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On-line sample concentration and determination of cationic alkaloids in human plasma by micelle to solvent stacking in capillary zone electrophoresis

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ABSTRACT

A sensitive method for the determination of three cationic alkaloids (berberine, palmatine and jatrorrhizine) from human plasma samples was developed by micelle to solvent stacking (MSS) in capillary zone electrophoresis (CZE). In MSS, the sample preconcentration mainly relies on the reversal in the effective electrophoretic mobility of the analytes at the boundary zone between the sample and CZE background solution (BGS). Under the optimized conditions, the sensitivity enhancement factors achieved in terms of corrected peak area were in the range from 47 to 53 for the alkaloids. The limits of detection (LODs) (S/N = 3) for berberine, palmatine and jatrorrhizine were 0.01, 0.01 and 0.02 μg/mL, respectively. The intraday (n = 6) and interday repeatabilities (n = 12) expressed as the relative standard deviations (RSDs) were less than 6.9% in terms of peak height and less than 7.3% in terms of corrected peak area, respectively. The recoveries of the method for the three alkaloids were in the range of 95.9–101.5% with peak height as the quantitative signal, and 92.6–103.6% with corrected peak area as the quantitative signal, respectively. The MSS-CZE method proved to be suitable for the analysis of the alkaloids in human plasma samples.

1. Introduction

Berberine, palmatine and jatrorrhizine are three well-known isoquinoline quaternary ammonium alkaloids and they are usually found in Traditional Chinese Medicines (TCMs) such as Rhizoma Coptidis (*Coptis chinensis* Franch), Cortex Phellodendron (*Phellodendron amurense* Rupr.) and Caulis Mahonia (*Mahonia fortunei* (Lindl.) Fedde). They possess antibiotic activity against bacteria, fungi and viruses, and have vasodilatory, sedative, hepatoprotective and antitumor effects [1–3]. The predominant clinical uses of these alkaloid preparations are for the treatment of various inflammatory diseases, such as bacterial diarrhea, gynecological inflammation, enteritis, respiratory tract infection, and urinary infection. So far, a variety of chromatographic methods have been reported for the qualitative and quantitative analysis of berberine, palmatine and jatrorrhizine in biological samples, such as high performance liquid chromatography–ultraviolet spectrometry (HPLC–UV) for plasma, blood, urine and bile samples [4–7], HPLC-fluorimetry for plasma samples [8], liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) for plasma and serum samples [9–11], liquid chromatography–tandem mass spectrometry (LC–MS/MS) for plasma samples [12–16] and GC–MS for plasma samples [17]. However, the previously described methods suffered from some disadvantages, such as the requirement for a large sample volume [4,9], long elution time [4,9,16] and expensive MS instrumentation and operations [9–17].

Capillary electrophoresis (CE) is considered to be an effective separation technique and has been widely used in different areas including chemistry, biology, medicine and pharmaceutics because of its high separation efficiency, short separation time and small amount of both sample and organic solvent consumptions [18,19]. However, when the most popular CE photometric detector is used, the main disadvantage of CE is its poor concentration sensitivity due to the short optical length of the capillary and small sample injection volume. To overcome this problem, some on-line preconcentration strategies have been developed to increase the sensitivity of CE, such as field amplification [20], dynamic pH junction [21], transient isotachophoresis (tITP) [22], isotachophoresis by acetonitrile stacking [23,24] and sweeping [25] or sweeping in couple with other techniques [26–29]. The more recent CE on-line preconcentration techniques include electrokinetic supercharging [30], analyte focusing by micelle collapse (AFMC) [31–33] and micelle to solvent stacking (MSS) [34–42]. The main advantage of the on-line preconcentration strategies is the ease of experimental manipulations without the need of modifications of the commercial CE instrumentation.

