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Separation and determination of strychnine and brucine in *Strychnos nux-vomica* L. and its preparation by nonaqueous capillary electrophoresis

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Abstract

An easy, rapid method for simultaneous determination of strychnine and brucine in *Strychnos nux-vomica* L. and its preparation was developed by nonaqueous capillary electrophoresis (NACE) without pretreatment for the first time. Optimum separation was achieved with a fused-silica capillary column (50 cm × 75 μm i.d.) and a running buffer containing 30 mM ammonium acetate, 1.0% acetic acid and 15% acetonitrile (ACN) in methanol medium. The applied voltage was 30.0 kV. The analytes were detected by UV at 214 nm. The effects of concentration of ammonium acetate, acetic acid and organic modifier on electrophoretic behavior of the analytes were studied. The established method with sophoridine as internal standard was linear in the range of 5–1000 mg/mL for both strychnine and brucine. The extracts of *Strychnos nux-vomica* and its preparation could be directly injected for determination with recoveries ranging from 94.5 to 104%.

Keywords: Strychnine; Brucine; Nonaqueous capillary electrophoresis; *Strychnos nux-vomica* L.

1. Introduction

Strychnos nux-vomica L. (Loganiaceae), a traditional Chinese medicine, is used as an anti-inflammatory and analgesic drug to relieve arthritic and traumatic pains [1]. It contains alkaloids, mainly strychnine and brucine [1]. It has been demonstrated that strychnine and brucine, which are stimulus for the central nervous system, cause the sense organ to be more sensitive, stimulate the visceral organs and urogenital tract [2]. Therapeutic dose was reported to produce a tonic effect on the alimentary canal, a limited amount of respiratory and vasomotor stimulation [2]. However, the margin between useful and dangerous doses is very narrow as it was reported to be fatal to man at doses of 30–90 mg [3,4]. Although they can improve the pulse, raise blood pressure and deepen respiration at low doses, they will result in the symptoms such as violent convulsion, great rise of blood pressure and even leading to lethal poisoning at high doses [5]. Therefore, a simple, rapid and sensitive method for the quantification of the two alkaloids in *S. nux-vomica* L. and its preparation is of interest.

TLC [6] and HPLC [7] have been the commonly used methods for the separation and determination of active constituents in *S. nux-vomica* L. and its preparation. But few of these methods are not entirely adequate because of poor accuracy, low resolution or the requirement of tedious pretreatment. High performance capillary electrophoresis (HPCE) has proved to be a highly efficient separation technique in pharmaceutical industry [8–12]. Meanwhile, unsatisfactory peak shapes were often obtained for alkaloids. According to our knowledge, there has been little research on *S. nux-vomica* L. and its preparation by CE methods [13–15] and there is no report about the determination of strychnine and brucine by nonaqueous capillary electrophoresis (NACE).

In recent years, nonaqueous capillary electrophoresis, which is based on the use of electrolyte solutions prepared from pure organic solvents, has become an active area of study. NACE offers a number of attractive features such as alteration of selectivity, reduced electrophoretic currents and improved mass spectrometric compatibility, solubility and stability of hydrophobic compounds. One of the most attractive features of

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organic solvents is that they greatly differ in physical and chemical properties (viscosity, dielectric constant, polarity, auto-protolysis constant, electric constant conductivity, etc.) with water, allowing a simple selective manipulation in NACE by changing the organic solvents or varying the proportions of two solvents [16,17]. Accordingly, NACE was successfully applied to analyze a large number of pharmaceuticals, including acidic and basic drugs, chiral compounds, peptides, ions and preservatives [18,20–24].

In this work, a nanoqueous capillary electrophoresis (NACE) method for the analysis of strychnine and brucine was presented because the solubility of strychnine and brucine in methanol (1 g/260 mL) is more than that in water (1 g/6400 mL) [25]. After optimization of the separation conditions, the two compounds can be quantified within 5 min with detection limits of 0.02 mg/L for strychnine and 0.01 mg/L for brucine. The method was applied to the analysis of the two alkaloids in *S. nux-vomica* L. and Yaotongning capsule with recoveries of 94.5–104% for strychnine and 95.8–101% for brucine.

