Qiang et al. 2009). More recently, we developed a method with this microfluidic device for screening of eight psychotropic drugs in human plasma coupled with solid-phase extraction (Sheng et al. 2010). In view of the strong UV absorption of narcotic drugs containing phenyl skeleton (Jong et al. 2009; Alnajjar et al. 2007), herein, a new approach for rapid and high-performance UV screening of narcotic drugs was designed on the microfluidic device by MECC. Using twelve common narcotic drugs as analytes (Fig. 1), baseline separation of $\mu g\,mL^{-1}$ concentrations of these drugs was achieved within several minutes. Coupling with liquid-liquid extraction, this MECC-UV microfluidic system showed low detection limits and could be successfully used to analyze twelve narcotic drugs in urine samples. This proposed system provided a promising choice for the rapid and multiple screening of narcotic drugs in clinics and forensic analysis.

EXPERIMENTAL

Chemicals and Materials

Narcotic drugs including morphine, 6-monoacetylmorphine, codeine, pholcodine, naloxone, acetylcodeine, thebaine, papaverine, noscapine, pethidine, lofexidine, and diphenoxylate with the purity >99.0% were obtained from Beijing Municipal Public Security Bureau. All aqueous solutions were prepared using $\geq 18\,M\Omega$ ultrapure water (Milli-Q, Millipore). Individual stock solutions containing $10.0\,mg\,mL^{-1}$ of each drug were prepared in water and stored at 4°C. The electrophoresis buffer was ultrasonicated for removing air bubbles prior to use. Urine samples were collected from Beijing Municipal Public Security Bureau, in which the presence of these twelve narcotic drugs was confirmed by gas chromatography/mass spectrometry. All other reagents were of analytical reagent grade. Fused-silica capillaries (360 μm o.d., 50 μm i.d.) were obtained from Yongnian Optical Fiber Factory (Hebei, China).

Instruments and Electrophoresis Procedure

The microfluidic device consisting of a printed circuit board as voltage supplier, a PMMA board as support substrate, and a 7.5-cm length fused-silica cappilary as microchannel was described previously (Qiang et al. 2009). A UV microfluidic workstation home-manufactured by cooperation with Beijing Cailu Scientific Instrument Limited Company was employed throughout this work. It was composed of an eight-port high-voltage power supply, a UV detector, and data processor. High-voltage power for sample injections and electrophoresis separation were performed by the high voltage module, which could monitor the real-time current and voltage. The determinations were carried out at a wavelength of 200 nm.

The electrophoresis buffer was composed of 5.0 mM borax (pH 9.5), 40.0 mM SDS, 1.0 M urea and 7.0% (v/v) 1-butanol. In the experiments, the separation channel was treated by rinsing with the electrophoresis buffer for several seconds with the aid of a vacuum pump prior to use, and then the sample reservoir was filled with 12.5 μL testing sample. The injection was performed by applying a high voltage to the sample reservoir and the waste reservoir float with the buffer reservoir at ground

potential. During the separation process, the high voltage was applied to the buffer reservoir and sample reservoir float while the waste reservoir was at ground potential. After detection, the microfluidic device could be renewed by neatly rinsing with the electrophoresis buffer via a vacuum pump.

Optimization

The electrophoresis buffer was composed of borax, SDS, urea, and 1-butanol, and their concentrations were optimized according to the resolution (R_s) of 12 drugs. When changing one parameter for optimization, others were at their optimal concentrations. The selection of an appropriate separation voltage was performed between 1000 V and 1600 V, and the migration time and separation efficiency was the deciding factor. The sampling times were investigated at 1, 2, and 3 seconds (s) with a sampling voltage of 90 V. The separation reproducibility was examined with ten separations of 10.0 $\mu g\,mL^{-1}$ analytes on the microfluidic device via run-to-run, device-to-device and day-to-day, respectively. The detection limits were calculated as the concentration of analytes at a signal-to-noise ratio (S/N) of 3.