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The AFMC method was first introduced by Quirino and Haddad [31] for the on-line sample preconcentration of both neutral and charged analytes. This technique relies on the use of a micellar carrier phase in the sample matrix that can collapse into a micellar dilution zone found in between the sample and the background solution (BGS). In AFMC, the sample is prepared in a matrix that contains the micelles and an electrolyte anion of high mobility, while the BGS is a lower conductivity solution relative to the sample matrix. The accumulated analytes were subsequently separated by MEKC [31,32] or capillary zone electrophoresis (CZE) [33]. AFMC was shown to provide hundreds fold improvement in detection sensitivity for some steroidal compounds and dialkyl phthalates [31,32]. Recently a new variation of AFMC termed as MSS has been developed by Quirino [34]. The crucial factor of MSS is the reversal of the effective electrophoretic mobility at the micelle to solvent stacking boundary (MSSB) between the micellar solution zone and the background solution (BGS). In MSS, the sample was prepared in a micellar solution without organic solvent while the BGS was modified by a large percentage of organic solvent. The analytes bound to the micelles were electrophoretically attracted to the MSSB containing the organic solvent and then the affinity of the analytes to the micelles was significantly lowered. As a result, the analytes then experienced an electrophoretic inversion or reversal, resulting in an analyte accumulation at the MSSB. MSS has been applied to the analysis of cationic [34–37] and anionic [38–40] analytes in CZE, and cationic analytes in both MEKC [41] and nonaqueous capillary electrophoresis (NACE) [42]. Among them, Zhu et al. developed an MSS-CZE method for the determination of two different cationic alkaloids (ephedrine and berberine) in human urine samples [36] and an MSS method in nonaqueous capillary electrophoresis (NACE) for the determination of berberine and jatrorrhizine in urine samples [42].

In this work, a simple and convenient approach by MSS in CZE was developed for the simultaneous preconcentration and determination of the three cationic alkaloids with high chemical structure similarity (berberine, palmatine and jatrorrhizine). The method was applied for the determination of the cationic alkaloids in human plasma samples. Compared with the previous work [36], the separation efficiency was improved and the three structurally similar alkaloids were well separated. To our best knowledge, few literatures have been reported for the simultaneous determination of berberine, palmatine and jatrorrhizine in biological fluids by CE.

2. Experimental

2.1. Apparatus

All CE experiments were performed on a Beckman P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA, USA) equipped with an auto sampler and a diode array detector (DAD). An uncoated fused-silica capillary (Yongnian Ruifeng Optical Fiber Factory, Hebei, China) of 50 cm (effective length, 40 cm) × 75 μm i.d. was used throughout the experiments. All of the operations were computer-controlled using Beckman P/ACE MDQ 32 karat software.

2.2. Reagents, chemicals and materials

Berberine hydrochloride, palmatine hydrochloride and jatrorrhizine hydrochloride (all >99%) were purchased from Chinese National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Sodium dodecyl sulfate (SDS) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium dihydrogen phosphate (NaH_2PO_4), sodium borate ($Na_2B_4O_7$), sodium acetate (NaAc), sodium citrate ($Na_3C_6H_5O_7$), ammonium acetate (NH_4Ac), ammonium bicarbonate (NH_4HCO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl), acetonitrile (ACN) and methanol (HPLC-grade) were from Kaitong Chemical Reagent Co. (Tianjin, China). All of the solvents were filtered through a 0.45 μm MicroScience membrane filter from Tianjin Automatic Science Instrument Co. (Tianjin, China). The BGS was prepared freshly everyday and sonicated for 10 min prior to use. Blank human plasma was obtained from the Baoding Blood Donor Service (Baoding, China).

A mixture stock solution containing 10.0 μg/mL each of berberine, palmatine and jatrorrhizine was prepared in methanol and stored in glass-stoppered bottles at 4 °C. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with 15 mM NH_4HCO_3 solution containing 6 mM SDS at pH 7.5 after the stock solution was dried under a mild stream of nitrogen.

2.3. Preparation of human plasma samples

For spiked plasma standards, a 100 μL aliquot of blank human plasma sample was mixed with 25 μL of an appropriate concentration of the mixture stock solution in a 1.0 mL centrifugal tube. Then, 175 μL acetonitrile was added and the mixture was vortexed for 3 min. After the sample was centrifuged at 10 000 rpm (5000 × *g*) for 10 min, the acetonitrile phase was separated, and then 200 μL of the supernatant was transferred to a 0.5-mL vial and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 200 μL of sample matrix (15 mM NH_4HCO_3 water solution containing 6 mM SDS at pH 7.5) for CE analysis.

For real human plasma samples, a 100 μL aliquot of human plasma sample was transferred to a 1.0 mL centrifugal tube, to which 200 μL acetonitrile was added. Then, the mixture was vortex-mixed for 3 min. Next, the sample was handled in the same way as described above for the plasma standards.

2.4. General electrophoresis procedure

The capillary was conditioned prior to use with 0.1 M NaOH at 20 psi for 10 min, followed with double-distilled water for 5 min, and finally with the BGS for 5 min. To ensure repeatability, the capillary was flushed between consecutive runs with 0.1 M NaOH at 20 psi for 3 min, then with double-distilled water for 3 min, and finally with the BGS for 5 min.

