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Combining poly (methacrylic acid-co-ethylene glycol dimethacrylate) monolith microextraction and on-line pre-concentration-capillary electrophoresis for analysis of ephedrine and pseudoephedrine in human plasma and urine

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Abstract

A method based on poly (methacrylic acid-co-ethylene glycol dimethacrylate) (MAA-EGDMA) monolith microextraction (PMME) and field-enhanced sample injection (FESI) pre-concentration technique was proposed for sensitive capillary electrophoresis-ultraviolet (CE-UV) analysis of ephedrine (E) and pseudoephedrine (PE) in human plasma and urine. The PMME device consisted of a regular plastic syringe (1 mL), a poly (MAA-EGDMA) monolithic capillary (2 cm × 530 μm I.D.) and a plastic pinhead connecting the former two components seamlessly. The extraction was achieved by driving the sample solution through the monolithic capillary tube using a syringe pump, for the desorption step, an aliquot of organic solvent, which normally provided an excellent medium to ensure direct compatibility for FESI in CE, was injected via the monolithic capillary and collected into a vial for subsequent analysis by CZE. The best separation was achieved using a buffer composed of 0.1 M phosphate electrolyte (pH 2.5) and 10% acetonitrile (v/v). The combination of both pre-concentration procedures allowed the detection limits of the analytes down to 5.3 ng/mL and 8.0 ng/mL in human plasma and urine, respectively. Excellent method of reproducibility was found over a linear range 50–5000 ng/mL in plasma and urine sample. Plasma and urine samples from volunteers receiving pseudoephedrine have also been successfully analysed.

Keywords: Poly (methacrylic acid-co-ethylene glycol dimethacrylate) monolith microextraction; Capillary electrophoresis; On-line concentration; Ephedrine; Pseudoephedrine

1. Introduction

With the development of modern sports games and the increasingly outstanding problems of doping abusing, it is of great importance to develop the fast and highly sensitive screening method for doping control. Stimulants like ephedrine (E) and pseudoephedrine (PE) are pairs of diastereoisomeric sympathomimetic amines that have human central nervous system stimulating properties [1] and are therefore included in the doping list of pharmacological forbidden substances indicated by the medical commission of the international Olympic committee. An athlete may be suspected as "positive" if his/her urine is found to contain high concentrations of these stimulants (for example, more than 10 μg/mL of ephedrine [2]).

Gas chromatography (GC) [3–5] and high performance liquid chromatography (HPLC) [6,7] have often been used for the determination of ephedrine derivatives. Capillary electrophoresis (CE) has also been introduced as a powerful complementary new technique to HPLC and GC for the separation of ephedrine derivatives in recent years [8–14] due to its excellent separation efficiency, short analysis time, minimal need of samples and solvents and high versatility in terms of separation modes. However, one of the main drawbacks of CE is its low sensitivity in terms of solute concentration compared to other separation techniques, which is due to both the small optical path length of the capillary used as detection cell and the small volumes (usually a few nanoliters) that can be injected.

