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Solid-phase microextraction coupled with capillary electrophoresis to determine ephedrine derivatives in water and urine using a sol–gel derived butyl methacrylate/silicone fiber

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Abstract

A sensitive method for determination of ephedrine derivatives using headspace solid-phase microextraction (SPME) with a novel fiber followed by capillary electrophoresis has been developed. The co-poly(butyl methacrylate/hydroxy-terminated silicone oil) (BMA/OH-TSO) was used as stationary phases with the aid of γ-methacryloxypropyltrimethoxysilane (KH-570) as bridge in SPME using sol–gel-coating method and cross-linking technology. It has high extraction efficiency for ephedrine derivatives in comparison with commercial poly(dimethylsiloxane) and poly(acrylate)-coated fiber. The coating exhibits good thermal and solvent stability as well as long lifetime. A simple and flexible device for desorption of analytes after headspace SPME was constructed. The effect of various experimental parameters for SPME (temperature, time, pH, ionic strength, desorption solvent, etc.) were discussed. Field amplified sample injection (FASI) was applied for on-line sample concentration and a sensitivity enhancement of two orders of magnitude was achieved. Linear ranges were found to be 20–5000 ng/ml. The detection limits for (1R,2S)-ephedrine, (1R,2R)-pseudoephedrine and (1S,2S)-pseudoephedrine were 3, 5 and 5 ng/ml, respectively. Relative standard deviation ($n = 6$) was found to be 4.96–7.57%. The method was successfully applied to the analysis of ephedrine derivatives in human urine.

Keywords: Capillary electrophoresis; Solid-phase microextraction; Sol–gel; Field-amplified sample injection; Ephedrine derivatives

1. Introduction

Ephedrine, pseudoephedrine are pairs of diastereoisomeric sympathomimetic amines known to have 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. Nowadays, the commission has adopted the following limits of concentration in urine above which an athlete is considered as "positive": for ephedrine 10 μg/ml [2]. Several analytical techniques, including gas chromatography (GC) [3–5] and high performance liquid chromatography (HPLC) [6,7], have been presented for determination of ephedrine derivatives. However, these methods often require a derivatization step or exotic mobile phases to allow for separation of the ephedrine derivatives. Capillary electrophoresis (CE) provides high efficiency, short analysis time, low operational cost and fast method development. It has been introduced as an important alternative technique for the separation of ephedrine derivatives in recent years [8–14]. However, one of the main drawbacks of CE is its low sensitivity in terms of solute concentration compared to other separation techniques like gas chromatography (GC) or high-performance liquid chromatography (HPLC). This low sensitivity is associated to both the short optical path-length of the capillary used as detection cell (for spectrophotometric detection) and the small volumes (usually a few nanoliters) that can be injected.

Solid-phase microextraction (SPME), introduced by Belardi and Pawliszyn [15], is a simple, solvent-waste free, time-efficient extraction technique. In recent years, SPME

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has been widely used for the determination of volatile organic compounds, semi-volatile chemicals in different fields, including the environment, food, natural products, pharmaceuticals, biology, toxicology, forensics, etc. [16–22]. SPME has frequently been used in conjunction with GC or LC, and its combination with CE has been presented in a few papers [23–29], but only two of them deal with biological samples [23,26].

In the previous studies about the use of SPME combined with CE, commercial SPME fibers [27–29], and custom-made fibers [23–25], et al., were used. However, most of them are normally prepared by mere physical deposition of the polymer coating on the surface of the fused-silica fiber (or its equivalent). The lack of proper chemical binding between the stationary phase and fused-silica fiber surface may result in the low solvent stability. Sol–gel coating technology can overcome this problem by providing efficient incorporation of organic components into the inorganic polymeric structure in solution under extraordinarily mild thermal conditions [30–32]. In our group, hydroxyl-crown ether [33], open crown ether [34], calixarene [35–37] have been first prepared with this technique. Owing to their inherent multifunctional properties and the features of sol–gel chemistry, these coatings exhibit good thermal, solvent stability and high extraction efficiency as well as long lifetime.

