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Short communication

Determination of five toxic alkaloids in two common herbal medicines with capillary electrophoresis

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Abstract

A method was developed for the determination of five highly toxic alkaloids in two commonly used herbal medicines by capillary electrophoresis, which had not been applied to the determination of *Aconitum* alkaloids before. The buffer contained 40 m*M* ammonium acetate and 0.1% acetic acid in 80% methanol. Five alkaloids can be determined in 15 min by a single run. The calibration curves showed a linear range from 2 to 200 mg/l for these alkaloids with correlation coefficients (R^2) between 0.9988 and 0.9999. Detection limits ($S/N=3$) varied from 0.85 to 1.90 mg/l. Recoveries ranged from 95 to 108.8%. The method can provide an effective tool for the strict control of these fetal herbal medicine components.

Keywords: Herbal medicines; Alkaloids; Aconitine; Hypaconitine; Mesaconitine; Strychnine; Brucine

1. Introduction

Aconite root (*Wutou*) and the seed of *Strychnos pierrian* (*Maqianzi*) are two typical Chinese medicines that can cause serious toxic effects in humans [1]. *Aconite* root contains various toxic alkaloids, including aconitine, hypaconitine and mesaconitine. The LD_{50} of aconitine for mice is 0.3 mg/kg. Usually, this herb exhibits cardiotonic, anti-inflammatory and analgesic effects [2]. The seed of *Strychnos pierrian* contains strychnine and brucine at concentrations of about 1 to 1.4% each. Both the strychnine and brucine are toxic, the LD_{50} of brucine in mice is 3.03 mg/kg. The herb can act as a stimulus for the nervous system [3], and it also acts as a stomachic, an expectorant and an anti-bacterial agent.

A main obstacle for the worldwide use of Chinese herbal medicines is how to quantitate the exact active components in herbs [4]. Moreover, almost all herbs need to be prepared before their use by patients. For example, the *Aconite* root should be boiled to convert *Aconitum* alkaloids to other innocuous alkaloids [5,6]. Therefore, efficient determination procedures are very important for the development of herbal medicine. The traditional methods for the determination of those alkaloids include colorimetry by adding dyes, thin-layer chromatography and liquid chromatography. However, none of those methods has provided the separation of all of those five alkaloids.

Capillary electrophoresis (CE) needs only a small

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amount of standards and can analyse samples rapidly. The strong separation efficiency of CE can provide better resolution and make itself one of the best tools in the separation and analysis of ingredients in herbal medicine. Many applications of CE in determinations of alkaloids [7–10] and other components [11–13] appeared recently. Several studies have reported methods for the determination of strychnine and brucine [3,14–16]. However, there has not been any report on the determination of *Aconitum* alkaloids (aconitine, hypaconitine and mesaconitine) based on CE to date. This research establishes a new method to determine five toxic alkaloids in both *Aconite* root and seeds of *Strychnos pierrian*. The combination of these two herbs is used as antirheumatic drug in Chinese herbal medicine.

2. Experimental

2.1. Chemicals and instrumentation

Brucine, aconitine, hypaconotine and mesaconotine were supplied by the National Institute for the Control of Pharmaceutical and Biological Products of China (NICPBP, Beijing, China). Strychnine was purchased from Sigma (St. Louis, MO, USA). Atropine (Fluka, Buchs, Switzerland) was used as internal standard (I.S.). All other reagents were of analytical grade. Water ($\geq$18 MΩ) used throughout the experiments was generated by a NANOpure ultrapure water system (Barnstead, IA, USA).

A laboratory-built CE system was comprised of Model CZE 1000R (Spellman, Plainview, NY, USA) high-voltage supply, and an SPD-10A UV–Vis detector from Shimadzu (Kyoto, Japan). Detection was at 200 nm because it provided more sensitive detection than longer wavelengths. The separation voltage was 15 kV. Data acquisition and recording of electropherograms were accomplished with a CSW Chromatography Station (CE Resources, Singapore).

Fused-silica capillaries (50 μm I.D.) were the products of Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillaries was 50.0 cm, and the effective length from the injection end to the detection window was 38.0 cm. The capillary was rinsed with 0.1 *M* NaOH for 15 min, followed by water and the appropriate electrolyte solution for 15 min each. The running electrolyte was refreshed after 10 runs.

2.2. Procedures

All standard solutions of alkaloids were prepared in methanol. Stock solutions were stored at 4 °C and renewed every week. The separation electrolyte was prepared daily, and contained 40 m*M* ammonium acetate and 0.1% acetic acid in 80% methanol. Samples were introduced into the capillary by hydrodynamic mode for 15 s (8 cm height).

A Chinese prepared medicine (CPM) (*Shen jin huo luo wan*, Mingshantang, China), which contains prepared *Aconite* root, prepared seed of *Strychnos pierrian* and other six herbs, was investigated. A 1-g amount of pulverized drug was immersed in 5 ml methanol at room temperature for 10 h. After 30 min ultrasonication, the solution was made up to volume with methanol and passed though 0.20-μm filters.