For MSS, sample was prepared in 15 mM NH_4HCO_3 water solution containing 6 mM SDS at pH 7.5. The BGS was 50 mM NH_4HCO_3 (pH 7.5) containing 40% methanol. The sample was introduced into the capillary by hydrodynamic injection at 0.5 psi for 30 s (3.6 cm, which is 9.0% of the effective capillary length). A short plug (at 0.5 psi for 5 s) of blank sample matrix was injected prior to the sample. The electrophoresis was performed at a constant voltage of 20.0 kV at 25 °C with the UV detection at 226 nm.

3. Results and discussion

3.1. MSS focusing theory

The model of the on-line sample concentration of the cationic alkaloids by MSS-CZE is illustrated in Fig. 1. Since the pK_a values of the alkaloids (berberine, palmatine and jatrorrhizine) are larger than 9.0 [43], they are organic cations when the pH is less than 9.0. In the first step, the capillary was filled with the BGS containing an organic solvent, i.e., 40% methanol. In Fig. 1A, a sample matrix with the same electrolyte and anionic micelles as the sample solution but without the cationic analytes was first injected as a short plug (0.5 psi, 5 s). Then, the cationic alkaloids dissolved in sample matrix were injected into the capillary as a long plug (0.5 psi, 30 s). The

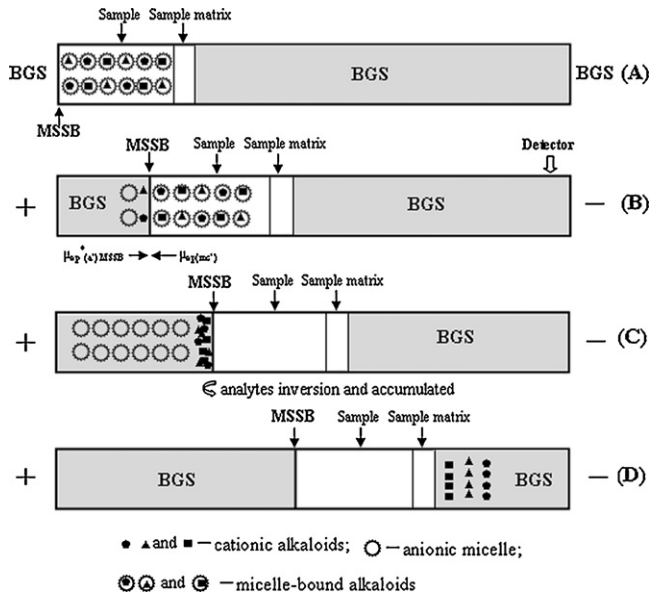

Fig. 1. Process of the on-line concentration of cationic analytes by MSS-CZE. (A) The capillary was conditioned with BGS and then a short plug (0.5 psi, 5 s) of sample matrix was injected followed by sample injection (0.5 psi, 30 s). (B) Application of positive voltage: transport of micelle-bound analytes to the MSSB by the anodically moving micelles. The micelles cross the MSSB boundary where their affinity to the analytes is lowered due to the organic modifier. (C) The reversal in the direction of the effective electrophoretic mobility causes the focusing of transported cations at the MSSB zone. The analytes were focused as more and more micelles traverse the MSSB and the analytes are accumulated along the boundary. (D) The analytes were separated by CZE and migrate to the detector.

micelle to solvent stacking boundary (MSSB) between the sample zone and BGS was created at the anodic end of the capillary. At this boundary, the effective electrophoretic mobility of the analyte ($\mu^*_{ep(a')MSSB}$) is given by Eq. (1) [38,44]:

$$\mu^*_{ep\ (a')MSSB} = \frac{1}{1+k}\mu_{ep(a')} + \frac{k}{1+k}\mu_{ep(mc)} \quad (1)$$

where $\mu_{ep(a')}$ is the electrophoretic mobility of the analyte a′, $\mu_{ep(mc)}$ is the electrophoretic mobility of the micelle, and k is the retention factor.

As presented in Fig. 1B, the micelles in the sample transported the bound analytes to the MSSB zone after the application of a positive voltage (i.e., +20.0 kV). The electroosmotic flow (EOF) was directed toward the detector. The effective electrophoretic velocity of the analytes in the sample ($\mu_{ep(a')}$) was directed toward the positive electrode (+) due to the negative micelles.