The goal of the present work is to develop a fiber with good thermal, solvent stability and high extraction efficiency for SPME-FASI-CE-UV to detect ephedrine derivatives at the very low concentration level. The effect of various experimental parameters for SPME ephedrine derivatives was optimized. Linearity, detection limits, and precision of the whole procedure were valuated. To our knowledge, this is the first report showing the great possibilities of the combined use of SPME, on-line sample preconcentration, and CE for ephedrine derivatives.

2. Experimental

2.1. Instrumention

All separation experiments were performed on a Beckman P/ACE^{TM} MDQ instrument (Beckman-coulter, Fullerton, CA, USA) equipped with a photo diode array detector (190–600 nm), automatic injector, a fluid cooled column cartridge (15–60 °C) and system Gold Date Station. The separations were performed in an uncoated fused silica capillary (60.5 cm × 75 μm i.d. × 360 μm o.d.) (Yongnian Photoconductive Fiber Factory, Hebei, China). The capillary was conditioned before use by successive washings for 10 min with 0.1 mol/l sodium hydroxide, and 10 min with water, followed by 10 min running buffer. Before each experiment, the capillary was rinsed with 0.1 mol/l sodium hydroxide for 2 min, water for 2 min, and running buffer for 3 min.

To mix various solution ingredients thoroughly, an Ultrasonator model KQ-50DE (Kunshan Ultrasonic Instrument Co. Ltd, shanghai, China) was used. A centrifuge model TGL-16C (Shanghai Anting Instrument Factory, Shanghai, China) was used to separate the sol solution from the precipitate. The fused-silica fiber (120 μm, o.d.) with protective polyimide coating was provided by the Academy of Post and Telecommunication, Wuhan, China.

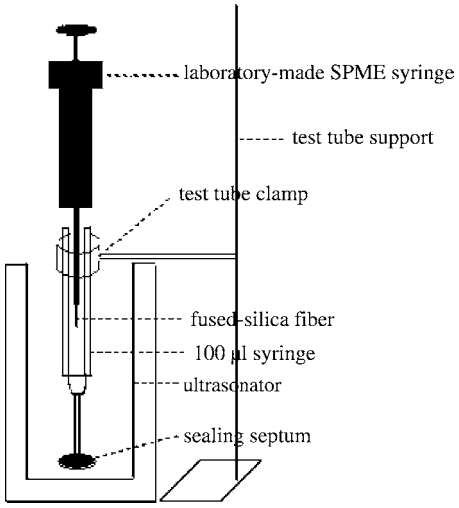

Fig. 1. Scheme of laboratory-made desorption device for SPME.

A magnetic stirrer DF-101B (Leqing, China) was employed for stirring the sample during extraction. A homemade SPME syringe with BMA/OH-TSO fiber was used to extract ephedrine derivatives from samples. The commercially available PDMS (100 μm) and PA (85 μm) coated fibers for comparison were obtained from Supelco (Bellefonte, PA, USA). A simple device was used for desorption of analytes from fiber (Fig. 1).

2.2. Reagents and materials

Hydroxy-terminated silicone oil (OH-TSO) was purchased from Chengdu Center for Applied Research of Silicone (Chengdu, China). γ-Methacryloxypropyltrimethoxysilane (KH-570), tetraethoxysilane and poly(methylhydrosiloxane) were obtained from the Chemical Plant of Wuhan University (wuhan, China). Butyl methacrylate (BMA) and benzophenone were purchased from Shanghai Chemical Factory (Shanghai, China). Trifluoroacetic acid (TFA) was purchased from Merck, Germany. (1R,2S)-Ephedrine were obtained from the National Institute for Control of Pharmaceutical and Biological products (Beijing, China), (1R,2R)-pseudoephedrine and (1S,2S)-pseudoephedrine were acquired from Aldrich (Milwaukee, WI, USA). β-Cyclodextrin (β-CD) was purchased from Shanghai Chemical Plant (Shanghai, China). All other chemicals used were analytical grade. CE buffers (the pH was measured using a pH meter (pHs-2, Shanghai Second Analytical Instrument Factory, Shanghai, China)) and sample solutions were prepared using deionized water from