Sample Pretreatment and Analysis

The sample pretreatment was carried out by means of liquid-liquid extraction with chloroform as the organic phase, which took about 10 min. Prior to analysis, urine samples were first filtered through 0.22 μm membranes and then the drugs were extracted with 1.0 mL of chloroform for 1.0 mL samples. In succession, the organic phase was evaporated under a gentle nitrogen stream at room temperature, and the residue was then dissolved in 100 μL electrophoresis buffer. Finally, the obtained sample solution was injected into the sample reservoir for analysis with a sampling voltage of 90 V for 2 s and a separation voltage of 1400 V. The extraction efficiency was calculated by dividing the concentration of the analyte obtained after extraction with its original concentration spiked in the urine sample.

RESULTS AND DISCUSSION

Optimization of Electrophoresis Buffer

The optimization procedure was carried out using a mixture of 12 narcotic drugs at 10 $\mu g\,mL^{-1}$, which was prepared with the electrophoresis buffer. The resolution between two adjacent drugs was considered as the function of optimization, and was defined as $2((t_M)_B\text{-}\ (t_M)_A)/(W_B + W_A)$, where t_M is the migration time and W is the full peak width (Ghowsi, Foley, and Gale 1990).

For MECC, SDS played an important role in the separation. As shown in Fig. 2A, when the concentration was at 30.0 mM, no evidence showed the probability of complete separation of these analytes. When the concentration increased up to 40.0 mM, the R_s values increased to above 1.0, which meant complete base-line separation. However, when the concentration continued increasing, sample zone broadening appeared due to the longer migration time and the Joule heating effect,

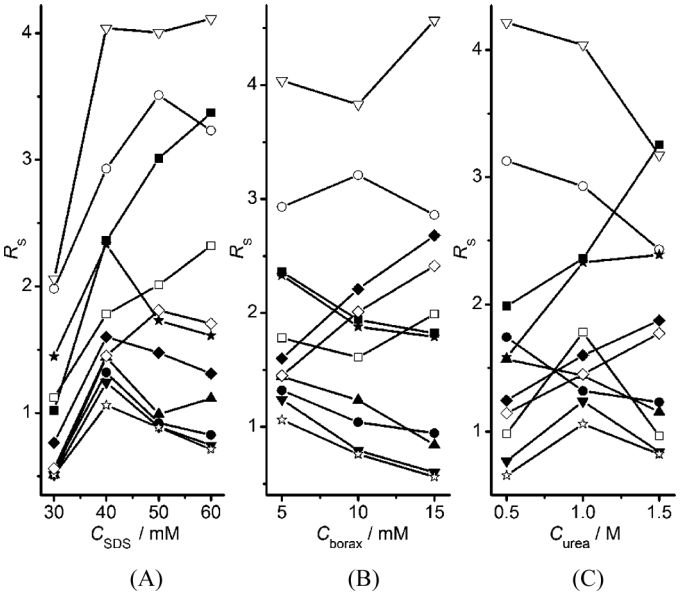

Figure 2. Effects of (A) SDS, (B) borax, and (C) urea concentrations on resolution for ■ morphine-6-monoacetylmorphine, ● 6-monoacetylmorphine-codeine, ▲ codeine-pholcodine, ▼ pholcodine-naloxone, ◆ naloxone-acetylcodeine, ○ acetylcodeine- thebaine, ☆ thebaine-papaverine, □ papaverine-noscapine, ★ noscapine-pethidine, ◇ pethidine- lofexidine, ▽ lofexidine-diphenoxylate. Separation voltage: 1400 V; sampling voltage: 90 V for 2 s. When changing one parameter, others are at their optimal values.

which resulted in poor resolution, stability, and repeatability. Thus, 40.0 mM was chosen as the optimal SDS concentration.

The borax concentration was tested between 5.0 mM and 15.0 mM. As an ionic strength supplier, low concentrations of borax showed low-lying buffer capacity and led to an unstable baseline. However, with the increase of borax concentration, larger migration time and sample zone broadening appeared, which were due to the thinner electrical double-layer and the suppressant electro-osmotic flow. Additionally, high concentrations of borax led to a steep increase of current in the separation channel. At the separation voltage of 1400 V, the currents through the separation channel were 6.5, 8.4, and 10.5 μA for 5.0, 10.0, and 15.0 mM borax, respectively, resulting in excessive Joule heating and a inadequate baseline. According to the results shown in Fig. 2B, an appropriate resolution was obtained at 5.0 mM borax, which was chosen as the optimal borax concentration of the electrophoresis buffer.