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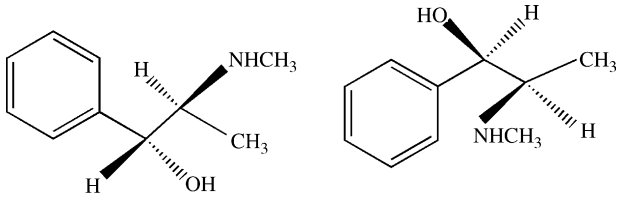
Thus various pre-concentration techniques, both electrophoresis-based and chromatography-based, were investigated to enhance the sensitivity [15–19]. Normal stacking mode (NSM), large volume sample stacking (LVSS) and field-enhanced sample injection (FESI) are the electrophoresis-based pre-concentration techniques most frequently used [20]. Among them, NSM is the simplest mode, which is done by hydrodynamic injecting large amounts of sample with electrical conductivity lower than that of the separation buffer. However, a limitation in NSM is the short optimum sample plug length that can be injected into the capillary without loss of separation efficiency. When the volume of sample introduced is greater than that found optimum in NSM (named as LVSS), the sample matrix must be pumped out from the capillary in order to preserve separation efficiency. Like NSM, LVSS is done by injecting the sample hydrodynamically but for a longer period of time. A polarity switching step has always been used to remove sample matrix in LVSS [21]. In order to eliminate the troublesome polarity switching step, LVSS without polarity switching has also been developed [22–26]. FESI is another widely used procedure for stacking, in which the sample (prepared in a low conductivity matrix) is injected using voltage [27]. However, stacking alone cannot improve the detection limits of CE when the analytes of interest are present in a complex matrix, e.g. biological and environmental samples, since real sample matrices often contain substances that would interfere with sample stacking, especially substances that increase the conductivity of the matrix. Thus, in dealing with real samples, a sample clean-up step before injection is inevitable to get rid of the sample matrix and enrich the analytes.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are chromatography-based pre-concentration techniques most commonly used for clean-up biological samples. However, for CE analysis, the analyte-containing extract obtained from LLE or SPE normally needs to be evaporated to dryness in a post-extraction step, and then reconstituted in a suitable medium, to ensure direct compatibility with the CE system [28,29]. These steps are tedious, time-consuming and also prone to loss of analytes. Solid-phase microextraction (SPME) was introduced as a simple, solvent-waste-free, time-efficient extraction technique, which can be combined off-line or on-line with CE [30–33]. As an attractive alternative method to conventional SPME, a novel poly (methacrylic acid-co-ethylene glycol dimethacrylate) (MAA-EGDMA) monolith microextraction (PMME) method was developed and successfully combined off-line with CE for analyzing real samples (i.e. urine samples) by our group recently [34,35]. In this work, the great possibilities of the combined use of PMME and on-line sample pre-concentration techniques was shown because only small volumes of organic solvent was needed for desorption of analytes in PMME, which provided an excellent medium to ensure direct compatibility for sample stacking in CE. Different on-line sample pre-concentration techniques such as NSM, LVSS and FESI are investigated. The best of these approaches is then combined with PMME and CE-UV for the analysis of ephedrine and pseudoephedrine (Fig. 1) in human plasma and urine at the low concentration level usually required.



Ephedrine Pseudoephedrine

Fig. 1. Structures of ephedrine and pseudoephedrine.

2. Experimental

2.1. Chemicals and materials

Ethylene glycol dimethacrylate (EGDMA) was purchased from Acros (Sweden). Methacrylic acid (MAA), 2,2′-azobis (2-methylpropionitrile) (AIBN), dodecanol and toluene were obtained from Shanghai Chemical Co. Ltd. (Shanghai, China) and were of analytical reagent grade. The poly (MAA-EGDMA) monolithic capillary tube was synthesized by a polymerization method described previously [36]. Double distilled water was used for all experiments.

Ephedrine, pseudoephedrine and berberine (used as internal standard, IS) were obtained from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). A stock standard solution of 1 mg/mL for each analyte was prepared in MeCN-H_2O (50:50, v/v). The composite standard containing 50 μg/mL of each analyte was prepared by diluting the stock solution with double distilled water.

2.2. Instrumental and analytical conditions

The configuration of the poly (MAA-EGDMA) monolith microextraction consists of a regular plastic syringe (1 mL), the poly (MAA-EGDMA) monolithic capillary tube (530 μm i.d. × 2 cm) and a plastic pinhead (one part of the whole syringe) [34]. One end of the pinhead coupled seamlessly with the syringe barrel, while on the other end of the pinhead, the metallic needles were removed and replaced by a 2 cm monolithic capillary tube (cut from the prepared monolithic capillary) with adhesive.

The CE analysis was performed on a CAPEL 105 CE system (LUMEX, Russia) equipped with a UV-Vis detector. Separations were carried out in an uncoated fused silica capillary (Yongnian Fiber Plant, Hebei, China) of 50 μm i.d. and an effective length of 51 cm (total length 60 cm). Data collection and manipulation were carried out on Chrom&Spec software for chromatography, Version 1.5 × (Ampersand). Before use, the capillary was rinsed sequentially with 1 mol/L NaOH (15 min), water (15 min), 1 mol/L HCl (15 min) and water (15 min), followed by conditioning with buffer for 15 min. Between runs, the capillary was rinsed sequentially with 1 mol/L NaOH (2 min), water (2 min) and running buffer (2 min). The UV absorbance detection was performed at 214 nm. The CZE system was operated using "normal" polarity (the cathode was located on the detector side). And all the separations were run at 25 °C at a constant voltage of 25 kV.