2. Experiments

2.1. Instruments

A Waters Quanta 4000 Capillary Electrophoresis System (Milford, MA, USA) controlled by a personal computer was used. Capillary electrophoresis was performed using a 50.0 cm (42.5 cm to the detector) × 75 μm i.d. fused silica capillary (Yongnian Photoconductive Fibre Factory, Hebei Province, China). Samples were introduced from the anodic end of the capillary by hydrodynamic injection by rasing the sample vial 10.0 cm for 5 s. Direct UV detection was employed at a wavelength of 214 nm. Data acquisition was carried out with a Maxima 820 chromatography workstation. The capillary was conditioned prior to use with 0.1 M NaOH for 10 min and distilled water for 5 min, followed by the electrophoresis buffer for 5 min; between two runnings, the capillary was equilibrated with running buffers for 2 min. All operations were performed at 25.0 ± 0.5 °C. To avoid buffer and sample evaporation, the buffer and sample reservoirs were capped.

2.2. Reagents

The strychnine, brucine and sophoridine (their structures are shown in Fig. 1) were purchased from Beijing Medicine Control Institute, China. Ammonium acetate and acetic acid were purchased from Tianjin First Chemical Factory. ACN was purchased from Tianjin Secondary Chemical Factory. Methanol was purchased from Shanghai Zhenxing First Chemical Factory. *S. nux-vomica* L. and Yaotongning capsule (Hebei Chengde Pharmaceutical Company) were purchased from Lanzhou Zhongyou Pharmaceutical Company, China. All reagents were of analytical grade.

2.3. Preparation of the stock solution and the electrolytes

Stock solutions of strychnine (1000 mg/L) and brucine (1000 mg/L) were prepared in methanol; sophoridine (1800 mg/

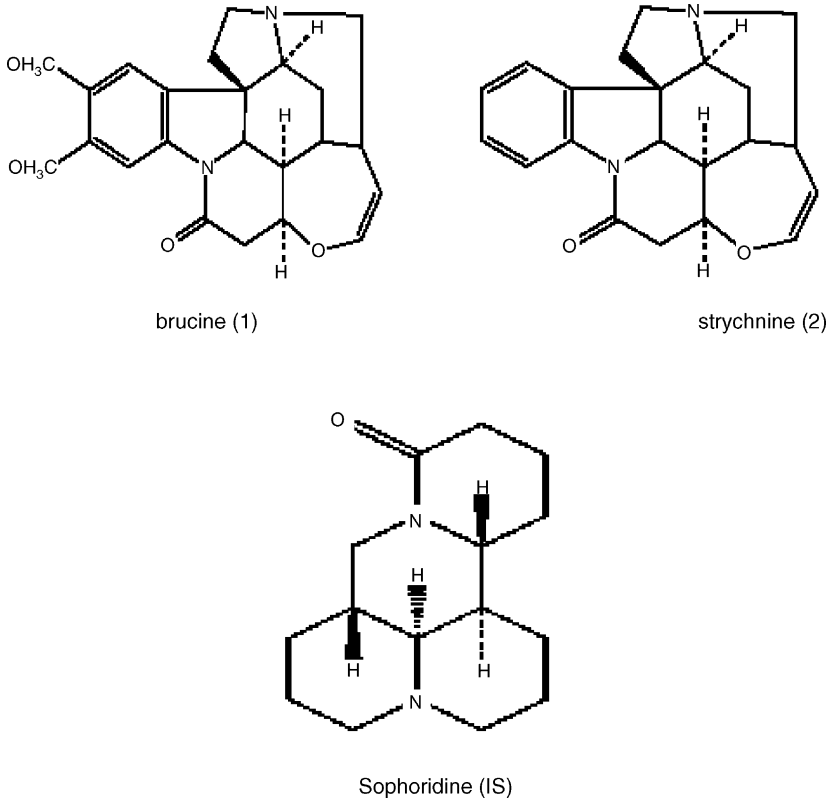

Fig. 1. The structures of the analytes.

L) as internal standard (IS) was prepared in methanol. Solutions of lower concentration were prepared by dilution of the stock solutions in the appropriate with methanol. The running buffer solutions were prepared by adding 15 mL 200 mM NH_4OAc (methanol medium), 1.0 mL acetic acid and 15 mL ACN in a 100 mL flask and diluting to 100 mL with methanol. All solutions for CE were filtered through a 0.45 μm filter.