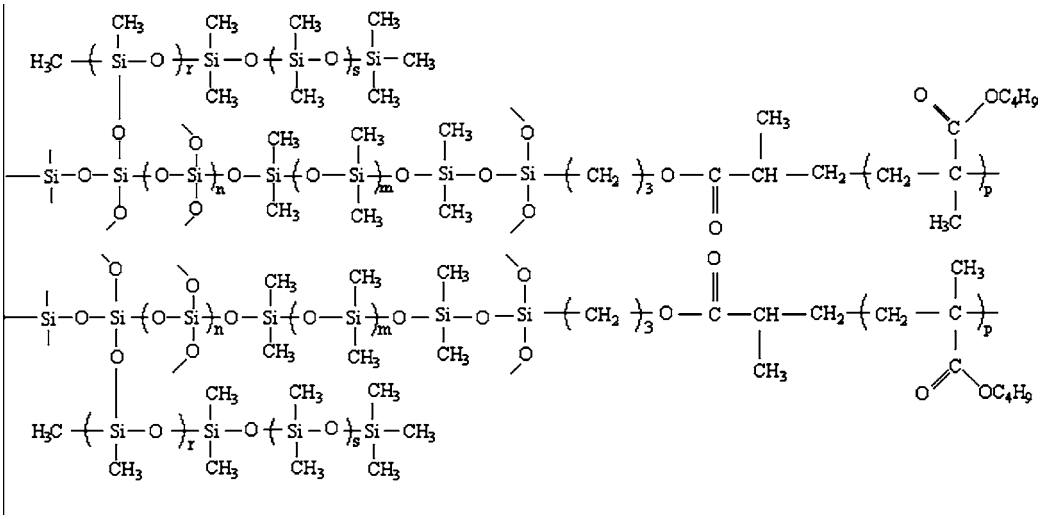

Fig. 2. The chemical structure of BMA/OH-TSO coating.

a Milli-Q water purification system (Bedford, MA, USA) and filtered through a 0.45 μm pore size filter.

2.3. Preparation of BMA/OH-TSO fiber

The pre-treating of the fiber can consult reference [33]. The sol solution was prepared as follows: 270 μl of BMA, 90 mg of OH-TSO, 100 μl of tetraethoxysilane, 50 μl of γ-methacryloxypropyltrimethoxylsilane, 10 mg of poly(methylhydrosiloxane) and 8 mg of benzophenone were dissolved in 100 μl of methylene chloride and mixed thoroughly by ultrasonic agitation in a plastic tube. A 70-μl volume of TFA containing 5% water was sequentially added to the resulting solution with ultrasonic agitation for another 5 min. The mixture was centrifuged at 12,000 rpm for 8 min. The top clear sol solution was collected for fiber coating. A sol–gel coating was formed on the bare outer surface of the fiber end (about 1 cm), after the fibers were dipped vertically into the sol–gel solution for 30 min. For each fiber, this coating process was repeated several times until the desired thickness of the coating was obtained. After that the fibers were irradiated under UV light for 30 min, then placed in a desiccator for 12 h at room temperature and conditioned at 250–320 °C under nitrogen protection for 2 h in the GC injection port. The final thickness of the coating was 70 μm. It was measured by microscope. The thickness is obtained by subtracting the original the radius of the bare fiber from the radius of the coated fiber. Fig. 2 shows the chemical structure of BMA/OH-TSO coating. An OH-TSO fiber was also coated for comparison by sol–gel technique with an identical preparation procedure except that BMA were not added.