3. Results and discussion

3.1. Optimization of running buffer system

Aqueous buffer systems with ammonium acetate were tried first. Although strychnine and brucine can be separated from other alkaloids, a poor resolution among *Aconitum* alkaloids was observed, and the resolution could not be improved by changing buffer pH values or replacing ammonium acetate by citric acid, glycolic acid or lactic acid. Subsequently, nonaqueous buffer systems were evaluated. A buffer containing 50% methanol could not achieve complete separation of aconitine, hypaconitine and mesaconitine, and a buffer containing 100% methanol gave a good separation, but a much longer analytical time was observed. So the buffer system with 80% methanol was selected as the optimum percentage of methanol.

To help the ionization of alkaloids, a small amount of acetic acid was added to the running buffer to maintain an acidic environment. The migration velocities of the alkaloids may change depending upon different ionization degrees under different acid concentrations. It is found that both 0.1 and 2%

acetic acid can achieve the separation of mesaconitine and aconitine with different orders, but 0.5% acetic acid led to co-migration of the two alkaloids. Because the high ionic strength caused an unstable baseline and large background noise in this buffer which contained a relatively high concentration of organic solvent, 0.1% acetic acid was applied in the following experiments.

The concentrations of ammonium acetate (20, 40, 50, 80 m*M*) were investigated also. 20 m*M* ammonium acetate caused too fast an electroosmotic flow (EOF) for baseline separation of the five alkaloids, meanwhile, the high ionic strength resulting from the high concentration of ammonium acetate also caused an unstable baseline. 40 m*M* ammonium acetate worked well based on our experiments. Baseline separation of alkaloids could still be accomplished with a sample loading of 20 s with 8 cm height, 15 s was set as the optimal sample loading time.

The inaccuracy resulting from the fluctuation of the EOF and sample loading among different runs can be minimized by adding an internal standard. Atropine was selected as internal standard because it can provide similar peak height and migration time comparing with other alkaloids. A typical electropherogram for the separation of five alkaloids and atropine is shown in Fig. 1. All the alkaloids were separated in less than 15 min.

3.2. Quantification

Standard mixtures of alkaloids at six concentration levels were measured and peak areas were calculated by comparing the peak areas of alkaloids with the peak area of atropine. Calibration curves of all the alkaloids exhibited good linear dynamic ranges from 2 to 200 mg/l with the correlation coefficients (R^2) between 0.9988 and 0.9999 (Table 1). The repeatabilities of peak areas (RSD, 0.92–2.02%) were measured by six injections of a solution containing 20 mg/l of each alkaloid. Detection limits ($S/N=3$) of the five alkaloids varied from 0.85 to 1.90 mg/l.

3.3. Sample analysis

Experimental data proved that the concentrations of alkaloids did not change after 30 min ultrasonication. The method was used for the determination of alkaloid concentrations in a CPM sample. A 1-g amount of medicine was extracted by methanol and condensed to 1 ml by evaporation under room temperature. The peaks of hypaconitine, strychnine and brucine were found (Fig. 2). The concentrations

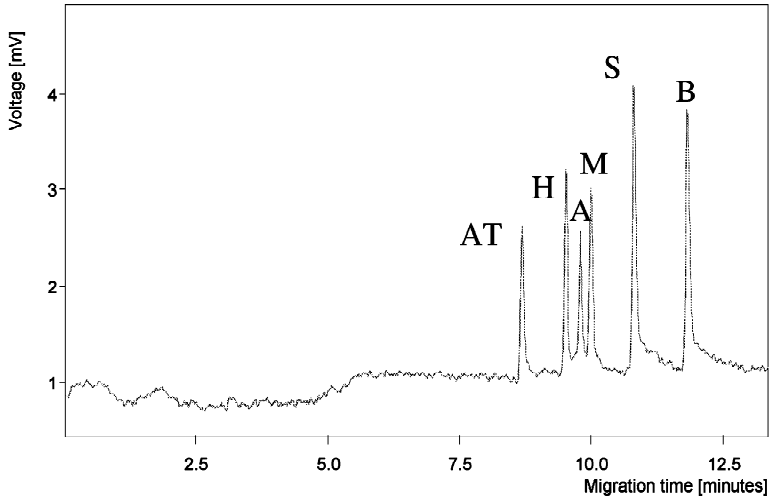

Fig. 1. Separation of alkaloids and internal standard. Buffer contains 40 m*M* ammonium acetate, 80% methanol and 0.1% acetic acid. Applied voltage, 15 kV. Sample introduction, hydrodynamic mode 8 cm for 10 s. Alkaloids: H, 30 mg/l hypaconitine; A, 20 mg/l aconitine; M, 30 mg/l mesaconitine; S, 30 mg/l strychnine; B, 30 mg/l brucine; AT, 20 mg/l atropine.