2.4. Sample preparation

2.4.1. NH_4OH–$CHCl_3$

One gram powdered *S. nux-vomica* L. was wetted with 1.0 mL ammonium hydroxide, then added 50 mL $CHCl_3$, capped and then shaken for 20 min. It was placed for 24 h and then filtered. The residue was washed three times with $CHCl_3$. The filtrate was combined and evaporated to near dryness. And then the residue dissolved with mixture methanol to 25 mL with methanol and spiked by 5.0 mL of sophoridine solution (4.5 mg of sophoridine/mL methanol).

2.4.2. NH_4OH–MeOH

One gram powdered *S. nux-vomica* L. was wetted with ammonium hydroxide (1.0 mL) for 10 min before being extracted with 7.0 mL methanol for 30 min in an ultrasonic bath and filtered. Extraction was repeated three times. The filtrate was combined. The mixture was diluted to 25 mL with methanol and spiked by 5.0 mL of sophoridine solution (4.5 mg of sophoridine/mL methanol). The method of extraction of Yaotongning capsule is the same as above.

2.4.3. 50% Ethanol

One gram powdered *S. nux-vomica* L. was soaked with 70 mL distilled water, boiled and then cooked for 30 min with weak fire. It was cooled and filtered water. The residue was washed three times with water. The filtrate was combined and evaporated to near dryness. And then the residue dissolved with mixture methanol to 25 mL with methanol and spiked by 5.0 mL of sophoridine solution (4.5 mg of sophoridine/mL methanol).

2.4.4. Water

One gram powdered *S. nux-vomica* L. was soaked with 50 mL distilled 50% ethanol, shaken for 20 min and then placed for 24 h. The ethanol was poured and filtered. The residue was washed three times with water. The filtrate was combined, extracted three times with $CHCl_3$ and then evaporated to near dryness. Finally, the residue dissolved with mixture methanol to 25 mL with methanol and spiked by 5.0 mL of sophoridine solution (4.5 mg of sophoridine/mL methanol).

All the solutions were passed through a 0.45-μm filter, and were injected directly into the capillary electrophoresis system. The measured results are listed in Table 1. From Table 1, it was found that the extraction efficiency of different solvents was very different. Though the extraction efficiency of NH_4OH–$CHCl_3$ was a little higher than that of NH_4OH–MeOH, but the method of NH_4OH–$CHCl_3$ was complicated than that of NH_4OH–MeOH.

Table 1
The extraction efficiency of different solvents

Solvent	Strychnine (%)	Brucine (%)
NH_4OH–$CHCl_3$	23.60	12.68
NH_4OH–MeOH	21.84	11.49
50% Ethanol	19.94	9.785
Water	14.88	8.941

So the NH_4OH–MeOH method was chosen for the optimum extraction.

2.5. Calculation

To achieve satisfactory separations, the NACE parameters were optimized taking the resolution and migration time as the principle figures of merit. In this work, the effects of acetic acid, ammonium acetate and ACN concentration, and voltage on migration behavior and resolution of the analytes were taken into consideration for obtaining optimized separation conditions. A univariate procedure was used for optimization of the above conditions. Medium values of the ranges were used as fixed parameter in preliminary univariate studies and the parameters were gradually adjusted to close-to-optimum value for the final univariate studies.

2.5.1. Calculation of resolution

The resolution R_s is calculated by the equation used in conventional chromatography:

$$R_s = \frac{2\,\Delta t_R}{W_1 + W_2} \tag{1}$$

where W_1 and W_2 are peak widths at the baseline and Δt_R is the difference in migration times.

2.5.2. Calculation of mobility

In this method, MeOH was used a neutral marker for determination electroosmotic flow mobility (EOF), so the EOF was calculated by Eq. (2):

$$\mu_{EOF} = \frac{lL}{V} \cdot \frac{1}{t_0} \tag{2}$$

where L is the total length of the capillary, l the effective length of the capillary, V the separation voltage and t_0 is the migration time of MeOH. So the effective mobility (μ_{eff}) of the analytes was calculated as:

$$\mu_{eff} = \mu_{EOF} + \frac{lL}{V} \cdot \frac{1}{t_m} \tag{3}$$

where t_m is the migration time of the analytes.