As a solubilization additive, urea was added to improve the separation capability of the electrophoresis buffer (Liu et al. 1999). As shown in Fig. 2C, the urea concentration (0.5–1.5 mM) was examined for obtaining the better resolution for all analytes, which was observed at a concentration of 1.0 mM.

As an organic additive, 1-butanol showed a great effect on the resolution of these drugs. Proper addition of 1-butanol could expand the migration-time window, improve the peak shape, and thus alter the separation efficiency (Huhn, Pütz, and Pyell 2008). When the 1-butanol concentration was 3.0%, 5.0%, and 7.0%, the R_s

value between thebaine and papaverine was 0.81, 0.93, and 1.06, respectively. Nevertheless, when the concentration rose up to 9.0%, the R_s went down to 0.96. Thus, with regards to the resolution, 7.0% of 1-butanol was selected as the optimal concentration. Therefore, the optimal electrophoresis buffer was composed of 5.0 mM borax (pH 9.5), 40.0 mM SDS, 1.0 M urea, and 7.0% 1-butanol.

Effect of Separation Voltage

The applied separation voltage controlled the separation process and the migration time of the analytes in the microchannel by altering the electro-osmotic flow. The resolution between two adjacent drugs and the migration time of each drug were measured as the function of the separation voltage between 1000 and 1600 V to evaluate the effect of separation voltage (Fig. 3). At the separation voltage of 1000 V, some of the analytes could not be completely separated due to the long migration time caused by low-lying electro-osmotic flow. Although shorter migration time showed at the separation voltage of 1600 V, high Joule heat was generated (the currents were 4.7, 5.6, 6.5, and 8.1 μA at the separation voltages of 1000, 1200, 1400, and 1600 V, respectively) and an unstable baseline appeared, resulting in poor resolution and reproducibility of the analysis. Finally the high separation voltage of 1400 V (187 V/cm of separation field strength) was selected to achieve base-line separation within 200 s. Moreover, the low separation voltage would benefit the design of low-cost power for separation of narcotic drugs.

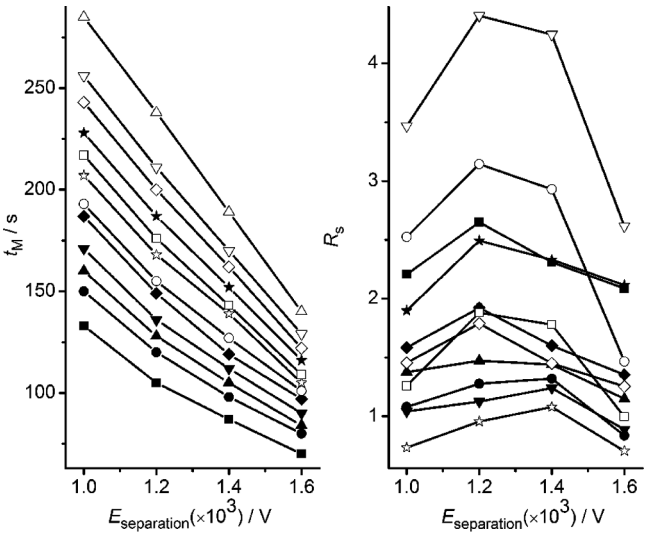

Figure 3. Effects of separation voltage on (A) migration time, and (B) resolution of 12 narcotic drugs. ■, ●, ▲, ▼, ◆, ○, ☆, □, ★, ◇, ▽, △ represents morphine, 6-monoacetylmorphine, codeine, pholcodine, naloxone, acetylcodeine, thebaine, papaverine, noscapine, pethidine, lofexidine, and diphenoxylate in (A), and resolution for sequentially adjacent drugs in (B), respectively. Electrophoresis conditions: 5.0 mM tetraborate buffer (pH 9.5) containing 40.0 mM SDS, 1.0 M urea and 7.0% 1-butanol. Sampling voltage: 90 V for 2 s.