2.4. SPME procedure

The 5 ml spiked sample was placed into a 10 ml vial containing a small magnetic stirring bar and mixed with a certain amount of sodium hydroxide and sodium chloride. To avoid sample evaporation, the vials were closed with butyl rubber stoppers wrapped with PTFE sealing tape, and then sealed with aluminum caps. The extraction was performed by exposing the coated fiber end to the headspace of the vial for an appropriate time. Care was taken to ensure that the fiber did not contact the liquid sample. After extraction, the fiber was then withdrawn into the needle followed by removal from the vial. The analytes extracted on fiber were then desorbed by immersing the fiber into 80 μl back-extraction solvent in a 100 μl syringe (the needle-point of syringe is clogged by septum) for a certain time (Fig. 1). Ultrasonic treatment was used to accelerate the desorption of analytes from the fiber. After the fiber was removed, the back-extraction solution was transferred to an injection vial for CE analysis; at the same time, the fiber was conditioned in a GC injector port at 260 °C for 5 min.

2.5. CE separation and detection

The separation conditions were as follows: run buffer of 150 mM phosphate background electropholyte at pH 2.5 with 17.5 mM β-CD, a potential of 25 kV and the capillary temperature of 20 °C. The injection of the samples was electroinjection (7 kV × 10 s). The diode array UV detection was performed at 192 nm, where the detection exhibits the best sensitivity for ephedrine derivatives.

3. Results and discussion

3.1. Optimization of SPME conditions

Experimental conditions, such as extraction modes, extraction temperature and time, pH, ionic strength and des-

orption conditions, were optimized before validating the analytical method.

Urine samples are complex mixtures and typically contain both organic and inorganic constituents. Headspace SPME can provide cleaner extracts and greater selectivity than direct SPME as interferences from non-volatile compounds can be avoided. Moreover, the lifetime of fibers is considerably increased because adverse effects due to harsh sample conditions could be minimized by isolating the fiber coating from direct contact with the sample matrix. A headspace method should be applied whenever possible in SPME of body fluids [19]. As semivolatile compounds, ephedrine derivatives could be extracted with headspace SPME by manipulating sampling conditions.

Ephedrine derivatives can dissolve in water, moreover, they can form hydrogen bonding with water, which make it difficult to volatilize. The effect of the temperature on the extraction was studied by exposing the fiber to the headspace of sample for 30 min at temperature ranging from 40 to 95 °C. Results showed that an extraction temperature of 90 °C exhibited the highest extraction efficiency. The temperature of 90 °C was adopted for further experiments.

Fig. 3 shows the effect of extraction time on the extraction efficiency. (1R,2S)-ephedrine ((−)-E), (1R,2R)-pseudoephedrine ((−)-PE) and (1S,2S)-pseudoephedrine ((+)-PE) reached equilibrium within 30 min. An extraction time of 30 min was chosen for the followed experiments.

Ephedrine derivatives are weak basic compounds. At a higher pH, the acid–base equilibrium of the analytes significantly shift towards the neutral form, which has a higher affinity for the fiber, thereby enhance the amount extracted. The extraction increased with increasing the amount of sodium hydroxide from 0.25 to 2.00 g, then, slightly decreased for 2.5 g. So 2.0 g of sodium hydroxide was favorable. It should

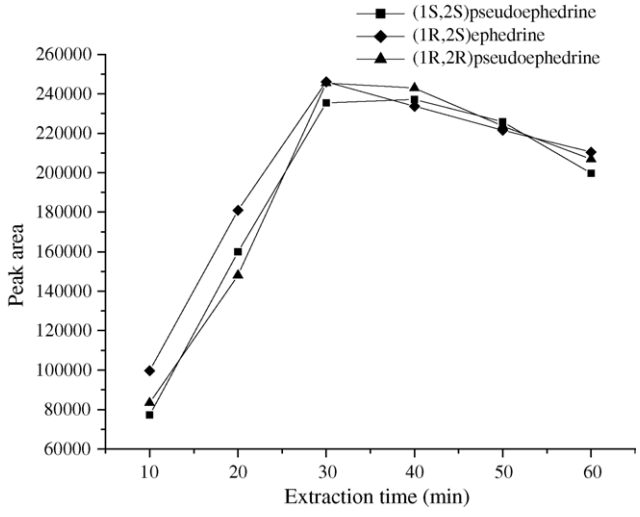

Fig. 3. The effect of time on the extraction efficiency of (−)-E, (±)-PE. (The figure was obtained with three replicate measurements.) Concentrations of analytes: 0.300 μg/ml. The run buffer contained 17.5 mM β-CD and 150 mM phosphate (pH 2.5) in the running voltage 25 kV at 20 °C. Sample injection: 7 kV × 10 s.

