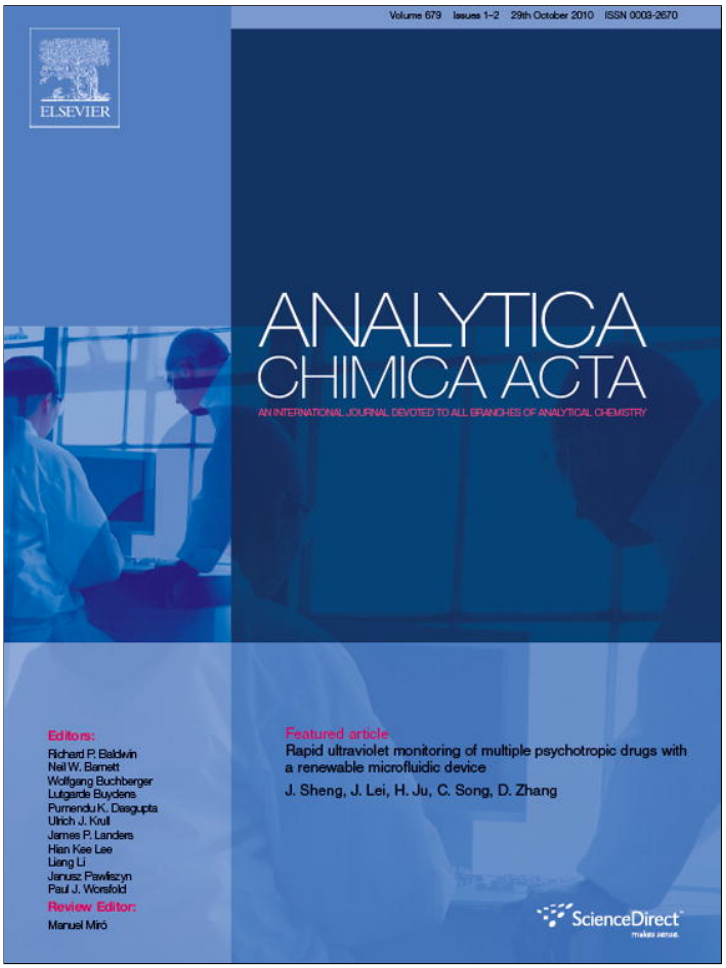

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Rapid ultraviolet monitoring of multiple psychotropic drugs with a renewable microfluidic device

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ABSTRACT

A rapid method for sensitive ultraviolet detection of multiple psychotropic drugs in human plasma was developed on a low-cost and expediently fabricated hybrid microfluidic device. The device was composed of one fused-silica capillary with a sampling fracture, a poly(methyl methacrylate) board with four reservoirs, and a printed circuit board. At the optimal separation and detection conditions, the baseline separation of three kinds of psychotropic drugs including barbiturates (phenobarbital and barbital), benzodiazepines (nitrazepam, clonazepam, chlordiazepoxide, alprazolam and diazepam) and tricyclic antidepressant drugs (amitriptyline) was achieved within 200 s with separation efficiency up to 3.80×10^5 plates m^{-1}. The linear ranges for ultraviolet detection were from 2.0 to 1000.0 $\mu g\,mL^{-1}$ for chlordiazepoxide and 1.0 to 1000.0 $\mu g\,mL^{-1}$ for other seven drugs. Combining with solid-phase extraction, this novel protocol could successfully be used to screen naturally existing psychotropic drugs in a known human plasma sample. The minimum detectable concentration was down to 27 $ng\,mL^{-1}$ for phenobarbital spiked in plasma. This work provided a promising way to initially screen different psychotropic drugs with high resolution, rapid separation and low-cost.

1. Introduction

Analysis of psychotropic drugs in human plasma is very important for therapeutic drug monitoring and forensic toxic judging [1–3]. The quantification and identification of psychotropic drugs have attracted considerable attentions in practical, social and environmental aspects [4]. Numerous methods based on liquid chromatography [5,6], spectrophotometry [7], immunoassay [8], electrochemistry [9,10], gas chromatography [11,12] and electrophoresis [13,14] have been developed for analysis of psychotropic drugs. Immunoassay method, such as capillary electrochemical enzyme immunoassay [8], needs specific antibodies of the analytes and relatively long analytical time. Although electrochemical biosensors show high sensitivity, great simplicity and inherent miniaturization, the narrow linear ranges limit their practical application [10]. High performance liquid chromatography has been considered as an efficient technique for separation and detection of these drugs [15,16], however, the high cost, polluting organic running phases and time-consuming analytical procedures prevent its prevalent applications [17,18]. More recently, an immunoassay for a major urinary metabolite of clonazepam has been reported on a microfluidic chip [19]. Therefore it is imperative to develop a rapid, reliable, sensitive, convenient and low-cost method for analyzing psychotropic drugs.