As shown in Fig. 1C, the direction of the effective electrophoretic mobility of the analytes was reversed from positive to negative electrode at the MSSB zone, and the focusing of the analytes occurred. In the MSSB, the retention factors of the analytes to the micelles were significantly lowered by the organic solvent modifier in the BGS. The negatively charged micelles continuously migrated toward anode but collapsed into the BGS where the concentration of the SDS drops below its critical micelle concentration (CMC). This can be explained as that the CMC of SDS could be markedly increased with the increase of the organic solvent content [36,38], and therefore the micelles would not be formed when 40% of methanol was added in the BGS. As a result, the effect of the micelle on the effective electrophoretic mobility almost disappeared and $\mu_{ep(a')}$ relied only on the electrophoretic mobility of the analyte.

At last, as shown in Fig. 1D, the contribution of the micelle interaction to electrophoretic mobility then almost disappeared and the focused zones then migrated to the negative polarity and detector where it separated according to normal CZE principles.

3.2. Optimization of the MSS-CZE conditions

3.2.1. Optimization of the BGS system

In order to optimize the MSS-CZE conditions, different BGS systems including sodium dihydrogen phosphate (NaH_2PO_4), sodium borate ($Na_2B_4O_7$), sodium acetate (NaAc), sodium citrate ($Na_3C_6H_5O_7$), ammonium acetate (NH_4Ac) and ammonium bicarbonate (NH_4HCO_3) at the pHs lower than the pK_a values of the alkaloids were examined for the separation of the three alkaloids. As a result, except for NH_4HCO_3, the baseline separations with good peak shapes could not be achieved with either NaH_2PO_4, $Na_2B_4O_7$, NaAc, $Na_3C_6H_5O_7$ or NH_4Ac as the BGS. NH_4HCO_3 had also been successfully used as the BGS in the MSS-CZE for hypolipidemic drugs in the previously published paper by Quirino et al. [34]. Since the overall peak-to-peak resolutions for the three alkaloids with NH_4HCO_3 were better than those obtained with other buffer systems, NH_4HCO_3 at pH 7.5 was selected as the BGS.

3.2.2. Effect of sample matrix injection prior to sample injection

In MSS, the accumulated analytes will migrate to the direction of cathode and detector after the reversal of effective electrophoretic mobility of the analytes in MSSB. However, some of the unretained alkaloid molecules, especially those near the boundary between the sample zone and BGS at the anodic side, are not transported by the micelles to the MSSB zone and therefore will directly migrate out of the sample zone toward the negative polarity and detector [36]. As a result, three small peaks (1′, 2′ and 3′) prior to their respective tall peaks corresponding to the same analytes 1 (berberine), 2 (palmatine) and 3 (jatrorrhizine) can be seen in Fig. 2A. This can be avoided by injecting a short plug (0.5 psi, 5 s) of sample matrix prior to the sample. In this sample matrix plug, the micelles could

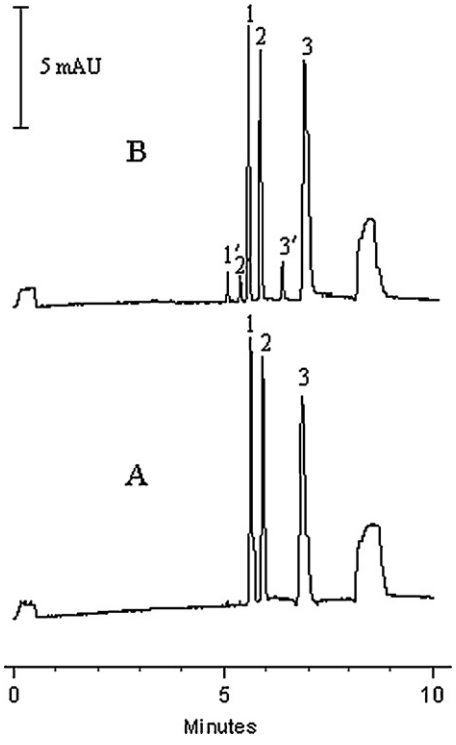

Fig. 2. The electropherograms obtained by MSS with the injection of sample matrix prior to sample injection (A) and without sample matrix injection (B). The sample matrix was 15 mM NH_4HCO_3 and 6 mM SDS (pH 7.5). (A) Injection at 0.5 psi for 5 s for sample matrix and then at 0.5 psi for 30 s for sample (5.0 μg/mL each of the alkaloids). Peak identification: 1 – berberine, 2 – palmatine, and 3 – jatrorrhizine. (B) Injection only for sample at 0.5 psi for 30 s (5.0 μg/mL each of the alkaloids). Peak identification: 1 and 1′, berberine; 2 and 2′, palmatine; 3 and 3′, jatrorrhizine.