be mentioned that this method is also suitable for extraction ephedrine hydrochloride and pseudoephedrine hydrochloride, as they are converted into its free-base form in the vial during extraction and become easy to volatilize without additional treatment. As the sol–gel BMA/OH-TSO coating is chemically bonded to the surface of the fused silica substrate, it demonstrated excellent pH stabilities over conventionally created coatings.

The influence of sodium chloride in the extraction extent, within the range 0–1.5 g, was evaluated. The amounts extracted increased with the addition of sodium chloride from 0 to 0.5 g; however, more sodium chloride was found to be negative. The reason is at a basic condition, the saturated salt hinders the shift of the ionized form of ephedrine derivatives towards the neutral form, which has a higher affinity for the fiber.

A simple device for SPME desorption was developed (Fig. 1). It has a variety of merits such as: (1) the device is easy to construct and operate. (2) The volume of back-extraction solvent from 1 to 100 μl can be obtained (by changing the type of desorption syringe) and can be almost entirely collected for CE injection. (3) Ultrasonic treatment can be used to accelerate the desorption of analytes from the fiber.

The desorption process was influenced by the temperature. To ensure the reproducibility, the temperature of water in the ultrasonator was kept at 20 °C with ice bag. The content of acetonitrile in back-extraction solvent (0–60% (v/v)) and back-extraction time (5–25 min) was optimized for BMA/OH-TSO fiber. Although the desorption efficiency increased with the increasing of content of acetonitrile, the reproducibility went to bad and current interruption happen frequency during CE separation. The content was determined to be 20% as a compromise of efficiency and the reproducibility. Effect of desorption time on the peak area of each compound was evaluated for 5, 10, 15, 20, 25 min. The analytes reached the back-extraction equilibrium at 20 min. Therefore, desorption time of 20 min was chosen.

Fig. 4 compares the extraction efficiency of BMA/OH-TSO fiber with laboratory-made sol–gel-derived OH-TSO fiber, commercial PDMS- and PA-coated fibers. The error bars were taken as the relative standard deviation of three replicate measurements. The three-dimensional network in the coating structure provides a higher surface area and sample capacity for BMA/OH-TSO and OH-TSO fiber, and thus the higher extraction efficiency than commercial PDMS and PA fiber. A better extraction efficiency of BMA/OH-TSO relative to OH-TSO was possibly due to the increased interactions between polymer and analytes through hydrogen bonding. For PDMS and PA, according to the principle of "like dissolve like", the polar analytes have higher affinities for polar coating than that for the non-polar coating, therefore PDMS fiber showed the lowest extraction efficiency.

The change of extraction efficiency of BMA/OH-TSO fiber in extracting ephedrine derivatives from the aqueous solution after being used for 1, 50, 100, and 150 times was investigated. To ensure reproducibility, the ratios of areas