Recently, microfluidic electrophoresis device (MED) has been developed as a powerful tool for analytical purpose due to its small consumption of reagents, short separation time, and high separation efficiency [20–22]. Furthermore, the fabrication of MED has also been simplified by using polymer materials such as polydimethylsiloxane [23], polymethylmethacrylate (PMMA) [24], or polycarbonate [25] as matrices and capillaries as microchannels [26,27]. With the capillary microchannel and a fracture sampling technique, our previous work designed a hybrid quartz capillary/PMMA microfluidic device (HMD) and developed a method for the screening of illicit drugs in human urine with ultraviolet (UV) detection coupling with liquid–liquid extraction [27]. However, different analytes have generally different affinities to the hydrophobic interior of the micelle, the micellar conditions need to be optimized for efficient separation and detection of other drug analytes.

Here, eight psychotropic drugs, including three different kinds: barbiturates, benzodiazepines and tricyclic antidepressants (Fig. 1), were selected as the analytes, and sodium dodecyl sulfate (SDS) was used as surfactant to form micelles. By combining the advantages

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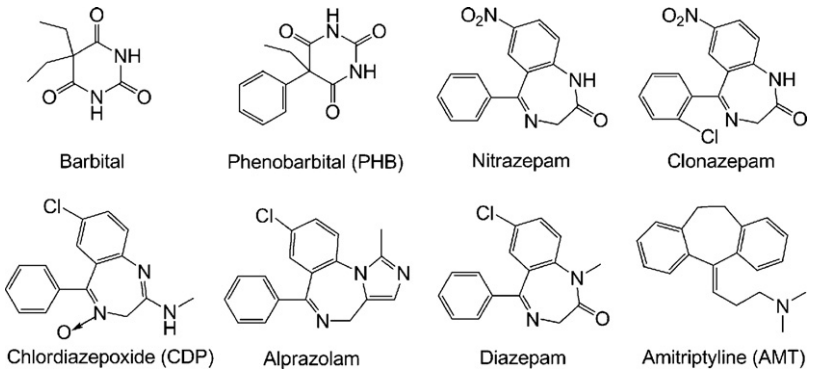

Fig. 1. Chemical structures of the selected psychotropic drugs.

of the HMD, the baseline separation of μg mL^{-1} concentrations of these psychotropic drugs was achieved within several minutes. The UV detection showed wide linear ranges. To our best knowledge, this was the first microfluidic technique for the separation and detection of psychotropic drugs. Furthermore, coupling with solid-phase extraction, this novel protocol could be successfully used to screen psychotropic drugs in human plasma. This proposed method provided a promising choice for therapeutic drug monitoring.

2. Experimental

2.1. Chemicals and materials

The psychotropic drugs including phenobarbital (PHB), barbital, nitrazepam, clonazepam, chlordiazepoxide (CDP), alprazolam, diazepam and amitriptyline (AMT) were obtained from Beijing Municipal Public Security Bureau. All aqueous solutions were prepared using ≥18 MΩ ultrapure water (Milli-Q, Millipore). Standard solutions of drugs were prepared in water at concentrations of 10.0 mg mL^{-1}, and stored at 4 °C. The electrophoresis buffer composed of 5.0 mM borax (pH 9.5), 30.0 mM SDS, 1.0 M urea and 7.0% (v/v) 1-butanol was dealt with ultrasonic for removing air bubbles prior to use. The phosphate buffer (0.2 M, pH 6.0) used during solid-phase extraction was prepared by mixing 0.2 M sodium dihydrogen phosphate with 0.2 M disodium hydrogen phosphate. Plasma samples were collected from Beijing Municipal Public Security Bureau, in which the presence of barbital (male, age 36), nitrazepam (female, age 28) and AMT (male, age 34) were confirmed, respectively. The samples were stored at −20 °C before analysis. All other chemicals were of analytical grade. Fused-silica capillaries (360 μm o.d., 50 μm i.d.) were obtained from Yongnian Optical Fiber Factory (Hebei, China).