pick up the unretained analytes, which could migrate out of the sample zone. Consequently, the small peaks disappeared in Fig. 2B and an improved peak profile of the analytes was obtained. Longer injection times (0.5 psi, 10 or 15 s) of the sample matrix plug were also tried. In both cases, all of the small peaks disappeared but no increase in peak height or improvement in peak shapes was observed. When a much longer injection of sample matrix plug (0.5 psi, 30 s) was applied, the analytes came out as broad peaks and their resolutions decreased. This result was in good agreement with those reported by Quirino et al. [38]. As a result, sample matrix injection was chosen at 0.5 psi for 5 s.

3.2.3. Effect of organic solvents in the BGS

The modification of the BGS by an organic solvent is critical in inducing MSS focusing effect, and the concentration of organic solvent has a significant effect on the peak shape and separation efficiency. The effect of the concentration of methanol in the BGS on the analysis and separation of the cationic alkaloids by MSS-CZE is shown in Fig. 3. The percentage of methanol was changed from 0% to 20%, 30%, 40% and 50% in the BGS of 50 mM NH_4HCO_3 (pH 7.5), respectively. It can be seen from Fig. 3 that small and broad peaks were found when no methanol was added and the resolutions of the alkaloids were improved when the percentage of methanol was increased. This can be explained as that the low percentage

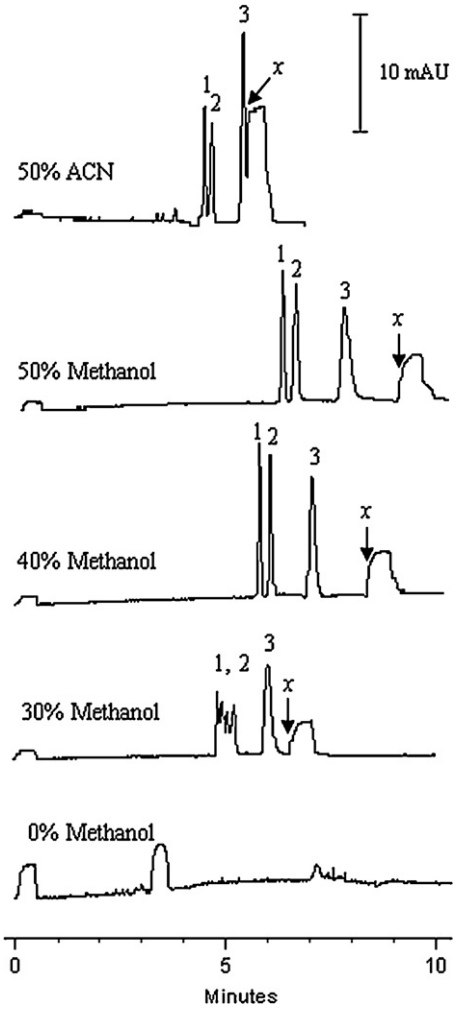

Fig. 3. Effect of organic solvent content in the BGS on MSS-CZE. The BGS consisted of 50 mM NH_4HCO_3 (pH 7.5) with different concentrations (0%, 30%, 40% and 50%) of methanol or 50% ACN. Other conditions are the same as in Fig. 2A. The *x* marks the start of the detection of the sample matrix.

of methanol might be not enough to lower the *k* at the MSSB. The enough high concentration of methanol in BGS could result in a sufficiently low *k* for the analytes that was necessary for MSS. With 40% or 50% methanol in BGS, sharp peaks and good resolutions for the analytes were observed. When methanol was used, the EOF could be reduced [39] and a larger retention time differences between the focused analytes and the sample matrix (*x* in Fig. 3) were achieved. In this work, another commonly used organic solvent in BGS, ACN, was also tested. However, the result showed that ACN was inferior to methanol. When ACN was used as the organic modifier, the sample matrix overlapped with the peak of jatrorrhizine, and the separation between berberine and palmatine was poor (see Fig. 3). The reason for this could be that unlike methanol, the EOF could not be reduced by ACN and this could result in a decreased retention time differences between the focused analytes and the sample matrix. Based on the above experimental results, 40% methanol in BGS was selected.