In NSM, the sample was injected at 30 mbar for 60 s; in FESI, the sample was injected by applying a voltage (10 kV) for 5 s. And separations were carried out using a running buffer composed of 0.1 M phosphate electrolyte at pH 2.5 and 10% acetonitrile (v/v). In LVSS without polarity switching, the sample was injected at 30 mbar for 300 s with the sample in the inlet position and then voltage (25 kV) applied with the electrophoresis buffer (0.1 M phosphate electrolyte with 2 mM CTAB at pH 1.9 and 20% acetonitrile v/v) at both ends of the capillary. Before use, all the electrolyte solutions were filtered through a 0.45 μm microfilter and degassed in an ultrasonic bath for 10 min. Sample plug lengths were estimated using the time in which a marker reached the detector window of the capillary with 30 mar pressure.

2.3. Poly (MAA-EGDMA) monolith microextraction procedure

The whole PMME procedure composed of precondition, sample loading, washing and desorption [34]. A programmable syringe pump (CP 2000, Silugao high-technology development Co. Ltd., Beijing, China) was employed for the delivery of solutions for PMME. For precondition, the syringe was filled with 0.3 mL methanol, which was then ejected via the monolithic capillary tube at 0.1 mL/min by the syringe pump, and then 0.2 mL phosphate buffer (10 mM, pH7.5) was ejected at 0.2 mL/min. After that, the sample solution was ejected at 0.2 mL/min in the same way. In order to eliminate the residual sample solution and the adsorbed sample matix, 0.1 mL phosphate buffer was kept to flow through the monolithic capillary tube at 0.2 mL/min. Then the residual solution in the pinhead and monolithic capillary tube was pushed out with an empty and clean syringe to avoid polluting the eluate. For desorption, 0.1 mL MeCN-HAc (100:0.2, v/v) was injected via the monolithic capillary tube at 0.1 mL/min and the eluate was collected into a vial for the subsequent analysis by CZE. For avoiding contamination, special syringe was used for injecting sample, buffer and desorption solution, respectively.

2.4. Sample preparation

Plasma and urine samples were collected from drug-free healthy volunteers. The plasma sample was centrifuged at 16,000 rpm for 5 min and the urine sample was centrifuged at 5000 rpm for 5 min to remove any precipitated materials. Ephedrine and pseudoephedrine were directly spiked into the supernatant of the biological samples. The plasma samples were diluted five times with 20 mM phosphate buffer (pH 7.5), and the urine samples were diluted with an equal volume of 20 mM phosphate buffer (pH 7.5). The obtained sample at the concentration range 50–5000 ng/mL was used for extraction. Berberine stock solution was diluted in water to the desired concentration and added to the eluate before CE analysis to minimize the variation resulted from the electrokinetic injection and the fluctuation of the electroosmotic flow. The plasma and urine samples from volunteers receiving pseudoephedrine were prepared in the same way without spiking. Triplicate injections of the sample were performed and relative peak areas (analyte area/berberine area) were used for quantification.

3. Results and discussion

3.1. Sample stacking techniques using normal polarity

In order to obtain a sample matrix with low conductivity and to provide a sensitivity increase as large as possible in NSM, different solutions containing MeCN-HAc (100:0.2, v/v), MeCN-H_2O-HAc (80:20:0.2, v/v/v) and MeCN-H_2O-HAc (50:50:0.2, v/v/v) were examined, since much acetonitrile will be beneficial for the complete desorption during PMME and the eluate was injected directly for CE analysis. It was observed that MeCN-HAc (100:0.2, v/v) and MeCN-H_2O-HAc (80:20:0.2, v/v/v) were precluded due to current breakdowns with short injection time (below 15 s). Thus, MeCN-H_2O-HAc (50:50:0.2, v/v/v) was used as sample matrix for injection. By using this solution, the sample could be injected in the capillary up to 60 s at 30 mbar (ca. 4% of the total volume of the capillary).