2.2. Equipments

The UV microfluidic workstation employed throughout this work and the fracture checkout apparatus were home-manufactured by cooperation with Beijing Cailu Scientific Instrument Limited Company. The workstation was composed of an eight-port high-voltage power supply, a UV detector and data processor [27]. The high-voltage modules enabled real-time current and voltage monitoring. Ultrasonic disintegrator with a 2-mm o.d. probe from Ningbo Scientz Biotechnology Co., Ltd. (Ningbo, China) was used to prepare the sampling fracture. Solid-phase extraction (SPE) was carried out on Gilson ASPEC XL (USA) using disposable Agilent AccuBOND ODS-C18 cartridges (500 mg, 3 mL, Beijing, China).

2.3. Fabrication of HMD

The fabrication of HMD was developed according to our previous procedure [27]. In brief, a printed circuit board (PCB, 85 mm × 35 mm × 1 mm for length × width × thickness) and a PMMA board (85 mm × 10 mm × 2 mm for length × width × thickness) with a groove (75 mm × 0.5 mm × 1.5 mm for length × width × depth) were firstly prepared with simple hot embossing [28], and then four holes were drilled on the PMMA board as buffer reservoir (BR, 3-mm i.d.), sample reservoir (SR, 3-mm i.d.), detection reservoir (DR, 2-mm i.d.) and waste reservoir (WR, 4-mm i.d.), respectively. The resulting PMMA board was glued on the PCB with the groove outside for fixing a pretreated fused-silica cappilary, and E1, E2 and E3 on PCB were connected with BR, SR and WR for applying separation and sampling voltages, respectively (Fig. 2A). After a 7.5-cm length capillary was scored with potsherd at 0.5 cm from one end and the polymer coat at 0.6 cm from another end was removed for UV detection, it was fixed on the groove. After the microfluidic device immerged into water and an ultrasonic probe was positioned over the capillary, the sampling fracture was formed at the score by the ultrasonic probe at the optimal conditions of 150 W with a pulse time of 2 s at a distance of 2 mm for 12 times [29,30]. With power greater than 200 W or the distance <1.5 mm, the capillary could be broken into pieces; while greater distance or less power could not form the fracture. The resulting fracture was

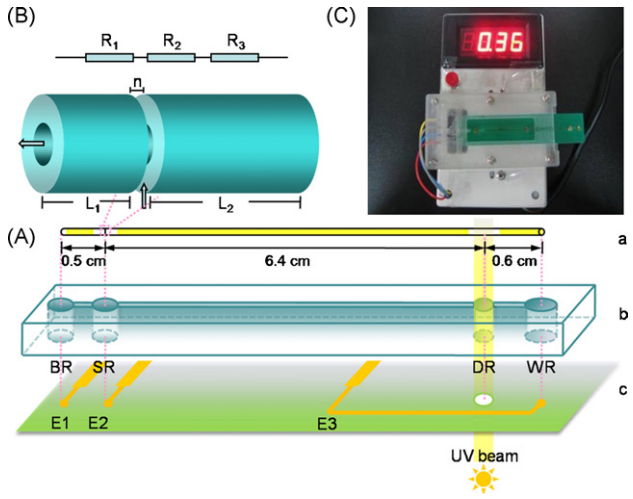

Fig. 2. (A) Scheme of the HMD: (a) the fused-silica capillary as separation and detection channels, (b) PMMA as support substrate, (c) PCB for high-voltage supply. BR, buffer reservoir; SR, sample reservoir; DR, detection reservoir; WR, waste reservoir; E1, E2 and E3, electrodes for applying sampling and separation voltages. (B) A simple equivalent circuit of the sampling fracture. (C) Sampling fracture test with a fracture checkout apparatus.

evaluated on a home-manufactured fracture checkout apparatus by applying a fixed voltage from E1 to E3 in 0.1 M phosphate buffer (pH 7.0). If the channel or fracture was blocked, the observational separation channel voltage at E1 to E3 or the partial voltage of sampling channel at E1 to E2 would be zero; otherwise, a designable value should be observed.

