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Determination of alkaloids in *Rhizoma Coptidis* by capillary electrophoresis with acidic potassium permanganate chemiluminescence detection

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**Abstract**

A simple and sensitive method based on capillary electrophoresis (CE) with acidic potassium permanganate chemiluminescence (CL) detection has been developed for the simultaneous determination of the three alkaloids (berberine, palmatine and jatrorrhizine) in *Rhizoma Coptidis*. A lab-built temperature control interface was used for CL detection. Experimental conditions for CE separation and CL detection were investigated in detail to acquire the optimum conditions. The optimized condition for the CL detection was $3.0 \times 10^{-4}$ M potassium permanganate in 3.0 M sulfuric acid solution injected by microinject pump. With 60 mM phosphate buffer saline (pH=8.0)-50% (v/v) methanol buffer as the running buffer, the three alkaloids were baseline separated within 13 min at a separation voltage of 18 kV. The calibration curves exhibit excellent linearity with the detection limits ranged from 0.81 to 4.11 µg/mL for the three alkaloids. This method was applied for the determination of the above three alkaloids in *Rhizoma Coptidis* with simple extraction procedures, and the assay results were satisfactory.

**Keyword:** Alkaloids; Capillary electrophoresis; Chemiluminescence; *Rhizoma Coptidis.*
1 Introduction

*Rhizoma Coptidis* is a common traditional Chinese medicine with the functions of clearing heat, drying dampness, and detoxication.\(^1\) Protoberberine alkaloids are main bioactive compositions of *Rhizoma Coptidis*, and have several pharmacological actions, including anti-inflammatory,\(^2\) hepatoprotective,\(^3\) antimicrobial,\(^4\) and antineoplastic effect.\(^5\) So the determination of protoberberine alkaloids in botanic drugs has significant meaning for the evaluation of their effects.

Various methods are available for the analysis of alkaloids in *Rhizoma Coptidis* and its pharmaceutical preparations, and rat plasma, mainly including 1HNMR,\(^6-8\) liquid chromatography (LC) with UV \(^9-11\) or MS \(^11-15\) detection, and capillary electrophoresis (CE) with UV \(^16-18\) or MS \(^19\) detection. While these methods have some individual disadvantages, it is still necessary to propose a simple and sensitive method for the determination of alkaloids in complicated samples.

Recently, chemiluminescence (CL)-based detection coupled to CE has attracted much interest due to its simplicity, low cost, and high sensitivity.\(^20-27\) Potassium permanganate, as a strong oxidant in acidic medium, has been commonly used for CL detection.\(^28-30\) CE-CL methods based on acidic potassium permanganate chemiluminescence system have also been developed for the determination of some alkaloids\(^31-32\) and catechol compounds.\(^33\)

To our knowledge, CE-CL method has not been used to determine alkaloids in botanic drugs previously. In this paper, a novel CE-CL method has been developed to simultaneously
determine the three protoberberine alkaloids (berberine, palmatine, and jatrorrhizine) in 
*Rhizoma Coptidis* using acidic potassium permanganate chemiluminescence system, and the 
assay results were satisfactory.

2 Experiments

2.1 Apparatus

The home-made CE-CL detection system is similar to the literature. The fabrication of 
the temperature control interface for CE-CL detection will be written in a patent. A 
high-voltage power supply (0-30 kV, Research Institute of Applied Physics, Shanghai) 
provides a separation voltage to drive the electrophoresis. Luminescence detector is ultra 
weak luminescence analyzer (Research Institute of Biophysics, Chinese Academy of Science, 
Beijing, China). Dual-channel microinject pump (Smith Medical Instrument Co., Ltd., 
Zhejiang, China) is used to inject CL reagent. A 60cm×75µm i.d. uncoated fused-silica 
capillary (Hebei Optical Fiber, China) is used for the separation. The outlet of the separation 
capillary was 10 cm lower than the other end to make the solution flow out of the separation 
capillary more easily and quickly. DF-2 collector-type magnetic heating stirrer (Changzhou 
National Equipment Institute, Jiangsu, China) is used to supply hot water. The pH510 meter 
(Eutech Instruments Pte Ltd., Singapore) is used to measure the pH value of the running 
buffer.