Effect of Sampling Time

The sampling quantity depended on the injection time and applied field strength. Under the injection field strength of 180 V/cm (90 V injection voltage), the effects of injection time on the UV adsorption of each drug and the R_s values between two adjacent drugs were examined (Fig. 4). At the injection time of 1 s, the adsorption was unstable. Considering both the sensitivity and the separation efficiency, the injection time of 2 s was selected for following experiments. Longer injection times could cause more sample being injected into the separation channel, resulting in the broadening of the sample zone, thus increasing the peak width and lowering the resolution.

Analytical Performance

The analytes were separated due to their different affinities to the hydrophobic interior of the micelle. Under optimized conditions: electrophoresis buffer, 5.0 mM borax containing 40.0 mM SDS, 1.0 M urea, and 7.0% 1-butanol; separation voltage, 1400 V; sampling time, 90 V for 2 s. Separation of the 12 narcotic drugs at 10.0 μg mL^{-1} was presented in Fig. 5. Among these drugs, morphine, 6-monoacetylmorphine, codeine, pholcodine, and thebaine were very similar narcotics, and they all shared the same carbon skeleton, only differing in the substituents on the two or six membered rings. The R_s values for morphine-6-monoacetylmorphine, 6-monoacetylmorphine-codeine, codeine-pholcodine, pholcodine-naloxone, naloxone-acetylcodeine,

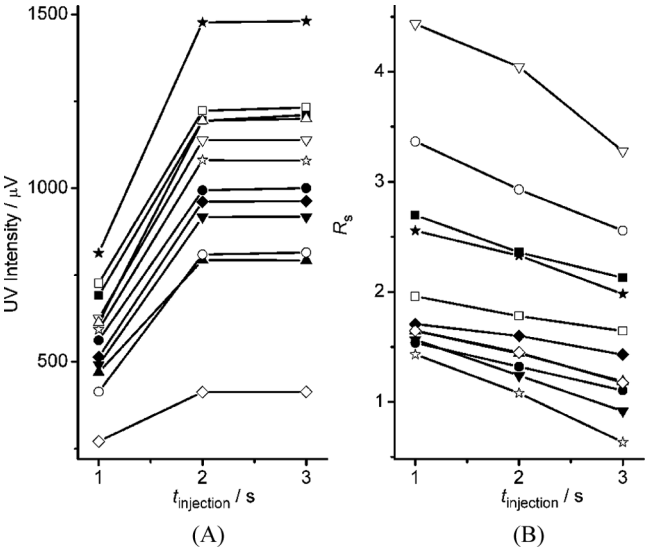

Figure 4. Effects of injection time on (A) UV intensity, and (B) resolution of 12 narcotic drugs. ■, ●, ▲, ▼, ◆, ○, ☆, □, ★, ◇, ▽, △ represents morphine, 6-monoacetylmorphine, codeine, pholcodine, naloxone, acetylcodeine, thebaine, papaverine, noscapine, pethidine, lofexidine, and diphenoxylate in (A), and resolution for sequentially adjacent drugs in (B), respectively. Separation voltage of 1400 V, other electrophoresis conditions are the same as in Fig. 3.