2.4. Sample pretreatment and electrophoresis procedure

The sample pretreatment procedure was carried out by means of solid-phase extraction, which took about 30 min for all steps from centrifugation to sample drying and resuspension. The plasma sample (2.0 mL) was first diluted with 6.0 mL of 0.2 M phosphate buffer, and centrifugated at 4000 rpm for 3 min at room temperature. The cartridge was activated and conditioned by sequential passage of 3.0 mL methanol, 3.0 mL ultrapure water, and 3.0 mL 0.2 M phosphate buffer (pH 6.0) at a rate of 5.0 mL min^{-1}. Then the upper solution of the sample was loaded onto the conditioned cartridge. The cartridge was then sequentially washed with 3.0 mL 0.2 M phosphate buffer (pH 6.0), 3.0 mL distilled water and 3.0 mL chloroform/acetone (50/50, v/v) mixture. Analyte elution was washed out with 3.0 mL ammonia/chloroform/isopropanol (2/78/20, v/v/v). The elution solvent was evaporated to dryness under nitrogen stream at 50 °C and the residue was re-dissolved in 100 μL electrophoresis buffer for analysis.

The electrophoresis buffer was composed of 5.0 mM borax (pH 9.5), 30.0 mM SDS, 1.0 M urea and 7.0% (v/v) 1-butanol. In the experiments, the separation channel was firstly rinsed with the electrophoresis buffer for several seconds with the aid of a vacuum pump, and then the sample reservoir was filled with 12.5 μL testing sample. The injection was performed by applying 90 V from E2 to E1 for 2 s with E3 floating, and the separation was performed by applying 1400 V from E1 to E3 with E2 floating.

3. Results and discussion

3.1. Properties of the HMD and examination of the fracture

UV detection is suitable for the determination of psychotropic drugs due to their UV absorption at 200 nm. The fused-silica capillary could provide a channel for separation and a window without cladding at DR for UV detection of these drugs. A fracture could be conveniently formed on the fused-silica capillary for sampling with help of a relatively low sampling voltage. Besides, due to the little dosage of analyte and miniaturization of separation channel, the capillary channel and sampling fracture could be neatly rinsed with the electrophoresis buffer via pump for next detection. That is, SR was firstly cleaned with electrophoresis buffer by filling in and sucking out for 3 times, and then the separation channel and sampling fracture were rinsed by pumping electrophoresis buffer into BR and SR at WR for several seconds. This proposal was more convenient and time saving than ordinary capillary electrophoresis. By combining solid-phase extraction with facture sampling, the rapid and sensitive UV detection of psychotropic drugs could be achieved on the renewable hybrid microfluidic device.

The home-made fracture checkout apparatus was used to examine the capability of the fracture based on the theory of a simple equivalent circuit model [31]. As described in Fig. 2B, the length L_1 and L_2 of microchannels were defined as resistor R_1 and R_3, while the width (n) of the fracture was as resistor R_2. The wider fracture showed smaller R_2 value, and thus the partial voltage of sampling channel would be smaller. A successful fracture generally showed the voltage of 0.35–0.38 V as shown in Fig. 2C, otherwise, a voltage of 0.25–0.27 V was observed. Only one device of 50 devices prepared in optimal conditions showed the voltage of 0.26 V, indicating an excellent reproducibility.

3.2. Optimization of electrophoresis buffer

The concentration of SDS played an important role in the separation and was chosen between 20.0 and 50.0 mM. When the concentration was lower than 20.0 mM, the analytical results showed poor resolution, stability and repeatability. With the increasing concentration of SDS, the retention times of analytes increased. Although the resolution also increased at low concentration, it decreased rapidly at the concentrations larger than 30.0 mM owing to the sample zone diffusion caused by longer migration-time and the Joule heat effect (Fig. 3A). Thus 30.0 mM SDS was added in the electrophoresis buffer, at which the resolution was good enough for the separation of eight psychotropic drugs.

The effect of borax concentration (ionic strength) on separation efficiency was shown in Fig. 3B. When its concentration was <5.0 mM, the buffer capacity was not enough for keeping a stable baseline. With the increasing borax concentration, the electrical double-layer became thinner. It might be one reason that the electroosmotic flow (EOF), which results from the ionization of the acidic silanol groups on the inside of the capillary and adsorption of cations near the negative charged surface [32], was suppressed [33], leading to larger migration time and broadened sample zone. Moreover, at the separation voltage of 1400 V, the current through the separation channel was 6.5, 8.3 and 10.3 μA for 5.0, 10.0 and 15.0 mM borax, respectively, indicating that high concentration produced large Joule heat ($P = VIt$, where P is the power, I is the current, V is the applied voltage, and t is the applied time). The non-linearly increased current could affect the micelle formation at different borate concentrations. Considering the analytical time, separation stability and buffer capacity, 5.0 mM of borax was used as the optimal concentration.