2.2 Chemicals and materials

Berberine hydrochloride, palmatine hydrochloride and jatrorrhizine hydrochloride were 
purchased from Chinese Chemical and Biological Drugs Institute (Beijing, China). *Rhizoma 
Coptidis* was obtained from Guoyitang of Fujian University of Traditional Chinese Medicine
(China). All other reagents were of analytical grade, and the water used in this study was doubly distilled.

Standard stock solutions of the three alkaloids at the concentration of 1.00 mg/mL were prepared in water and stored in refrigerator. All standard solutions were diluted to the desired concentration with water just prior to use. The phosphate buffer saline (PBS) was prepared by mixing Na$_2$HPO$_4$ solution with NaH$_2$PO$_4$ solution. Before use, all solutions were filtered through a 0.22 µm polypropylene filter film.

2.3 Sample preparation

Air-dried *Rhizoma Coptidis* was dried in the drying oven for 4 h at 50 °C and finely powdered. 1.00 g powder of *Rhizoma Coptidis* was extracted with 2×10 mL 80% ethanol solution by sonication for 30 min. The extracts were combined and centrifuged for 15 min at 3000 rpm. The extract was concentrated up to the volume less than 10 mL by rotary evaporator, and then it was transferred into volumetric flask, adding water to a final volume of 10.0 mL and stored in refrigerator at 4 °C. The sample extract was diluted to proper concentration with water and filtered through a 0.22 µm polypropylene filter film just prior to its analysis. Peak identification was performed by standard addition methods.

2.4 Procedures

A new capillary was rinsed with 1 M HCl, water, 1 M NaOH, water, and running buffer for 20 min, respectively. A used capillary was sequentially rinsed with 0.1 M NaOH, water, and running buffer for 5 min before use. The separation capillary was then equilibrated 5 min with the running buffer by voltage mode. Electrophoresis separations were performed at 18 kV, and sample was injected electrokinetically at 18 kV for 8s. The injection speed of
microinject pump was 6.0 mL/h. The electrophoresis buffer consisted of 60 mM PBS (pH 8.0) with 50% (v/v) methanol. The CL reagent was $3.0 \times 10^{-4}$ M potassium permanganate in 3.0 M sulfuric acid solution. The detection temperature was 30 °C.

3 Result and discussion

3.1 Optimization of CE conditions

3.1.1 Selection of running buffer

The running buffer was a critical factor for the separation efficiency of electrophoresis. In this paper, two buffer solutions, such as borate buffer and phosphate buffer saline (PBS) were investigated, respectively. Experimental results showed that the best resolution and sensitivity of the three alkaloids were obtained by using PBS as the running buffer. Therefore, PBS was chosen as the running buffer to separate alkaloids.

3.1.2 Effect of running buffer pH

Running buffer pH was also a key factor because it affected the capillary surface charge characteristics and the charge of the analytes. 60 mM PBS-50% (v/v) methanol running buffer with different pH were tested the effects on the separation of alkaloids. As shown in Fig.1, the migration time decreased with the increasing of pH value. The three alkaloids were well separated at pH 8.0. Therefore a 60 mM PBS-50% (v/v) methanol buffer of pH 8.0 was chosen as running buffer.

3.1.3 Effect of PBS concentration

The concentration of running buffer mainly affected the Zeta potential of capillary wall, viscosity and diffusion coefficient of solution, which resulted in changing the migration rate and the resolution of analytes. Several PBS-50% (v/v) methanol running buffers of pH 8.0
with the concentration ranging from 40 to 70 mM were studied to examine the effects. The results showed that the resolution increased with increasing buffer concentration, but Joule heating became obvious (see Fig. 2). Considering the resolution and sensitivity, a 60 mM PBS-50% (v/v) methanol running buffer with pH 8.0 was chosen in this work.