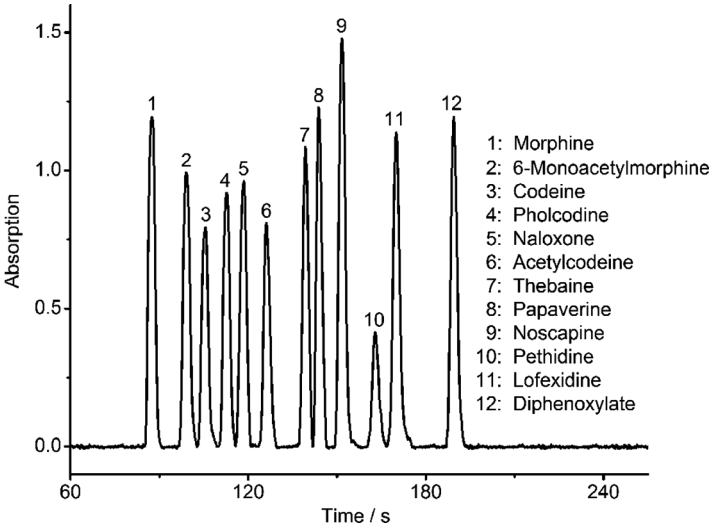

Figure 5. Electropherogram for 12 narcotic drugs at 10.0 μg mL^{-1} in 5.0 mM tetraborate buffer (pH 9.5) containing 40.0 mM SDS, 1.0 M urea and 7.0% 1-butanol. Separation voltage: 1400 V; sampling voltage: 90 V for 2 s.

acetylcodeine-thebaine, thebaine-papaverine, papaverine-noscapine, noscapine-pethidine, pethidine-lofexidine, and lofexidine-diphenoxylate were 2.36, 1.32, 1.44, 1.24, 1.60, 2.93, 1.06, 1.78, 2.33, 1.45, and 4.04, respectively, indicating that the narcotic drugs were separated satisfactorily on this MECC microfluidic device. The theoretical plate numbers (N), defined as 5.54 $(t_M/W_{1/2})^2$ ($W_{1/2}$ was the peak width at the half-maximum points) (Jorgenson and Lukacs 1981), were in the range from 1.01×10^5 to 5.14×10^5 plates m^{-1}, demonstrating good separation efficiency.

The baseline separation was achieved within 200 s, presenting a rapid separation when compared with the retention times of at least 8 min for papaverine (Lombardo-Agüí, Cruces-Blanco, and García-Campaña 2009), 10.0 min for noscapine, 10.3 min for thebaine, 14.9 min for codeine, and 22.2 min for morphine (Reid et al. 2007) by capillary electrophoresis, and 6.7 min for thebaine, 6.8 min for codeine and 7.7 min for morphine by gas chromatography (Lewis, Johnson, and Hattrup 2005). The precision of the whole method was evaluated in term of repeatability. Both the relative standard deviations (RSDs) ($n = 10$) of t_M and peak areas, which were respectively represented for identification and quantification, were assessed for run-to-run, day-to-day, and device-to-device, and the results are summarized in Table 1. All the RSDs were less than 4.3%, demonstrating that the microfluidic device exhibited a promising stability and repeatability of identification and quantification for the narcotic drugs.

The linear range and detection limit of each drug were investigated using the peak area as a function of concentration prepared by gradual dilution of standard solution with electrophoresis buffer. The standard curves were linear in the ranges of 1.0 to 1500.0 μg mL^{-1} for acetylcodeine; 1.0 to 1000.0 μg mL^{-1} for 6-monoacetylmorphine, codeine and naloxone; 1.0 to 750.0 μg mL^{-1} for morphine, pholcodine,

Table 1. Separation efficiency and reproducibility of microfluidic device for narcotic drugs at 10.0 μg mL^{-1} ($n = 10$)

Analyte	$N/10^5$ plates m^{-1}	R_s	RSD (%) of t_M			RSD (%) of peak area		
			Run-to-run	Device-to-device	Day-to-day	Run-to-run	Device-to-device	Day-to-day
Morphine	1.01	2.36	0.3	1.2	0.5	0.7	1.4	1.2
6-Monoacetylmorphine	1.25	1.32	0.6	1.6	0.9	1.4	2.9	2.2
Codeine	1.46	1.44	1.1	2.1	1.5	2.4	3.2	2.9
Pholcodine	1.73	1.24	0.8	1.5	1.2	1.9	2.8	2.4
Naloxone	1.97	1.60	0.8	2.1	1.6	2.1	3.3	2.9
Acetylcodeine	2.28	2.93	0.7	1.9	1.3	2.0	3.4	2.8
Thebaine	3.33	1.06	1.0	1.8	1.5	2.5	3.9	3.3
Papaverine	3.75	1.78	0.4	1.6	0.8	1.8	2.9	2.5
Noscapine	3.29	2.33	0.7	2.0	1.4	2.3	3.8	3.1
Pethidine	3.51	1.45	0.6	2.3	1.6	2.1	4.1	3.5
Lofexidine	3.75	4.04	0.7	1.9	1.4	2.5	3.8	3.2
Diphenoxylate	5.14	–	0.9	2.3	1.5	2.8	4.3	3.4