Urea, as a solubilization additive, was used to increase the dipolar selectivity of the electrophoresis buffer for the separation of hydrophobic analytes [34]. Thus it was added into the electrophoresis buffer to improve the separation of these drugs. As shown in Fig. 3C, the optimal concentration of urea was at 1.0 M. At other urea concentrations these eight psychotropic drugs could not be completely separated.

1-Butanol, an organic additive, could expand the migration-time window, alter separation selectivity, achieve gradient elution and even improve the peak shape in the separation of these drugs. When the concentration of 1-butanol rose up to 7.0%, all analytes could be well separated with the lowest resolution of 1.10 (Fig. 3D). Higher concentration of 1-bunotal, beyond the miscibility limit of 1-bunotal with aqueous phase, led to unstable baseline and thus decreased the resolution. Thus, the optimal electrophoresis buffer was composed of 5.0 mM borax (pH 9.5), 30.0 mM SDS, 1.0 M urea and 7.0% 1-butanol.

3.3. Separation voltage and sampling conditions

The separation voltage altered the electric field and EOF, and affected the migration-time (t_M) and separation efficiency. Low separation voltage resulted in long t_M due to the low-lying electrophoresis and EOF, while high separation voltage led to high Joule heat in separation channel, thus lowered the separation efficiency. At the separation voltages of 1000, 1200, 1400 and 1600 V the currents were 4.7, 5.6, 6.5 and 8.1 μA, respectively. To evaluate the effect of separation voltage, the resolution (R_s) between two adjacent drugs and the t_M of each drug were measured as the function of the separation voltage between 1000 and 1600 V (Fig. 4). Here R_s is defined as $2((t_M)_B - (t_M)_A)/(W_B + W_A)$, where W is the full peak width [35]. At the separation voltage of 1400 V, eight analytes could

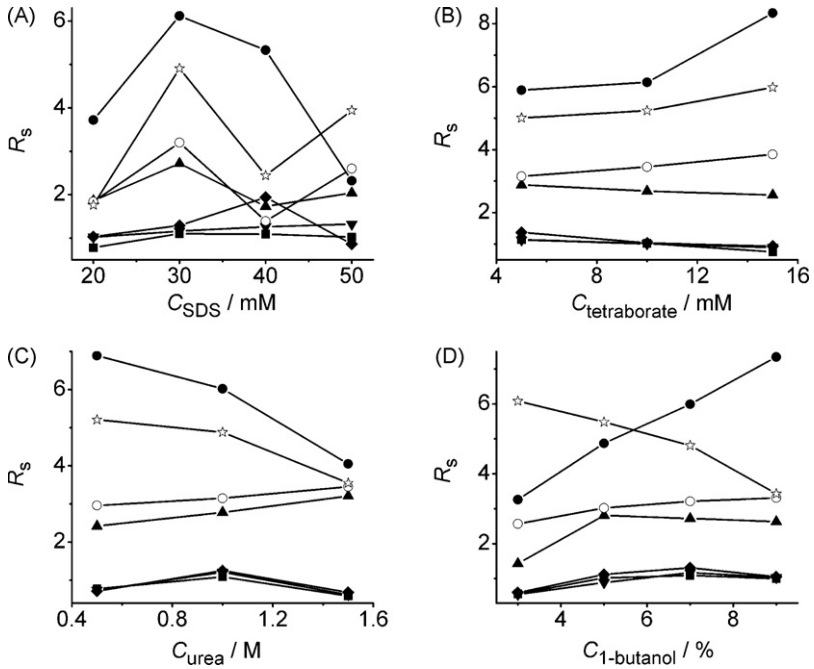

Fig. 3. Effects of (A) SDS, (B) borax, (C) 1-butanol and (D) urea concentrations on resolution for eight psychotropic drugs (■ PHB–barbital, ● barbital–nitrazepam, ▲ nitrazepam–clonazepam, ▼ clonazepam–CDP, ♦ CDP–alprazolam, ○ alprazolam–diazepam, ☆ diazepam–AMT). Separation voltage: 1400 V; sampling voltage: 90 V for 2 s. When changing one parameter, others are at their optimal concentrations.

be completely separated with R_S values more than 1.10, at which the total separation time was <200 s. Thus, it was selected as separation voltage to obtain fast separation and acceptable resolution.