LVSS without polarity switching has also been used in the present work. The optimized buffer for LVSS and separation is composed of 0.1 M phosphate solution (pH 1.9) with 2 mM CTAB and 20% acetonitrile (v/v). After the sample in a low conductivity matrix was injected, the application of voltage will cause focusing of the sample ions at the concentration boundary, while sample matrix is removed by the slow anodic EOF [26]. Several sample matrices containing MeCN-HAc (100:0.2, v/v), MeCN-H_2O-HAc (80:20:0.2, v/v/v), and MeCN-H_2O-HAc (50:50:0.2, v/v/v) were tested. Current breakdowns for longer injections were observed with the matrix of MeCN-HAc (100:0.2) and MeCN-H_2O-HAc (80:20:0.2, v/v/v). Thus, MeCN-H_2O-HAc (50:50:0.2, v/v/v) was used as sample matrix for injection. Injection time was optimized as 300 s at 30 mbar (ca. 20% of the total volume of the capillary). Longer injections were not possible due to loss of current and resolution [26].

In FESI, only charged analytes or neutral analytes associated to micelles can be concentrated by electrokinetic injection. Therefore, the sample matrix should have a low conductivity and bring about simultaneous protonation of the solutes (pKa of ephedrine is around 9.6 [37]). Different solutions containing MeCN-HAc (100:0.2, v/v), MeCN-H_2O-HAc (80:20:0.2, v/v/v) and MeCN-H_2O-HAc (50:50:0.2, v/v/v) were examined. It was found that the highest signal/noise ratio was yielded with the solution of MeCN-HAc (100:0.2), probably because this solution provides the best conditions for solute ionization together with low conductivity of the sample matrix. Samples could be electrokinetically injected up to 5 s at 10 kV under these conditions. A decrease of resolution was observed with longer injection time.

Table 1 shows the main figures of merit of NSM, LVSS and FESI using the optimum conditions, including resolution, efficiency of peaks, and the increase in sensitivity for ephedrine and peseudoephedrine (0.5 mg/L) compared to that without stacking. As can be concluded by comparing the three procedures, in general, FESI provided the best results in terms of sensitivity improvement (more than 200 compared to that without stacking), separation efficiencies (above 175,000) and resolution (1.65). The electropherograms obtained after stacking of 0.5 mg/L sample by means of NSM, LVSS and FESI are shown

Table 1
Figures of merit of the CE-UV analysis of ephedrine (E) and pseudoephedrine (PE) using different stacking techniques

		Compound	
		E	PE
NSM	Rs(n, $n+1$)	1.83	
	NTP/m[a]	239000	291000
	Fold[b]	19	18
LVSS	Rs(n, $n+1$)	2.44	
	NTP/m[a]	89000	96000
	Fold[b]	62	60
FESI	Rs(n, $n+1$)	1.65	
	NTP/m[a]	175000	234000
	Fold[b]	220	215

Data given for 0.5 mg/L.
[a] Number of theoretical plates per meter of column.
[b] Increase in sensitivity calculated by comparing the peak height to that without stacking.

in Fig. 2B, C and D, respectively. For comparison, Fig. 2A shows the electropherogram obtained by injecting 50 mg/L sample without stacking. As it can be seen, FESI clearly provides better sensitivity than the other procedures. And this procedure was selected for the subsequent experiments.

3.2. Optimization of the poly (MAA-EGDMA) monolith microextraction conditions

Several factors that influence the microextraction efficiency such as desorption conditions, extraction equilibrium profiles, the pH and salt concentration of the sample matrix were optimized.