3.2.4. Effect of the concentration of NH_4HCO_3 in BGS

The effect of the concentration of NH_4HCO_3 in BGS was investigated by changing its concentration in the range from 15 to 75 mM (pH 7.5) while the methanol concentration being kept the same at 40%. The results showed that both peak heights and peak areas were almost the same when the NH_4HCO_3 concentration was increased from 15 to 50 mM, but the peak heights became slightly decreased with some peak broadening when the NH_4HCO_3 concentration was larger than 60 mM. Based on the above experimental result, the concentration of NH_4HCO_3 in BGS was chosen as 50 mM NH_4HCO_3.

3.2.5. Effect of the SDS concentration in sample matrix

To explore the effect of the SDS concentration in sample matrix on the separation, different concentrations of SDS (3.0, 5.0, 6.0, 8.0, 10.0, 20.0 and 30.0 mM) were prepared in 15 mM NH_4HCO_3 (pH 7.5) and used as the sample matrix for investigation. The BGS was 50 mM NH_4HCO_3 containing 40% methanol. As illustrated in Fig. 4, with increased SDS concentration in sample matrix, both the peak heights and peak areas increased first and then decreased. The concentration corresponding to the optimum focusing efficiency is 6 mM. At the SDS concentration of 20 mM and higher, the peak heights greatly decreased and the peaks became broader with a prolonged migration time. This could be explained that the reversal in the effective electrophoretic mobility at the MSSB could not occur for the micelle-bound analytes when the high concentration of SDS was used. Therefore, 6 mM SDS was selected for further studies.

3.2.6. Effect of the concentration of NH_4HCO_3 in sample matrix

With 50 mM NH_4HCO_3 containing 40% methanol as the BGS, the effect of the concentration of the NH_4HCO_3 in the sample matrix was studied by changing its concentration to 5, 10, 15, 30, and 60 mM. As a result, the peak shapes and the resolutions between the analytes were good at 10 or 15 mM NH_4HCO_3. But the peak shapes deteriorated and the resolutions between the peaks decreased when the NH_4HCO_3 concentration was 30 mM. At 60 mM NH_4HCO_3, the separations became more deteriorated with a broad peak of unresolved berberine and palmatine. The currents of the sample matrix with 30 and 60 mM NH_4HCO_3 were 36.9 and 42.1 μA, respectively. With increased concentration of the NH_4HCO_3 in the sample matrix, there was an increased difference in conductivity between the sample matrix and BGS zone (current, 31.9 μA). This could result in destacking in the sample zone and therefore lead to a deteriorated separation for the analytes [35]. For the sample matrix with 15 mM NH_4HCO_3, the ratio of the current of the BGS (30.1 μA) to the sample matrix (31.9 μA) is about 1, indicating that the difference in the conductivity between the sample matrix and BGS was negligible. Consequently, 15 mM NH_4HCO_3 was chosen.

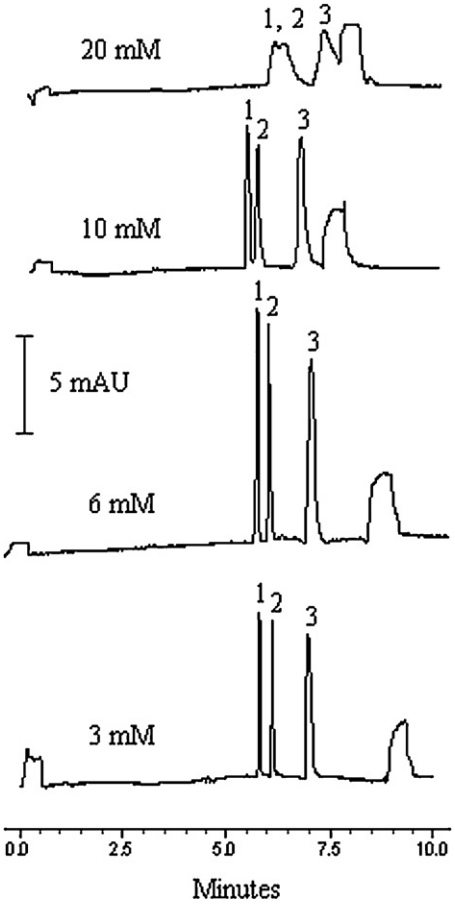

Fig. 4. Effect of SDS concentration in the sample matrix on MSS-CZE. The sample containing 5.0 μg/mL each of the alkaloids was prepared in 15 mM NH_4HCO_3 (pH 7.5) and different concentrations (3.0, 6.0, 10.0, and 20.0 mM) of SDS. Other conditions are the same as in Fig. 2A.