The sampling voltage and time influenced the detection sensitivity and separation efficiency. The peak area of each drug and the R_S value between two adjacent drugs were measured as functions of sampling conditions. At the sampling voltages of <90 V, a longer time was needed to obtain enough sensitivity. At the sampling voltage of 90 V, the peak height turned out to be a constant value after 2 s. Although high sampling voltage and long sampling time led to large peak area and highly sensitive detection, serious broadening of sample zone resulted in low separation efficiency. Considering both the sensitivity and the separation efficiency, a sampling time of 2 s at 90 V was used for detection of psychotropic drugs. Both the low sampling voltage and the relatively low separation voltage would benefit the design of low-cost power for separation of psychotropic drugs.

3.4. Separation of psychotropic drugs

Under optimal conditions the electropherogram for eight psychotropic drugs at 10.0 μg mL^{-1} was shown in Fig. 5. The analytes were separated due to their different affinities to the hydrophobic interior of the micelle [36]. The R_S values for PHB–barbital, barbital–nitrazepam, nitrazepam–clonazepam, clonazepam–CDP, CDP–alprazolam, alprazolam–diazepam, diazepam–AMT were

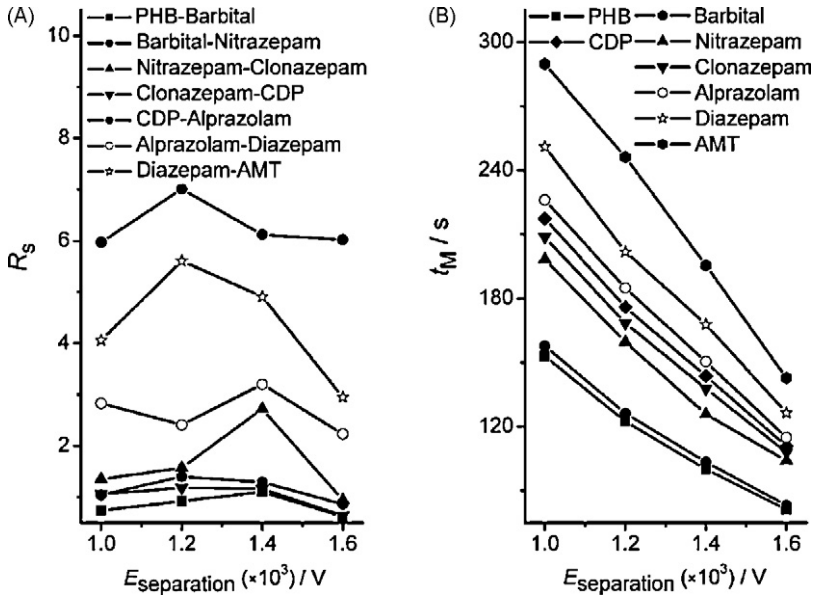

Fig. 4. Effects of separation voltage on (A) resolution and (B) migration-time for eight psychotropic drugs at 10.0 μg mL^{-1} with a sampling voltage of 90 V for 2 s.

Table 1
Separation efficiency and reproducibility of the HMD for psychotropic 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
PHB	3.09	1.10	0.6	1.1	0.8	0.8	2.0	1.2
Barbital	3.39	6.12	1.1	3.9	1.5	3.3	6.8	4.1
Nitrazepam	2.95	2.72	0.8	2.5	0.9	2.7	4.6	3.5
Clonazepam	2.72	1.16	0.9	2.7	1.1	3.2	7.4	4.3
CDP	2.32	1.29	1.2	2.6	1.4	2.0	4.2	3.1
Alprazolam	2.61	3.20	0.4	3.2	0.7	2.6	5.3	3.4
Diazepam	2.80	4.91	0.9	1.5	1.3	2.3	6.2	3.2
AMT	3.80	–	0.7	2.5	1.2	2.2	7.1	3.3

Table 2
Linear ranges and detection limits of the HMD for psychotropic drugs.