3.1.4 Effect of organic additives

Organic additives were commonly added to the CE separation system to improve the separation efficiency. In this work, three kinds of organic additives, such as methanol, ethanol, and acetonitrile, have been selected for investigating their effects on the separation of the three alkaloids. The results indicated that berberine and palmatine were not separated well when ethanol and acetonitrile were used as additives. Methanol showed an improvement in resolution, so methanol was chose as organic additive. The resolution of the three alkaloids increased with increasing the content of methanol, which is due to that methanol can decrease the velocity of electroosmotic flow to improve the resolution (see Fig. 3). However, too high methanol concentration can cause the running buffer to instability. When the content of methanol was up to 50% (v/v), the three alkaloids were well separated. So 50% (v/v) methanol was selected as the optimal additive concentration for the subsequent experiment.

3.1.5 Effect of separation voltage and injection time

Separation voltage had direct impact on the field strength, migration time, column efficiency, and Joule heat. The effect of separation voltage ranging from 14 to 22 kV on the separation and CL intensity of the three alkaloids was investigated in this work. When the separation voltage was lower than 14 kV, the analysis time was too long, and CL intensity was lower. With the increase of separation voltage, separation efficiency decreased, but CL
intensity increased. When the separation voltage was up to 18 kV, the three alkaloids were separated well and CL intensity was higher. Therefore, a separation voltage of 18 kV was carried out for further studies.

The effect of injection time on CL intensity was investigated by varying injection time from 5 to 15s at 18 kV. The results showed that the CL intensity increased with increasing injection time, but the separation efficiency decreased, and the peak broadening became more severe. Then, 8s of injection time (at 18 kV) was selected for the following studies.

3.2 Optimization of CL conditions

Detection temperature was a key parameter to the CL intensity and separation efficiency. As shown in Fig.4, the CL intensity increased with the increasing of detection temperature. When detection temperature was at 30°C, the CL intensity was stronger and the three alkaloids were baseline separated. When the detection temperature was continuously increased, the CL intensity wasn’t increased obviously. Meanwhile, the baseline was unstable. So the optimal detection temperature was 30°C.

The effect of potassium permanganate concentration on the CL intensity was shown in Fig.5. The concentration of potassium permanganate varied from $2.0 \times 10^{-4}$ to $4.0 \times 10^{-4}$ M, the CL intensity firstly increased and then decreased with the increasing of potassium permanganate concentration. This phenomenon could be due to the absorption of the emitted light by the colored potassium permanganate. The maximum CL intensity was obtained when potassium permanganate concentration was $3.0 \times 10^{-4}$ M. Therefore, $3.0 \times 10^{-4}$ M potassium permanganate was selected for the following experiments.
Sulfuric acid concentration tremendously influenced the oxidation ability of potassium permanganate, which result in the change of CL intensity. Experiments were performed using sulfuric acid at different concentration (from 2.0 to 4.0 M). The results were shown in Fig. 6. The CL intensity increased rapidly with the increasing of sulfuric acid concentration and then decreased gradually with the continuously increasing of sulfuric acid concentration. Thus, the optimum concentration of sulfuric acid was 3.0 M, at which the maximum CL intensity could be obtained.

Owing to CL reagent injected by a medical microinject pump, the flow rate of CL solution also affected the CL intensity. If the flow rate was too low to provide sufficient CL reagent, the CL reaction was insufficient. While the flow rate was too high, the analytes from the end of capillary have not yet been completely oxidized by potassium permanganate, so the strongest CL intensity could not be detected. The influence of the flow rate of CL solution on CL intensity was studied in this work. The results illustrated that the CL intensity increased tremendously with the increasing of the flow rate of CL solution and then decreased rapidly with the continuously increasing of the flow rate of CL solution. The maximum CL intensity was obtained when the flow rate of CL solution was 6.0 mL/h, so the optimum flow rate of CL solution was 6.0 mL/h in this work.

3.3 Analytical performance

Under the optimized conditions, the three alkaloids could be completely separated within 13 min (see Fig. 7(1)). The precision of the proposed method was studied by reduplicate injecting a standard mixture solution within a day (intra-day) and in 5 days (inter-day). Intra- and inter-day precisions for the peak height and migration time of three
alkaloids expressed as relative standard deviation (RSD) were between 2.8% and 4.9% (See Table 1), which showed the method had a good precision.