3. Results and discussion

3.1. Choice of organic solvent and electrolyte

In selecting the proper solvent for NACE, several characteristics of the solvents should be considered including

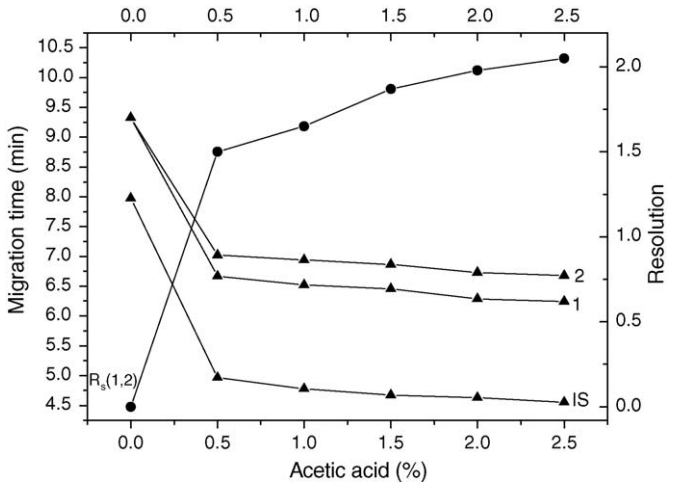

Fig. 2. Effect of acetic acid concentration on the migration time and resolution of strychnine and brucine. Buffer: acetic acid, concentration 0–2.5%, 30 mM ammonium acetate and 10% ACN. Applied voltage: 25 kV. Cartridge temperature: 25.0 ± 0.5 °C. Detection: 214 nm. Strychnine (1) and brucine (2). (▲) Migration time; (●) resolution.

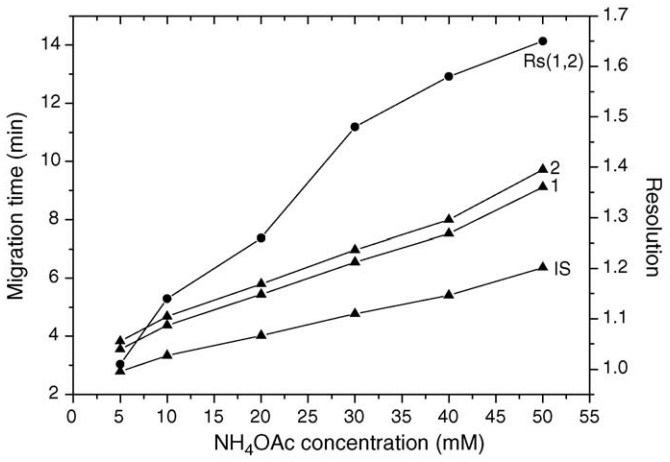

Fig. 3. Effect of ammonium acetate concentration on the migration time and resolution of strychnine and brucine. Buffer: 1.0% acetic acid, 5–50 mM ammonium acetate and 10% ACN. The experiment conditions are same as Fig. 2. (▲) Migration time; (●) resolution. Strychnine (1) and brucine (2).

viscosity, dielectric constant, electrical and thermal conductivity, self-dissociation constant, polarity and boiling point. The most polar solvents like methanol, ACN, formamide, *N*-methylformamide (NMF), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO) and mixtures of methanol and ACN are commonly used in NACE. Bjornsdottir and Cherkaoui reported that methanol and ACN were the best solvents when UV detection was selected [18,19]. In addition, electrophoretic medium containing a mixture of solvents was found particularly advantageous to achieve high selectivity. Moreover, ammonium acetate is the most common electrolyte in NACE system. Therefore, the first investigations were carried out using 30 mM ammonium acetate and 10% ACN (v/v) buffer with methanol as solvent.

3.2. Effect of concentration of acetic acid

The acidity of the electrophoretic medium is also a governing factor in separation of ionizable analytes because it determines the extent of ionization of each individual analyte in NACE. In this paper, the acidity of buffer was adjusted using acetic acid. To verify the effect of acetic acid concentration (0–2.5%, v/v) on the resolution and migration time of the analytes, experiments were performed with 30 mM ammonium acetate and 10% (v/v) ACN as the electrophoretic medium, and applied voltage 25 kV (Fig. 2). As shown in Fig. 2, the migration time of IS, strychnine and brucine decreased markedly in the concentration range of 0–0.5%, when concentration of acetic acid was more than 0.5%, the migration times of IS, strychnine and brucine slightly altered. But, the resolution of strychnine and brucine increased significantly in the concentration range of 0–2.5%. Considering the total analysis time and resolution, 1.0% acetic acid concentration was chosen as the optimum.