Analyte	Linear ranges (μg mL^{-1})	Detection limit (μg mL^{-1})	Extraction efficiency (%)	Minimum detectable concentration in plasma (ng mL^{-1})
PHB	1.0–1000.0	0.486	90.4 ± 3.3	27
Barbital	1.0–1000.0	0.699	71.1 ± 2.8	49
Nitrazepam	1.0–1000.0	0.588	76.0 ± 3.7	39
Clonazepam	1.0–1000.0	0.565	72.2 ± 2.1	39
CDP	2.0–1000.0	1.186	88.5 ± 2.6	67
Alprazolam	1.0–1000.0	0.678	66.7 ± 3.1	51
Diazepam	1.0–1000.0	0.625	98.0 ± 2.5	32
AMT	1.0–1000.0	0.897	87.3 ± 2.2	51

greater than 1.10 with a maximal value of 6.12. The baseline separation could be completed within 200 s, which was obviously shorter than 12 min for capillary electrophoresis [37] and 15 min for high performance liquid chromatography [15]. The excellent performance in separation enhanced greatly the ability of the HMD to analyze complex psychotropic samples in practical application.

The theoretical plate numbers (N) and separation reproducibility of these psychotropic drugs were summarized in Table 1. Here, N is defined as 5.54 $(t_M/W_{1/2})^2$, and $W_{1/2}$ is the peak width at the half-maximum points [38]. The N values ranging from 2.32×10^5 to 3.80×10^5 plates m^{-1} showed good separation efficiency of the proposed HMD. The relative standard deviations (RSDs) (n = 10) of t_M were <1.2% for run-to-run, 1.5% for day-to-day and 3.9% for device-to-device, indicating acceptable separation and fabrication reproducibility of the HMD. The RSDs of peak areas measured at the concentration of 10 μg mL^{-1} analytes (n = 10) were from 0.8% to 3.3% for run-to-run, from 1.2% to 4.3% for day-to-day and from 2.0% to 7.4% for device-to-device. These results showed that the designed HMD exhibited the promising application for identification and quantification in the initial screening of psychotropic drugs.

The calibration curves for UV detection of psychotropic drugs in electrophoresis buffer showed the linear ranges from 1.0 to 1000.0 μg mL^{-1} for PHB, barbital, nitrazepam, clonazepam, alprazolam, diazepam and AMT, and 2.0 to 1000.0 μg mL^{-1} for CDP with the relative coefficients ranging from 0.9996 to 0.9999 (Table 2). The upper limit was much higher than 3 μg mL^{-1} of PHB for capillary electrochemical enzyme immunoassay [8] and 0.58 μg mL^{-1} of PHB for cytochrome P450 2B4 modified biosensor [10]. The detection limits at the signal-to-noise ratio of 3 were 0.486, 0.699, 0.588, 0.565, 1.186, 0.678, 0.625, and 0.897 μg mL^{-1} for PHB, barbital, nitrazepam, clonazepam, CDP, alprazolam, diazepam and AMT, respectively.

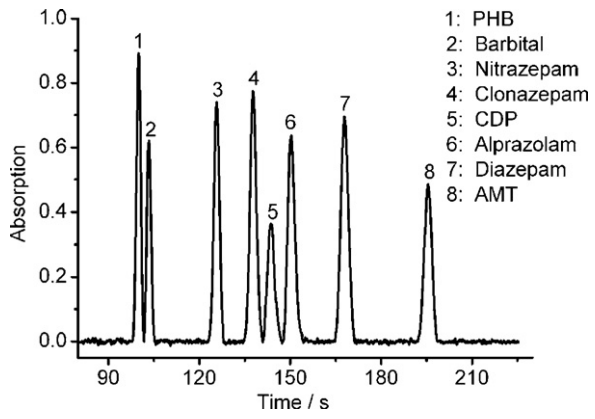

Fig. 5. Electropherogram for eight psychotropic drugs at 10.0 μg mL^{-1} in 5.0 mM borax buffer (pH 9.5) containing 30.0 mM SDS, 1.0 M urea and 7.0% 1-butanol. Separation voltage: 1400 V; sampling voltage: 90 V for 2 s.