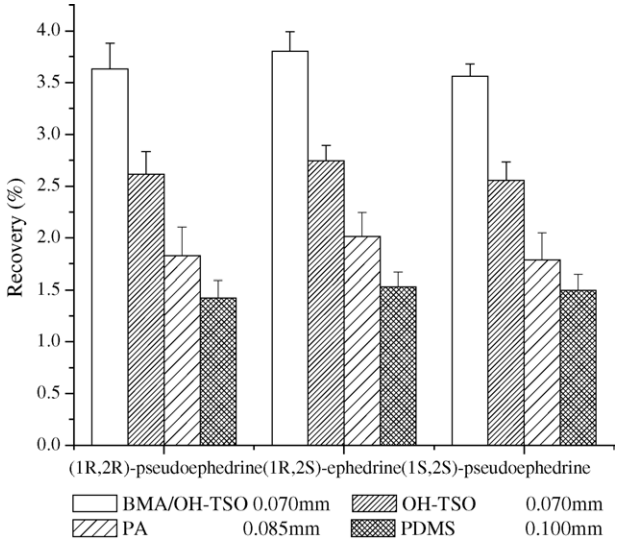

Fig. 4. Comparison of the extraction efficiency of BMA/OH-TSO with commercial PDMS, PA fibers. (The figure was obtained with three replicate measurements.) The optimized SPME conditions: temperature of 90 °C, time of 30 min, 2.00 g sodium hydroxide and 0.50 g sodium chloride for headspace extraction; 20% acetonitrile aqueous phase solvent with desorption time of 20 min for desorption. Concentrations of analytes and CE conditions were as described in Fig. 3.

obtained by the comparison of the areas in the electropherogram measured by headspace SPME with the corresponding peak areas gained by direct CE separation of 40 μl of a solution with the same concentration, were used for representing the extraction performance of the fiber. The extraction efficiencies had no obvious decline after being used 150 times. Being chemically bonded to the substrate, the fibers are inherently stable in organic solvents.

3.2. Field amplification sample injection

Electroinjection of samples (prepared in a low-concentration buffer or water) into a column filled with higher concentration of the same buffer results in an electric field at the injection point that is much stronger than the electric field in the column. Charged analytes are injected at high velocity and stacked at the interface between the low-conductivity zone and the running buffer. Consequently, analytes are effectively concentrated. In our experiment, electroosmotic flow was suppressed simply by adjusting pH at 2.5 allowing sample stacking and separation to proceed continuously without intermediate polarity switching. Table 1 shows the performance of FASI procedure. A concentration factor of about 80-fold compared to the conventional electrokinetic sample injection, 120-fold compared to hydrodynamic injection model had been achieved by FASI.

3.3. Validation of the SPME-CE

The method was validated for the determination of (−)-E and (±)-PE in aqueous phase. The data of linear corre-

Table 1
Performance of FASI procedure

Compound	Migration time (min)	R.S.D. (%) ($n = 6$)[a]				Rs[b]	LOD (μg/ml)	FASI area (7 kV × 10 s)[a]	HI area (0.5 psi × 5 s)[c]	ESI area (7 kV × 10 s)[d]
		Migration time		Peak areas (A)						
		Intraday	Interday	Intraday	Interday					
(−)-PE	12.8	0.52	2.11	4.60	5.87	–	7.00×10^{-3}	1.44×10^5	2.87×10^5	4.05×10^5
(−)-E	13.0	0.48	1.94	3.15	4.54	1.35	5.00×10^{-3}	1.38×10^5	3.02×10^5	4.31×10^5
(+)-PE	13.6	0.67	2.75	4.24	5.38	3.46	5.00×10^{-3}	1.41×10^5	2.80×10^5	4.01×10^5

[a] Sample concentrations of analytes in water: 0.400 μg/ml.
[b] Rs: peak resolution; sample concentrations of analytes in water: 0.400 μg/ml.
[c] HI: hydrodynamic injection model; sample concentrations of analytes in buffer: 100 μg/ml.
[d] ESI: electrokinetic sample injection; sample concentrations of analytes in buffer: 100 μg/ml.