thebaine, papaverine, noscapine, lofexidine, and diphenoxylate; and 2.0 to 750.0 μg mL^{-1} for pethidine, with the correlation coefficients ranging from 0.9994 to 0.9999 (Table 2). The detection limits considered as the minimum analyte concentrations at a signal-to-noise ratio of 3 were 0.377, 0.454, 0.567, 0.490, 0.468, 0.556, 0.414, 0.366, 0.304, 1.087, 0.395, and 0.377 μg mL^{-1} for morphine, 6-monoacetylmorphine, codeine, pholcodine, naloxone, acetylcodeine, thebaine, papaverine, noscapine, pethidine, lofexidine, and diphenoxylate, respectively, which were much lower than 10 μg mL^{-1} for cocaine on CE microchip with electrochemiluminescent detection (Du and Wang 2008). Furthermore, lower detection limits could be obtained by adopting some pre-enrichment techniques.

Table 2. Linear ranges and detection limits of microfluidic device for narcotic drugs

Analytes	Linear ranges/ μg mL^{-1}	Detection limit/ μg mL^{-1}	Extraction efficiency (%)	Minimum detectable concentration in urine/ng mL^{-1}
Morphine	1.0–750.0	0.377	82.3 ± 4.6	45
6-Monoacetylmorphine	1.0–1000.0	0.454	52.5 ± 2.8	86
Codeine	1.0–1000.0	0.567	93.7 ± 2.1	60
Pholcodine	1.0–750.0	0.490	58.6 ± 3.3	84
Naloxone	1.0–1000.0	0.468	48.1 ± 4.2	97
Acetylcodeine	1.0–1500.0	0.556	83.3 ± 3.8	66
Thebaine	1.0–750.0	0.414	88.6 ± 2.6	47
Papaverine	1.0–750.0	0.366	63.2 ± 3.5	58
Noscapine	1.0–750.0	0.304	92.6 ± 3.9	33
Pethidine	2.0–750.0	1.087	54.6 ± 2.8	199
Lofexidine	1.0–750.0	0.395	77.3 ± 3.7	51
Diphenoxylate	1.0–750.0	0.377	72.8 ± 4.1	52

Analysis of Human Urine Samples

In order to demonstrate the practical application of the detection of narcotic drugs by the proposed MECC method on the microfluidic device, urine samples were analyzed. First, liquid-liquid extraction was employed to extract the analytes from urine samples prior to analysis. The extraction efficiency, calculated by dividing the concentration of the analyte obtained after extraction with the concentration of the analyte originally spiked in the urine sample, was shown in Table 2. In virtue of the enrichment caused by extraction, the minimum detectable concentrations of narcotic drugs in real urine samples were 45, 86, 60, 84, 97, 66, 47, 58, 33, 199, 51, and 52 ng mL^{-1} for morphine, 6-monoacetylmorphine, codeine, pholcodine, naloxone, acetylcodeine, thebaine, papaverine, noscapine, pethidine, lofexidine, and diphenoxylate, respectively. Since the cut-off level of morphine/opiates was 300 ng mL^{-1}, a standard by the American National Institute on Drug Abuse, this MECC–UV microfluidic system coupled with liquid-liquid extraction pretreatment showed enough sensitivity for screening of narcotic drugs in urine samples.