3.3. Effect of concentration of ammonium acetate

Buffer concentration has markedly affected the separation because it could influence the EOF and the viscosity of the electrolyte. In order to obtain the best resolution of the two analytes, the effect of the concentration of ammonium acetate was investigated in the range from 5 to 50 mM with 10% (v/v) ACN, 1.0% acetic acid and 25 kV applied voltage. As illustrated in Fig. 3, the migration time and the separation of the two analytes were significantly improved with the increasing of ammonium acetic concentration. From Fig. 4, it was found their detection sensitivities increased with the ammonium acetate concentration increasing, and the most detection sensitivity was obtained in the concentration range of 30–40 mM. When the ammonium acetate

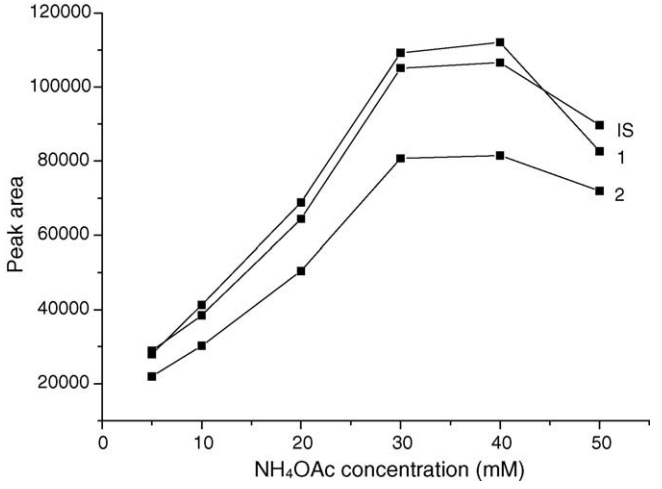

Fig. 4. Effect of ammonium acetate concentration on the peak area of strychnine and brucine. Buffer: 1.0% acetic acid, 5–50 mM ammonium acetate and 10% ACN. The experiment conditions are same as Fig. 2. (▲) Peak area. Strychnine (1) and brucine (2).

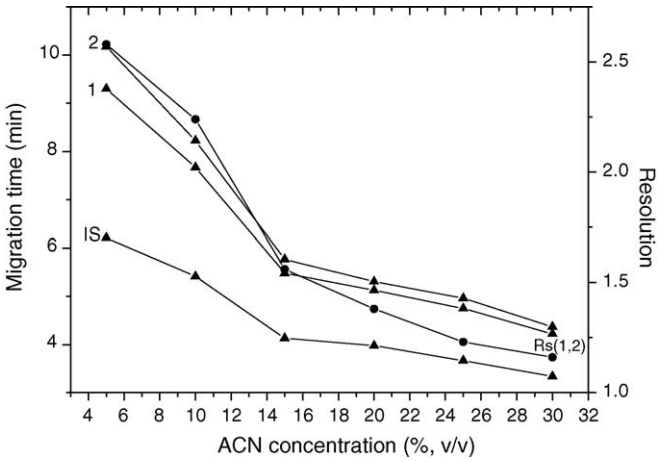

Fig. 5. Effect of ACN concentration on the migration time and resolution of strychnine and brucine. Buffer: 1.0% acetic acid, 30 mM ammonium acetate and 5–30% ACN. Other conditions are same as Fig. 2. (▲) Migration time; (●) resolution. Strychnine (1) and brucine (2).