3.2.7. *Effect of the sample injection time*

The effect of sample injection time was studied by injecting at 0.5 psi with changed injection time from 5 to 10, 20, 30, 45, 60, 90 and 120 s. The results showed that both the peak heights and peak areas were increased when the injection time was increased from 5 to 30 s, and no further increase in peak height was observed at the injection time of 45 or 60 s compared with 30 s. Moreover, when the injection time was more than 60 s, the peak heights slightly decreased and the peak shapes became broader, probably due to the diffusion effect. Therefore, an injection of 30-s duration was chosen.

In summary, the optimal experimental conditions for MSS were selected as follows: BGS: 50 mM NH_4HCO_3 (pH 7.5) containing 40% methanol; sample matrix: 15 mM NH_4HCO_3 containing 6 mM SDS (pH 7.5); injection time: at 0.5 psi for 5 s for sample matrix and then at 0.5 psi for 30 s for sample.

3.3. *Analytical performance*

According to the literature [40], the sensitivity enhancement factors (SEFs) of MSS were calculated in terms of corrected peak area (peak area/migration time [39]) using Eq. (2):

$$\text{SEF} = \frac{\text{peak response in MSS}}{\text{peak response in typical injection}} \times \frac{[\text{concentration in typical injection}]}{[\text{concentration in MSS injection}]} \quad (2)$$

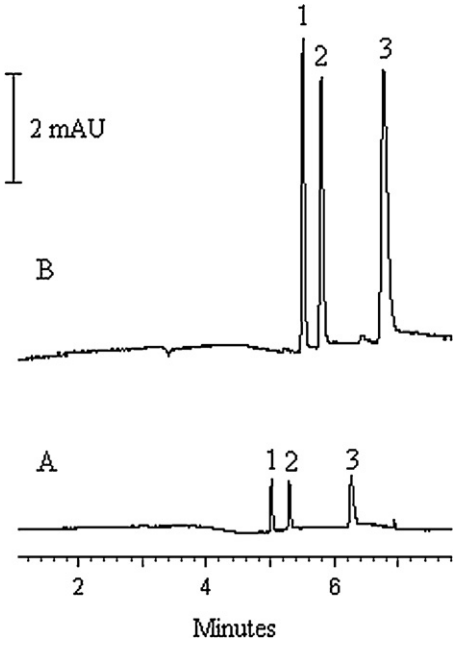

Fig. 5. Comparison of the electropherograms obtained by typical CZE (A) and MSS-CZE (B). (A) Typical CZE: 10.0 μg/mL standard solution of each alkaloid prepared in BGS; injection at 0.5 psi for 5 s. (B) MSS-CZE: 2.0 μg/mL in sample matrix; injection at 0.5 psi for 30 s. Other conditions are the same as in Fig. 2A.

where, the peak response is the corrected peak area. Fig. 5 shows a comparison of the electropherograms for the three cationic alkaloids between the typical CZE (A) and MSS-CZE (B). Compared with the sensitivity obtained with typical sample injection in CZE (0.5 psi, 5 s), the SEFs in terms of corrected peak area were 50, 47 and 53, respectively.

Table 1 lists the linearity (the linear range in both the peak height and corrected peak area as the quantification signal and the determination coefficients (r^2)), limits of detection (LODs), and intraday and interday repeatabilities for the MSS-CZE of the compounds. The linearity was evaluated by plotting both the peak height and corrected peak area of the analytes against their corresponding concentrations. The calibration curves exhibit a good linear behavior over the concentration range of 0.1–10.0 μg/mL with r^2 higher than 0.996 for both the peak height and corrected peak area. The LODs (S/N = 3) obtained for berberine, palmatine and jatrorrhizine were 0.01, 0.01 and 0.02 μg/mL, respectively.

The repeatabilities of the method for the analytes were evaluated in terms of intraday and interday relative standard deviations (RSDs) by parallel extracting and determining the analytes from the spiked human plasma samples at the concentration level of 1.0 μg/mL in the same day (n = 5) and on three consecutive days (n = 15). The intraday RSDs obtained were 3.9–5.8% in peak height and 4.5–6.9% in corrected peak area, and the interday RSDs were 5.3–6.5% in peak height and 5.6–7.3% in corrected peak area, respectively.