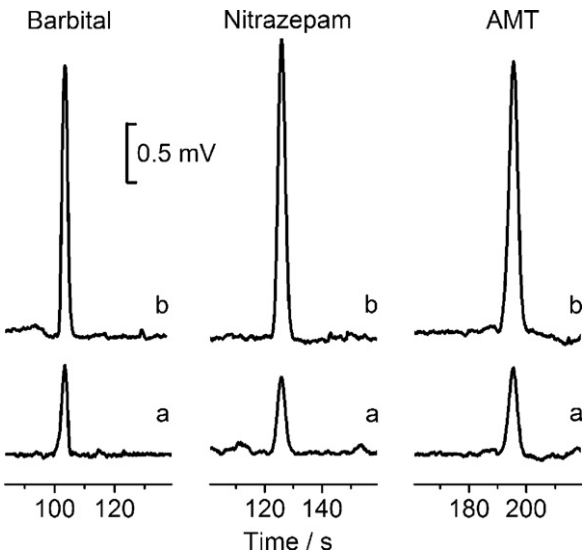

Fig. 6. Electropherograms for barbital-positive, nitrazepam-positive and AMT-positive plasma samples (a) before and (b) after spiking 2.2 μg mL^{-1} barbital, nitrazepam or AMT, respectively.

The interference experiment was carried out by adding another six commonly used psychotropic drugs (secobarbital, estazolam, imipramine, triazolam, doxepin and clozapine) into the testing solution containing the primary eight drugs. All resolutions were more than 1.0, indicating good separation.

3.5. Minimum detectable concentrations in real samples

Solid-phase extraction was employed to extract the plasma sample prior to analysis. The extraction efficiencies were evaluated by dividing the peak areas obtained after extraction in the spiked plasma with the peak areas obtained by direct injection of equal amount of the drugs in electrophoresis buffer (Table 2). Normal plasma sample did not show detectable peak after extraction. The values of extraction efficiency for spiked plasma samples were up to 98.0%, indicating that phosphate buffer was adequate for degrading the protein–drug complexes of plasma sample. Coupling with the preconcentration by extraction, the minimum detectable concentrations of psychotropic drugs in spiked plasma samples were 27, 49, 39, 39, 67, 51, 32 and 51 ng mL^{-1} for PHB, barbital, nitrazepam, clonazepam, CDP, alprazolam, diazepam and AMT at the signal-to-noise ratio of 3, respectively, which were lower than the cut-off value of 100 ng mL^{-1} for the clinical therapy and 500 ng mL^{-1} for toxic concentrations [39], demonstrating the compatibility of the microdevice with these commercial SPE cartridges. Thus, the designed HMD was suitable for screening of psychotropic drugs in plasma sample, indicating a promising choice for therapeutic drug monitoring.

3.6. Application in plasma samples

The designed method was used to analyze real human plasma samples, which had been individually confirmed as barbital-positive, nitrazepam-positive and AMT-positive by gas chromatography/mass spectrometry. Prior to analysis, the plasma samples were extracted by means of solid-phase extraction. Fig. 6 shows the electropherograms for analysis of these plasma samples. After spiking the standard solutions of barbital, nitrazepam and AMT into these samples, respectively, the electropherograms showed the enhanced absorption peaks at unchangeable retention times. From the peak areas, the values of extraction efficiency and the calibration curves, the concentrations of barbital, nitrazepam and AMT in the samples were measured to be 915, 700 and 803 ng mL^{-1} with the RSD (n = 5) of 3.5%, 3.1% and 2.8%, respectively. The concentrations of the spiked 2.2 μg mL^{-1} barbital, nitrazepam and AMT were measured to be 100%, 102.7% and 103.6% of the spiked concentrations, respectively. Thus, the designed microfluidic device and proposed method had good performance in the separation and detection of these psychotropic drugs in human plasma.

4. Conclusions

A rapid and low-cost method was developed for identification and quantification of psychotropic drugs on a renewable hybrid microfluidic device with UV detection. Three kinds of drugs including barbiturates (phenobarbital and barbital), benzodiazepines (nitrazepam, clonazepam, chlordiazepoxide, alprazolam and diazepam) and tricyclic antidepressant drugs (amitriptyline) were used as the analyte models. Coupling with the advantages of HMD and fracture sampling, eight psychotropic drugs could be rapidly separated with high resolution and wide linear ranges. With the aid of solid-phase extraction, this novel protocol was successfully used to initially screen psychotropic drugs in human plasma. Although a follow-up confirmation test is needed to definitively identify the drugs, this sensitive, rapid, reliable and low-cost approach could be used to analyze multiple psychotropic drugs and offered a powerful way for therapeutic drug monitoring. In future, integrating the extraction and concentration procedures on a chip could further reduce the analytic time and increase the detection sensitivity.

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