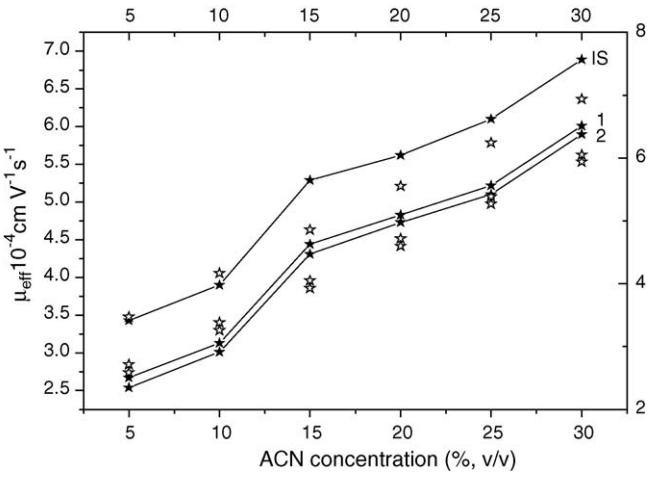

Fig. 6. Effect of ACN concentration on the effective mobility of the analytes. Buffer: 1.0% acetic acid, 30 mM ammonium acetate and 5–30% ACN. Other conditions are same as Fig. 2. (⋆) Experiment results; (☆) simulating results. Strychnine (1) and brucine (2).

concentration was more than 40 mM the detection sensitivity decreased markedly. Considering the resolution and detection sensitivity, 30 mM ammonium acetate was selected for subsequent experiments.

3.4. Influence of ACN concentration

In previous studies concerning the application of NACE to the analysis of pharmaceutical drugs, it was demonstrated that the organic solvent composition has a critical effect on resolution,