Table 2
Linear correlation, linear range, correlation coefficients (R), limits of detection and R.S.D. for the analysis of ephedrine derivatives

Compound	Linear correlation[a]	Linear range (μg/ml)	Correlation coefficient (R)	LOD (μg/ml)		R.S.D. (%) ($n = 6$)[c]	Recovery (%)
				Detected[b]	In literature		
(−)-E	$Y = 54104 + 502002X$	0.02–5	0.996	3.00×10^{-3}	0.017[7], 1.74[8], 1.00[9], 2.00[12], 2.60×10^{-3} [14]	7.57	90.4[d], 97.5[e]
(−)-PE	$Y = 47096 + 440330X$	0.02–5	0.997	5.00×10^{-3}	500[3], 1.00[9], 2.80×10^{-3} [14]	4.96	88.7[d], 98.6[e]
(+)-PE	$Y = 53647 + 443492X$	0.02–5	0.996	5.00×10^{-3}	500[3], 1.00[9], 2.80×10^{-3} [14]	5.62	91.4[d], 94.7[e]

[a] The linear correlations were obtained by the injection of seven spiked standards solvent after SPME, where Y and X is the peak area and the concentration of each analyte, respectively.
[b] Detection of limits is the concentration of analytes that give a signal equal to thrice the peak-to-peak noise level of the analytes.
[c] R.S.D. for relative peak areas was obtained from six repetitive analyses of a standard solvent spiked by three analytes without internal standard.
[d] The recovery of the three compounds at limit of quantification (concentrations of analytes: (−)-PE and (+)-PE at 15.0 ng/ml; (−)-E at 10.0 ng/ml) ($n = 5$).
[e] The recovery of the three compounds at 0.200 μg/ml ($n = 5$).

lation, linear range, correlation coefficient, limit of detection of the method (LOD), and the relative standard deviation (R.S.D.) of peak area are summarized in Table 2. The studies presented herein demonstrate that by combing SPME, field-amplified sample injection and low wavelength UV detection, yields highly improved detection sensitivity. Drug levels of 5 ng/ml can be monitored by CE/diode array UV, this being comparable to these typically measured with GC-MS [6], HPLC-MS [7], CE-MS [10] and CE-LIF [14]. The sensitivity is sufficient for confirmatory testing of most urinary drugs of abuse without the needing of expensive detection, such as LIF detector, or mass spectrum.

In this paper, the reliability of the method for the analysis of ephedrine derivatives in urine by standard addition was evaluated. Recovery has been estimated as: (the amount found in the spiked sample – the amount found in the sample) × 100/the amount added.

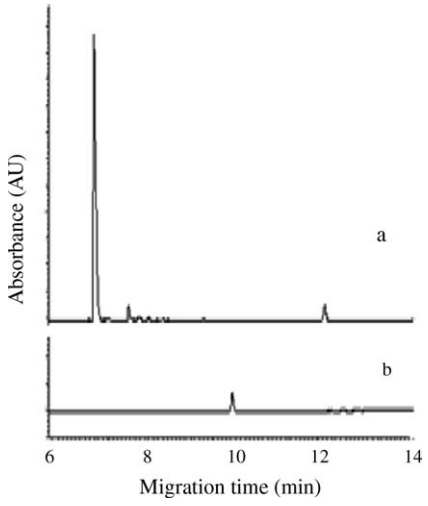

Fig. 5. Separation of blank urine sample (a) with directly injected; (b) after SPME followed by CE separation. SPME and CE conditions were as described in Fig. 4.

3.4. Analytical applications

The developed SPME-CE system was applied to the analysis of ephedrine derivatives in urine samples. Urine is a highly variable matrix as diet and liquid intake vary urine ionic strength and pH to a great degree. Testing of accuracy and precision in different urine matrices should therefore be included in the validation of a SPME method. The urine samples, which were used as different urine matrices, were collected from five healthy individuals. The intra-assay relative standard deviations were between 4.38 and 7.76%