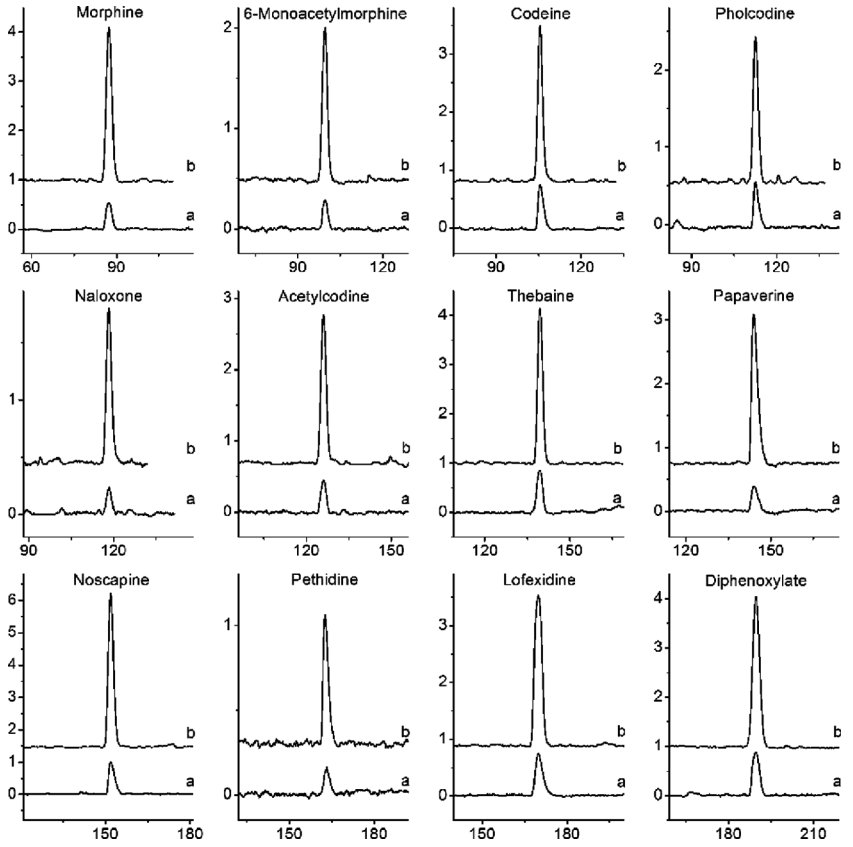

Figure 6. Electropherograms for 12 narcotic drug-positive urine samples (a) before and (b) after spiking 2.5 μg mL^{-1} drugs, respectively.

Figure 6 shows the electropherograms for analysis of the 12 narcotic drug-positive urine samples, which was confirmed by gas chromatography/mass spectrometry. Electropherograms (a) present the detection of original urine samples, while (b) present the samples spiked with $2.5\,\mu g\,mL^{-1}$ of the individual standard solutions of drugs. According to the enhanced absorption peaks at unchangeable retention times and the peak areas, the samples were identified and measured to contain 538, 540, 1039, 1090, 495, 686, 883, 508, 732, 616, 630, and $1014\,ng\,mL^{-1}$ of morphine, 6-monoacetylmorphine, codeine, pholcodine, naloxone, acetylcodeine, thebaine, papaverine, noscapine, pethidine, lofexidine, and diphenoxylate, respectively. The recoveries of the $2.5\,\mu g\,mL^{-1}$ spiked drugs were 100.8%, 98.8%, 102.8%, 96.0%, 96.8%, 101.2%, 95.6%, 100.8%, 96.4%, 100.4%, 97.2%, and 99.2%, respectively, indicating accuracy for identification and quantification of narcotic drugs in human urine samples with the proposed MECC method on the microfluidic device.

CONCLUSIONS

The present work outlined a successful development and application of the MECC–UV method for simultaneous separation of 12 narcotic drugs on a microfluidic device. Combined with the advantages of quartz capillary and MECC, high-performance separation and fast screening of narcotic drugs were obtained on the designed microfluidic device with high sensitivity, good repeatability and wide linear ranges. With the aid of liquid-liquid extraction, this novel protocol was successfully used to screen narcotic drugs in human urine samples. This MECC-UV microfluidic system could be used as a rapid, sensitive, reliable, and low-cost method for routine monitoring of narcotic drugs in clinical and forensic analysis.

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