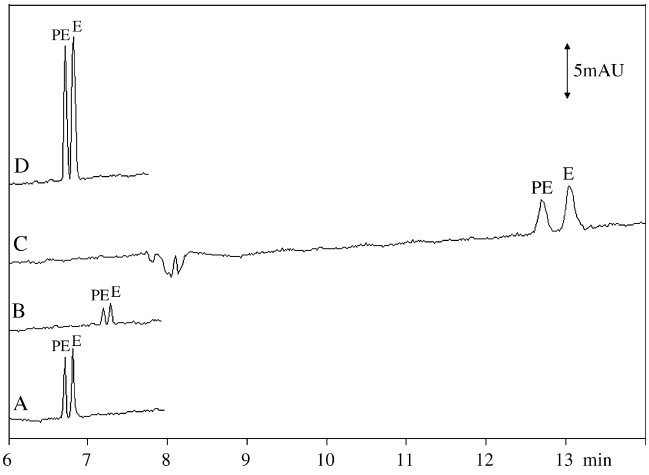


Fig. 2. CE analysis of ephedrine and pseudoephedrine carried out with: (A) no stacking; (B) NSM stacking; (C) LVSS; and (D) FESI stacking. Carrier electrolyte for (A); (B); and (D): 0.1 M phosphate electropholyte (pH 2.5)/10% acetonitrile; and for (C) 2 mM CTAB in 0.1 M phosphate solution (pH 1.9)/20% acetonitrile. (A) No stacking: 50 mg/L sample in MeCN, hydrodynamic injection for 5 s at 30 mbar. (B) NSM stacking: 0.5 mg/L sample in MeCN-H_2O-HAc (50:50:0.2, v/v/v), hydrodynamic injection for 60 s at 30 mbar. (C) LVSS: 0.5 mg/L sample in MeCN-H_2O-HAc (50:50:0.2, v/v/v), hydrodynamic injection for 300 s at 30 mbar. (D) FESI stacking: 0.5 mg/L sample in MeCN-HAc (100:0.2, v/v), electrokinetic injection for 5 s at 10 kV.

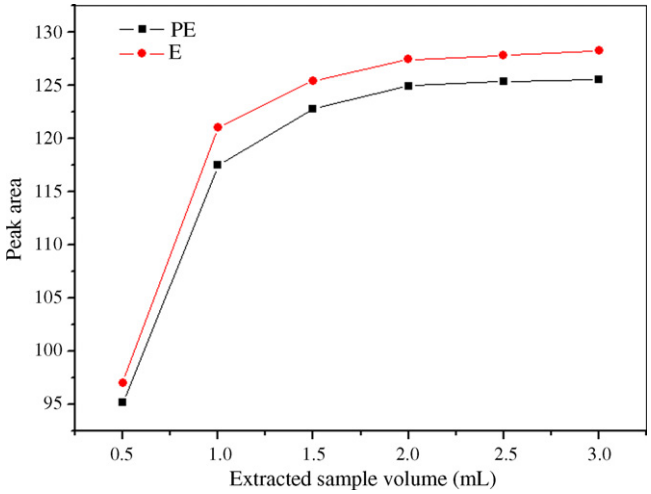


Fig. 3. The extracted sample volume profile of E and PE for the polymer monolith microextraction (PMME). Sample solution consisted of E and PE spiked at 1 mg/L. Extraction conditions and CE conditions outlined in Section 2.

To ensure direct compatibility with CE system, MeCN-HAc (100:0.2, v/v) was used as the desorption solution. A thorough desorption could be achieved with 0.1 mL of the solvent. No peak was detected in the following blank analysis. It was also found that much more eluate was needed for complete desorption of the analytes with reducing the content of acetonitrile in the eluate. Further increasing the volume of the eluate was not preferred for resulting in the decrease in the detection sensitivity.

The extraction equilibrium profiles were monitored by increasing the volume of the extracted sample from 0.5 mL to 3 mL at a constant extraction flow rate. As shown in Fig. 3, the peak areas increased with increasing the volume of the extracted sample up to 1.5 mL. In order to shorten extraction time, a sample volume of 1 mL was selected for subsequent analysis with satisfactory sensitivity achieved.