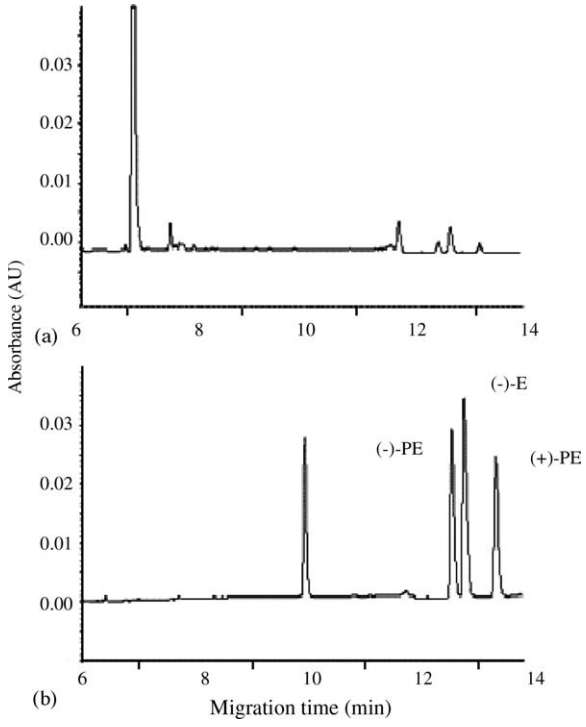

Fig. 6. Separation of spiked urine sample (a) with directly injected (concentration of each analyte: 5.00 μg/ml); (b) after SPME (concentration of each analyte: 0.25 μg/ml) followed by CE separation. SPME and CE conditions were as described in Fig. 4.

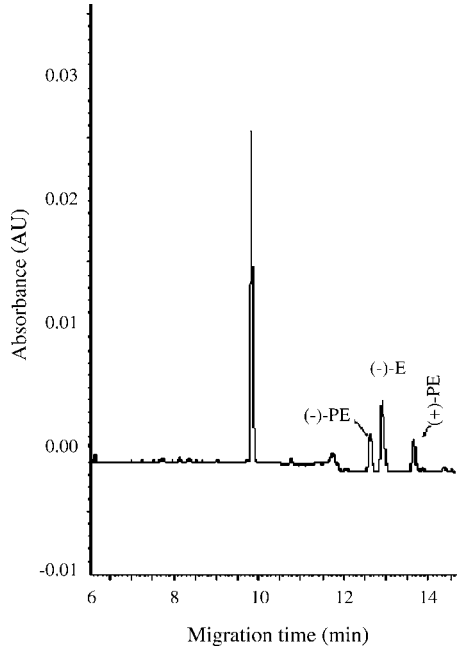

Fig. 7. Separation of spiked urine sample after SPME (concentration of each analyte: 0.015 μg/ml) followed by CE separation. SPME and CE conditions were as described in Fig. 4.

(concentration of analytes: 0.3 μg/ml; $n = 5$), which were in concurrence with the results shown in Table 2. The variable urine matrixes were compensated for by adding sodium hydroxide (2.0 g/vial) and sodium chloride (0.4 g/vial) to the urine sample before SPME. The method was found to be highly reproducible and robust towards natural variations in the sample matrix.

Fig. 5 shows a comparison of electropherogram of blank urine sample with directly injection (a) and after SPME (b). The electropherogram for the extracts is cleaner than that of direct injection since most of the matrix in urine was removed after SPME. Separation of spiked urine sample with directly injection (a) and after SPME (b) was shown in Fig. 6. As can be seen from the Figure, SPME plays an important role in eliminating the complexity of the matrix and concentrating sample. Urine sample spiked with 5.00 μg/ml analytes was used for direct injection as no measurable peak was obtained when it was spiked with 0.500 μg/ml analytes. Separation of spiked urine sample at the limit of quantification after SPME was shown in Fig. 7.

4. Concluding remarks

SPME followed by CE with field amplified sample injection was used successfully for the separation and determination of ephedrine derivatives in water and human urine. The analytes could be reliably analyzed using optimized conditions, with excellent selectivity (in urine sample), low limits of detection (3–5 ng/ml) and wide method linearity (20–5000 ng/ml). In view of the simplicity, sensitivity, the present method is recommendable for doping control. BMA/OH-TSO fiber prepared by sol–gel method and cross-linking technology exhibits high solvent stability, high extraction efficiency and long lifetime, which demonstrates it is a promising coating for SPME in combination with CE and HPLC.

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