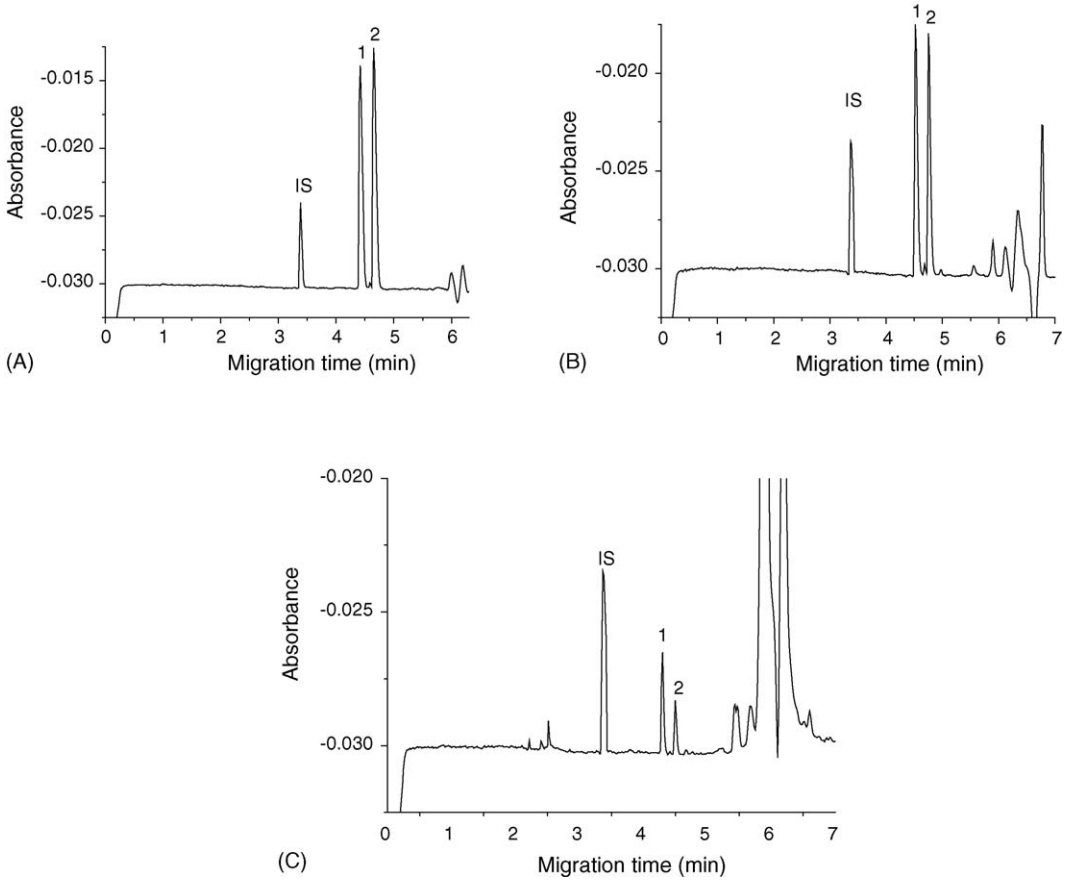

Fig. 7. The electropherograms of the standards mixture solution and the samples. (A) The standards mixture. (B) Extract of *Strychnos nux-vomica* L. (C) Extract of Yaotongning capsule. Buffer: 1.0% acetic acid, 30 mM ammonium acetate and 15% ACN. Applied voltage: 30 kV. Other conditions are same as Fig. 2. Strychnine (1) and brucine (2).

Table 2
Simulation parameters of Eq. (4)

Compound	A	B	R
IS	2.782	0.1385	0.9834
1	2.047	0.1335	0.9838
2	1.916	0.1342	0.9841

efficiency and migration time [18,19]. Thus, the ACN percentage in buffer was examined between 5 and 30%. The effect of ACN concentration on migration time and resolution of the analytes is shown in Fig. 5. As shown in Fig. 5, the migration times of the two analytes and resolution were considerably decreased when the ACN percentage in buffer was increased. This behavior is mainly due to the modification of dielectric constant to viscosity ratio. When the concentration of ACN was more than 15%, impurity peak between strychnine and brucine overlapped partly with that of brucine. Considering the analysis time and the resolution of the impurity peak and brucine, 15% (v/v) ACN was a good compromise for a rapid separation with a high resolution.

Furthermore, it was found that the effective mobility of the analytes was a function of acetonitrile concentration (c, %). By simulating the experiment results, Eq. (4) was proposed to explain the influence of acentonitrile concentration on the effective mobility of the analytes.

$$\mu_{\text{eff}} = A + Bc \quad (4)$$

where A and B are constants. In Fig. 6, the scatter graph and line graph represent the experiment and simulating results, respectively. The simulating parameters are listed in Table 2. A high correlation relationship (correlation coefficients >0.98) between the experiment and simulating results was obtained. As Eq. (4) indicated, the effective mobility of the analytes increased with the increasing of ACN concentration. This can be explained by the change of the viscosity and relative dielectric constant of the buffer in the MeOH–ACN mobile phase, which resulted in the change of the electrophoretic behavior of the analytes.

3.5. Effect of applied voltage

High voltage is required in CE to reduce the analysis time. In this paper, the effect of applied voltage was tested in the range of 20–30 kV. The results showed that the migration times were shortened and resolution of the two analytes decreased with increased applied voltage (resolution from 1.67 to 1.32), and the separation efficiency was improved. Based on the experiment, 30 kV was selected for a complete separation of all compounds in a short time with an acceptable electric current (66 μA).

Table 4
Contents of the analytes in samples (n = 3, mg/g)

Sample	Strychnine	R.S.D. (%)	Brucine	R.S.D. (%)
Strychnos nux-vomica L. (mg/g)	21.9	1.58	11.7	1.62
Yaotongning capsule (mg/capsule)	1.27	2.03	0.581	2.24

Table 5
Recovery of the two analytes (n = 6)

Sample	Strychnine		Brucine	
	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)
Strychnos nux-vomica	500	98.8	500	96.1
	250	104	250	100
	100	94.5	100	99.7
R.S.D. (%)		4.80		2.20
Yaotontning capsule	250	95.7	250	95.8
	100	97.0	100	97.7
	50	102	50	101
R.S.D. (%)		3.39		3.27

According to that mentioned above, the best resolution was obtained with an electrolyte containing 30 mM ammonium acetate, 1.0% acetic acid, 15% ACN and 30 kV applied voltage. The typical electropherograms for a standard mixture under the optimum conditions are shown in Fig. 7A. The two analytes were well separated within 5 min.

3.6. Linearity, repeatability and detection limit

An eight-point calibration curve for each alkaloid was established by peak area ratio of analyte to IS against the concentration ratio of the analyte to IS. Each solution was spiked with an IS concentration of 900 mg/L. The calibration curves exhibit excellent linear behavior over the concentration range (5–1000 mg/L for strychnine, and 5–1000 mg/L for brucine). The results are given in Table 3.

The detection limit was defined as the concentration where the signal-to-noise ratio is 3. The detection limits are also given in Table 2.