The sample pH, which influenced the molecule form of the analytes, relates closely to the interactions between analytes and the extraction phase. As can be seen from Fig. 4, the extraction efficiency is highest around pH 7.5 and decreases at pH lower or higher than it. The explanation might be based on the fact that ephedrine and pseudoephedrine are extracted by the monolithic column mostly by hydrophobic interaction and ion-exchange interaction, which arise from the polymer bone structure and its acidic pendant groups [38]. It is obvious that the highest extraction efficiency resulted from the equilibrium of the two interactions, and thus pH 7.5 was selected for the subsequent analysis.

The effect of salt was also examined by adding sodium chloride to the sample solution in the range 0–100 mM. The obtained results revealed that there was a slight decrease in extraction efficiency with the increase in sodium chloride concentration. This behavior can be explained by the existence of the ion-exchange interaction between analytes and the extraction phase. Thus, PMME was performed without salt addition to the sample solutions.

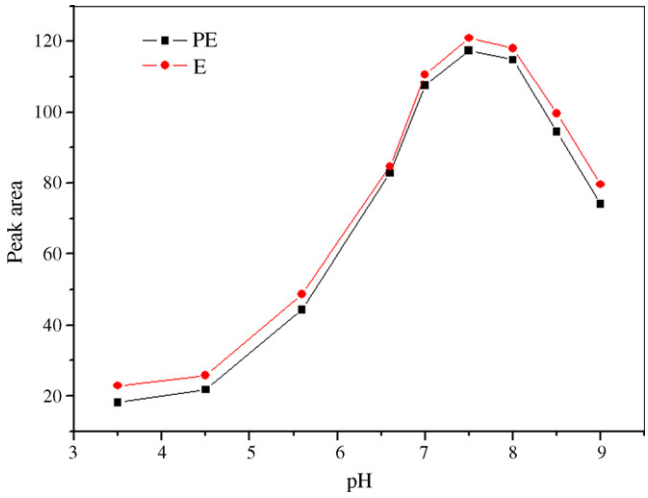


Fig. 4. Optimization of pH of the sample matrix. E and PE were spiked in 0.01 mol/L phosphate buffer solution at different pH at 1 mg/L. Extraction conditions and CE conditions outlined in Section 2.

3.3. Analysis of ephedrine and pseudoephedrine in human plasma and urine

For biological samples, it was important to apply a wash step immediately after the extraction, which ensures the reduction of the matrix interference. After extraction, 0.1 mL solution of 10 mM phosphate buffer (pH 7.5) was kept to flow through the capillary as the wash step. It was demonstrated that the extraction efficiency of the analytes was uninfluenced by the wash step. The electropherograms obtained by PMME-CE of blank and analytes spiked human plasma and urine samples as well as the direct CE analysis of the analytes spiked human plasma and urine samples are shown in Fig. 5. Comparing the electropherogram obtained by PMME-CE (Fig. 5A(c) and B(c)) to that of the direct injection of analytes spiked plasma and urine samples (Fig. 5A(a) and 5B(a)), a dramatic peak height enhancement was found, indicating both the remarkable pre-concentration ability of the monolithic column and the excellent medium provided by PMME for direct compatibility for FESI in CE. And no interferential peak was observed in influencing the quantification of the analytes both in plasma and urine sample (shown in Fig. 5A(b), A(c) and B(b), B(c)). The results showed that the PMME method, which integrated the pre-concentration and removal of sample matrix as a whole, is ideal for plasma and urine sample analysis.

The stability of ephedrine and pseudoephedrine in human plasma and urine was evaluated by comparing the relative peak area obtained at different time intervals with those of a freshly prepared one. It was found that ephedrine and pseudoephedrine were stable in plasma and urine for at least 12 h, which is also consistent with the results of the published literature [39].

Plasma and urine sample were spiked over a range of concentrations (50–5000 ng/ml) with ephedrine and pseudoephedrine. After extraction, a constant amount of 20 mg/L berberine was added as internal standard (IS). Linear regression analyses were performed using ratios of peak areas of analytes to that of IS against the respective analytes concentration. The results are listed in Table 2. The regression coefficients (r) were better than 0.9991, and the detection and quantification limits were also calculated with the signal-to-noise ratio set at 3 and 10, respectively. The obtained sensitivity, which is much better than that of the commonly used LLE-CE-UV system (LOD of E is 1000 ng/mL) [9] and comparable to that of the typically measured with HPLC–MS (LOD of E is 17 ng/mL) [7] and CE-LIF (LOD of E is 2.6 ng/mL) [14], was found to be more than adequate for the usual analytical requirements [2].