Table 1
Calibration curves, reproducibilities and limits of detection

Alkaloid	Calibration curve	Correlation coefficient (R^2)	Detection limit (mg/l)	RSD (%) (n=6)
Hypaconitine	$y=0.2124x-0.4179$[a]	0.9999	1.57	1.61
Aconitine	$y=0.2082x-0.3287$	0.9998	1.59	2.02
Mesaconitine	$y=0.2253x-0.4668$	0.9996	1.90	1.27
Strychnine	$y=0.6037x-1.8333$	0.9988	0.87	0.92
Brucine	$y=0.6095x-1.6937$	0.9990	0.85	1.20

[a] x, Concentration of alkaloids (mg/l); y, peak area (mV s).

of alkaloids in the original CPM sample were 0.60 g/kg for strychnine, 0.29 g/kg for brucine and 6.0 mg/kg for hypaconitine. The sample solution was spiked with 20 mg/l each of the alkaloids before the extraction. Recoveries of alkaloids were 99.5, 104.1, 95.0, 107.3 and 108.8% for hypaconitine, aconitine, mesaconitine, strychnine and brucine, respectively.

As mentioned previously in the Introduction, the *Aconite* root in the CPM should have been prepared to reduce its toxicity. Our result showed that the hydrolysis of toxic alkaloids does cause very low concentrations of those alkaloids. To confirm this, the CPM sample was extracted with 1 *M* hydrochloric acid, and then the solution was extracted by chloroform. No distinct peaks for *Aconitum* alkaloids were found from this CPM sample while clear peaks of *Aconitum* alkaloids were acquired in the application of the same extract procedure to an unprepared *Radix aconiti carmichaeli* (*Fu Zi*, the lateral root of *Aconitum carmichael)* sample (Fig. 3).

4. Conclusion

The developed capillary electrophoresis method has been successfully applied to the determination of five toxic alkaloids in both CPM and crude herb. To our knowledge, this is the first report about determination of *Aconitum* alkaloids by capillary electrophoresis. All of the alkaloids investigated can be determined in a 15-min run. The method showed advantages of simplicity in operation, speediness of

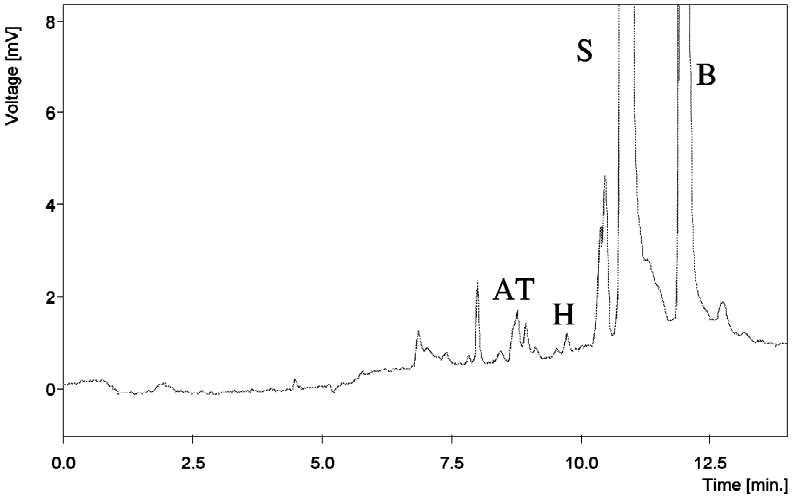

Fig. 2. Electropherogram of CPM sample: 1 g of CPM was extracted into 1 ml methanol with 10 mg/l atropine. Other conditions as in Fig. 1.

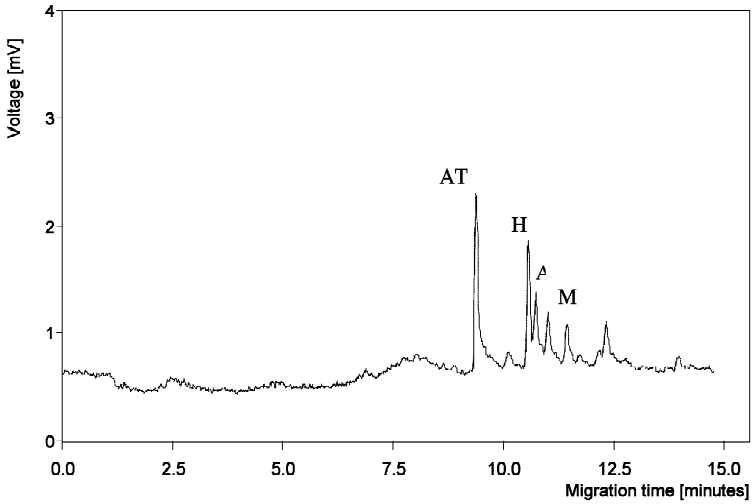

Fig. 3. Electropherogram of an unprepared *Radix aconiti carmichaeli* sample: 1 g of the crude drug was extracted by 1 *M* HCl and into 5 ml chloroform finally with 20 mg/l atropine. Other conditions as in Fig. 1.

separation, economy in the cost of reagents and potential applicability in CE–mass spectrometric analysis in the future.

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