The repeatability of the migration time and the peak area ratio of the analyte to IS of strychnine and brucine in the experiment were determined by repeated injection (n = 6) of a standard mixture solution of 500, 250 and 100 mg/L of strychnine and brucine under the optimum conditions, respectively. The relative standard deviations (R.S.D.) of the migration time and peak area

Table 3
The results of regression analysis on calibration curves and the detection limits

Analyte	Regression equation, $Y = a + bx$[a]	Correlation coefficient	Linear range (mg/L)	Detection limit (mg/L)[b]
Strychnine	$Y = -0.00331 + 4.333x$	0.9999	5–1000	0.02
Brucine	$Y = 0.00217 + 4.741x$	0.9999	5–1000	0.01

[a] Y and x stand for the peak area ratio and the concentration ratio of the analyte to internal, respectively.
[b] The detection limit was defined as the concentration where the signal-to-noise ratio is 3.

Table 6
The comparison of the present method to others

	Sample preparation	Capillary length (cm)	Buffer conditions	Analysis time (min)	Line range (μg/mL)	Detection limit (μg/mL)/λ (nm)	Analytes/ application
CZE [13]	A home-made pressurized liquid extraction (PLE)	76.0 cm × 75 μm i.d. uncoated fused-silica capillary tube	50 mM ammonium acetate, pH 3.1 (adjusted by acetic acid)	15	0–25	2.06/254	Berberine and strychnine/medicinal plants and their preparations
CZE [14]	Sample was wetted with ammonium hydroxide before soaked with chloroform and then heated under reflux. Chloroform was dried in vacuum, and the residue was dissolved in methanol	57.5 cm × 75 μm i.d. uncoated fused-silica capillary tube	20 mM ammonium acetate containing 0.2 mM acetic acid (pH 3.64)	5	5–100	0.04–0.06/214	Strychnine and brucine/*Strychnos nux-vomica* L. and Zhishangxiaoyu pills
Field-enhanced stacking CZE [15]	Sample was extracted with distilled water in an ultrasonic bath	47 cm × 75 μm i.d. uncoated fused-silica capillary tube	100 mM NaH_2PO_4 (pH 4.5)	5	0.08–1.2; 0.07-1.05	0.001–0.014/ Diode-array	Strychnine and brucine/*Strychnos nux-vomica* L.
NACE (the present method)	Sample was soaked with ammonium hydroxide (10 min), and then extracted with methanol for in an ultrasonic bath	50 cm × 75 μm i.d. uncoated fused-silica capillary tube	30 mM ammonium acetate, 1.0% acetic acid and 15% *acetonitrile* methanol medium.	5	5–1000	0.02–0.01/214	Strychnine and brucine/*Strychnos nux-vomica* L. and Yaotongning capsules

ratio of analyte to IS of each peak were 1.1–1.6 and 1.8–2.6% (intra-day), and 2.6–3.7 and 3.2–4.8% (inter-day), respectively.

3.7. Application and recovery

The optimum conditions were applied to the separation and determination of strychnine and brucine in the extracts of *S. nux-vomica* L. and its preparation. A typical electropherogram obtained is shown in Fig. 7B and C. The peaks were identified by comparison with previously obtained migration times and standard spike.

The contents of the analytes found in the *S. nux-vomica* L. and its preparation together with their relative standard deviations (R.S.D.) are given in Table 4.

In order to determine recovery, strychnine and brucine were added to the samples in known concentrations, and were then analyzed according to the proposed procedure. The results are listed in Table 5.

The present method was compared to others. The compared results are listed in Table 6. From Table 6, it was observed that the present method was applicable in the analysis of strychnine and brucine, and it was easy to use for the analysis of *S. nux-vomica* L. and its preparation. Especially, it is very simple and sensitive.

4. Conclusion

These results demonstrate that the NACE method proposed in this study was useful, simple and repeatable for identification and determination of analytes strychnine and brucine in *S. nux-vomica* L. and its preparation using direct on-column UV detection. The proposed NACE method is a good alternative for simultaneous analysis of these bioactive components in *S. nux-vomica* L. and its preparation. As an efficient technique it may be used for the investigation of other *S. nux-vomica* L. preparation in Chinese traditional herbs.

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