The average recovery of the analytes from spiked samples was calculated by comparing the obtained peak areas with those of spiked aqueous solution. And the average recovery was found to be 100% and 95% for E and PE in plasma. For urine samples, it was 72% and 68% for E and PE, respectively. The results confirmed that the plasma matrix (e.g., protein) hardly affected the extraction under the optimized conditions, while the relatively low recovery in urine may be due to the effect of urine matrix (e.g., inorganic salt). For further validating the method, analytes spiked in urine obtained from different volunteers were analyzed

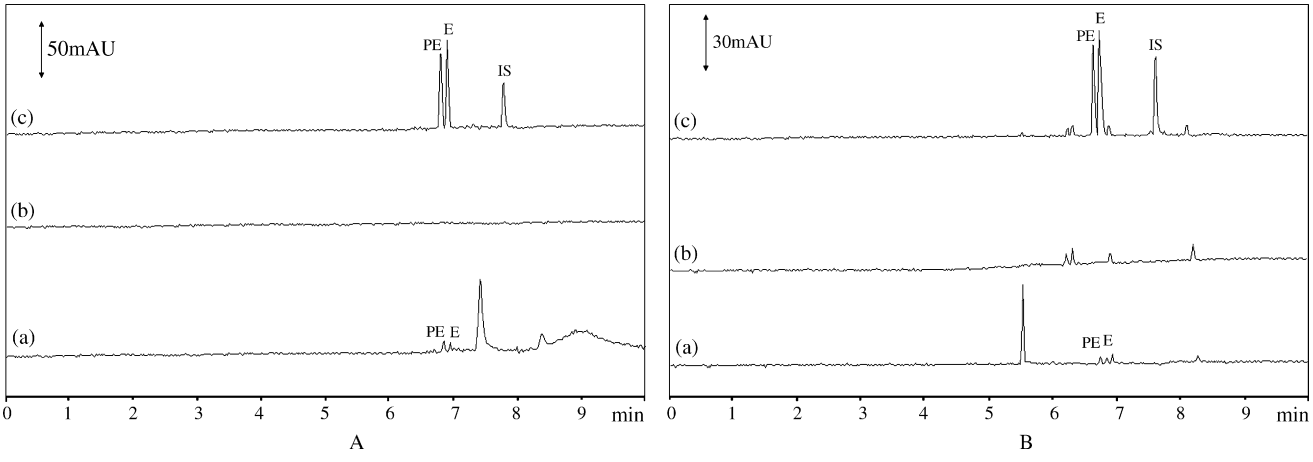


Fig. 5. Electropherograms obtained by PMME-CE of spiked plasma (A (c)) and urine (B (c)) at 0.5 mg/L; PMME-CE of blank plasma (A (b)) and urine (B (b)) sample; and directly analysis of spiked plasma (A (a)) and urine (B (a)) at 5 mg/L by CE without treatment. Berberine (IS) was added after PMME with the concentration of 2 mg/L. Extraction conditions and CE conditions outlined in Section 2.

Table 2
Calibration parameters for the polymer monolith microextraction of the ephedrine and pseudoephedrine from plasma and urine samples

	Compounds	Linear range (ng/mL)	Calibration curves			LOD[a] (ng/mL)	LOQ[a] (ng/mL)
			Slope	Intercept	r		
Plasma samples	E	50–5000	2.34	0.15	0.9995	5.3	17.7
	PE	50–5000	2.25	0.18	0.9994	5.8	19.5
Urine samples	E	50–5000	1.61	0.079	0.9991	8.0	26.6
	PE	50–5000	1.54	0.13	0.9991	8.4	28.0

Number of data point for calibration curves is 8 and three repetition per point. Extraction conditions and CE conditions outlined in Section 2.
[a] Refer to concentrations in the diluted body fluids as mentioned in Section 2.4.