3.4. *Recoveries of the method for human plasma samples*

The applicability of the developed MSS-CZE method was examined by applying it for the analysis of human plasma samples. Under the optimum conditions established above, the recovery experiments were carried out by the determination of the three alkaloids in spiked plasma samples. The plasma samples were spiked with a mixture of the three cationic alkaloids (berberine, palmatine and jatrorrhizine) at three different concentration levels (0.2, 1.0 and 5.0 μg/mL, respectively) according to the procedures described in

Table 1
Linearity, limits of detection (LODs) and intraday and interday repeatabilities of the method.

Alkaloids	Intraday RSD% (n = 5)		Interday RSD% (n = 15)		Linear range (μg/mL)	r^2		LOD (S/N = 3) (μg/mL)
	Peak height	Corrected peak area[a]	Peak height	Corrected peak area		Peak height	Corrected peak area	
Berberine	3.9	4.5	5.9	6.1	0.1–10.0	0.998	0.998	0.01
Palmatine	4.3	5.2	5.3	5.6	0.1–10.0	0.999	0.997	0.01
Jatrorrhizine	5.8	6.9	6.5	7.3	0.1–10.0	0.997	0.996	0.02

[a] Corrected peak area = peak area/migration time [35].

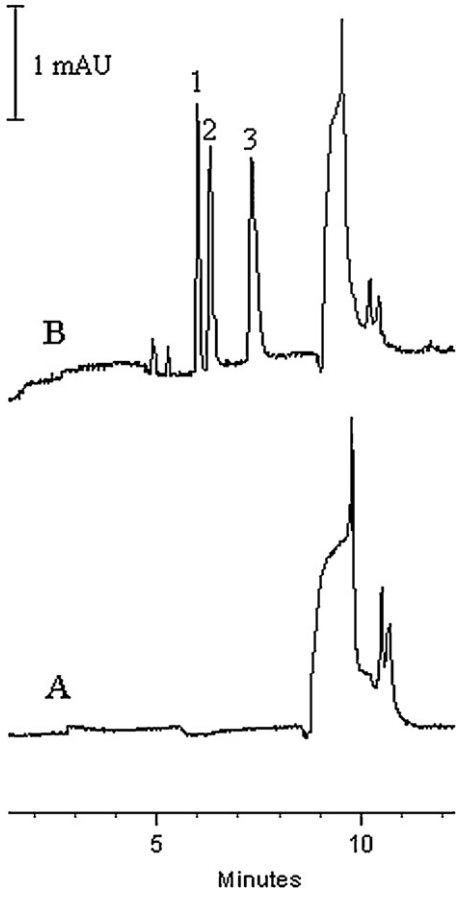

Fig. 6. MSS-CZE electropherograms of human plasma blank (A) and human plasma sample spiked at 1.0 μg/mL each of the alkaloids (B). Other conditions are the same as in Fig. 2A.

Section 2.3. Each level was prepared in triplicate and each sample was extracted and determined three times. The results are presented in Table 2. The recoveries of the method for the three alkaloids were 95.9–101.5% in peak height and 92.6–103.6% in corrected peak area, respectively. A typical electropherogram for the spiked human plasma sample containing each of the alkaloids at 1.0 μg/mL is presented in Fig. 6.

Table 2
Recoveries of the method for the determinations of the three cationic alkaloids in spiked human plasma samples.

Alkaloids	Spiked (μg/mL)	Peak height		Corrected peak area	
		Recovery%	RSD%	Recovery%	RSD%
Berberine	0.2	97.9	5.8	95.0	6.9
	1.0	96.5	5.2	92.6	6.3
	5.0	95.9	6.0	98.9	6.7
Palmatine	0.2	100.1	6.5	99.1	6.0
	1.0	98.0	4.8	94.6	5.3
	5.0	97.5	5.1	103.5	7.9
Jatrorrhizine	0.2	96.9	6.6	95.1	5.7
	1.0	99.5	7.3	102.0	8.1
	5.0	101.5	6.3	103.6	5.9

4. Concluding remarks

In this work, a MSS-CZE method was developed for the simultaneous stacking and determination of three cationic alkaloids in human plasma. Compared with conventional CZE method, a 47- to 53-fold sensitivity increase in corrected peak area was obtained. The method has a good precision and sensitivity, and is suitable for the analysis of the alkaloids in human plasma samples

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