Table 3
Intra-day and inter-day precision of relative peak areas at three different concentrations for the polymer monolith microextraction of ephedrine and pseudoephedrine from plasma and urine samples

Compounds	Concentration (ng/mL)	Precision (RSD, %)			
		Plasma sample		Urine sample	
		Intra-day ($n=5$)	Inter-day ($n=5$)	Intra-day ($n=5$)	Inter-day ($n=5$)
E	50	2.6	3.5	4.0	5.9
	100	1.9	3.8	2.5	4.5
	2000	2.4	3.2	3.5	5.8
PE	50	2.8	4.0	3.8	4.9
	100	2.0	4.1	3.9	4.3
	2000	2.5	3.6	2.6	4.2

The intra-day precision were calculated by performing 5 extraction of independently prepared plasma and urine samples with analytes spiked at three different concentrations over a day. Inter-day precisions were accessed by performing 5 extraction of independently prepared plasma and urine samples with analytes spiked at three different concentrations for continuous five days. Extraction conditions and CE conditions outlined in Section 2.

and the relative standard deviation (RSD) for five measurements was below 8.7%, which confirmed that the method for extraction of E and PE from urine sample was robust and reliable.

The reproducibility of the developed method was determined by the inter-day and intra-day precision. Three levels of sample concentrations were tested. As shown in Table 3, the intra-day and inter-day precisions of the relative peak areas, which were calculated as relative standard deviation for five measurements, are lower than 4.0% and 5.9%, respectively.

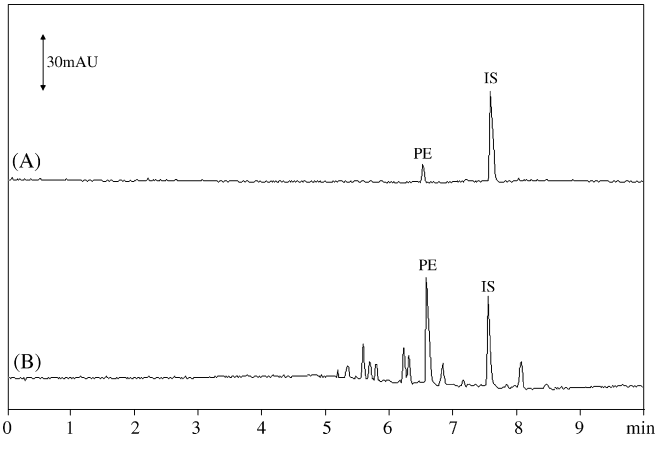


Fig. 6. Electropherograms obtained by PMME-CE of a plasma sample from a volunteer after 1 h of the administration of 60 mg pseudoephedrine sustained relief preparation (A); and a urine sample from a volunteer after 24 h of the administration of 60 mg pseudoephedrine sustained relief preparation (B). Berberine (IS) was added after PMME with the concentration of 2 mg/L. Extraction conditions and CE conditions outlined in Section 2.

Plasma and urine samples from volunteers receiving pseudoephedrine have also been analysed under the optimized conditions. Method of standard addition was used to confirm the identity of the peaks. The electropherogram was shown in Fig. 6. Successful analysis was accomplished with the quantification result as 82.2 ng/mL psedoephedrine in plasma (1 h after an oral administration of 60 mg pseudoephedrine sustained relief preparation) and 626.7 ng/mL psedoephedrine in urine (24 h after an oral administration of 60 mg pseudoephedrine sustained relief preparation). The results confirmed that the PMME combined with CE system could be directly employed for the determination of pseudoephedrine in clinical samples.

4. Conclusions

Poly (methacrylic acid-co-ethylene glycol dimethacrylate) monolith microextraction followed by on-line preconcentration-capillary electrophoresis is able to extract and detect ephedrine and pseudoephedrine in human plasma and urine. In view of the simplicity, low cost, rapidness and sensitivity, the present method is recommendable.

The method exhibits good precision, reproducibility and linear response over a wide concentration range. Moreover, since the extract by PMME can be directly analyzed by CZE with sample stacking, the PMME method is rapid and easy to use compared with the other extraction method coupled with CZE.

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