The CL linearity and detection limits (S/N =3) were determined by testing a series of alkaloid standard solutions. The results of regression analysis on calibration curves and detection limits are presented in Table 2. The calibration curves exhibit excellent linearity with the limits of detection (LOD) ranged from 0.81 µg/mL to 4.11 µg/mL for the three alkaloids. The comparison of the proposed method with other reported methods in respect of linearity and LODs was made to evaluate the performance of the method. As listed in Table 3, in comparison with the reported methods used for the determination of berberine, the sensitivity of the proposed method is better than HNMR and CE-UV method, but inferior to UPLC-UV and UPLC-UV/MS methods. However, UPLC needs unfavorable pH modifier which will potentially lead to column contamination and damage. In addition, MS detector is more expensive and complicated than CL detector.

### 3.4 Sample analysis and recovery

Since the contents of the alkaloids are different in *Rhizoma Coptidis*, the sample extract was diluted to 400, 100 and 1 times to determine berberine, palmatine and jatrorrhizine respectively by CE-CL under the optimum conditions. The typical electropherograms of the samples were shown in Fig.7. The contents of berberine, palmatine, and jatrorrhizine, measured by external standard method, were 14.3 mg/g, 3.49 mg/g, and 0.064 mg/g, respectively. The result of the determination of berberine is different from those given by other reported methods (see Table 3), which may be due to that the diversities in geographical cultivations make the content of active alkaloids quite different from each other.
Five independent sample solutions of *Rhizoma Coptidis* in parallel were prepared and analyzed to evaluate the repeatability. The RSDs of the three alkaloids measurements were 3.5% for berberine, 2.6% for palmatine, and 5.5% for jatrorrhizine, respectively. Sample stability was also monitored by analyzing sample solutions for 3 days at an interval of every 12 h. The storage stability (RSD) of the measurements for the three alkaloids was less than 5.8%. These results indicate the developed method has good repeatability and stability.

Accurate amounts of the three alkaloids were added to the diluted extract of *Rhizoma Coptidis*, and the recoveries were obtained using their peak height from the calibration curve under the same conditions. The recoveries were ranging from 71.2% to 110% (see Table 4), and RSD values were less than 5.6%, which indicates the developed method has good accuracy and is appropriate for the analysis.

**4 Conclusions**

In this work, a sensitive CE-CL method based on acidic potassium permanganate chemiluminescence system has been proposed to simultaneously determine the three alkaloids (berberine, palmatine, and jatrorrhizine) in *Rhizoma Coptidis*. Compared with LC, the developed CE–CL technique was less expensive, simpler, and more rapid. If on-line preconcentration techniques in CE are utilized to improve the sensitivity, this method can be extended to the determination of other ultra-trace bioactive components in medicinal plants.

**Acknowledgments**

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References


1137-1143.

5628-5634.


855,329-335.


Table Captions

**Table 1** The precision of the CE-CL method

**Table 2** The analytical performance of the CE-CL method

**Table 3** Determination of berberine in *Rhizoma Coptidis* by different methods

**Table 4** Determination results of the recovery for this method (*n* = 3)

Figure captions:

**Fig. 1** Effect of pH on the separation of alkaloids. CE conditions: fused silica capillary: 60cm×75µm i.d.; separation voltage: 18 kV; injection time: 8s (at 18 kV). Running buffer: 60mM PBS-50% (v/v) methanol. CL conditions: 3.0×10^{-4} M potassium permanganate; 3.0 M sulfuric acid; the flow rate of CL solution: 6.0mL/h; detection temperature: 30°C. 1. berberine; 2. palmatine; 3. jatrorrhizine.

**Fig. 2** Effect of PBS concentration on the separation of alkaloids. Running buffer: PBS (pH=8.0)-50% (v/v) methanol, other conditions as in Fig. 1. 1. berberine; 2. palmatine; 3. jatrorrhizine.

**Fig. 3** Effect of methanol concentration on the separation of alkaloids. Running buffer: 60mM PBS (pH=8.0)-methanol; other conditions as in Fig. 1. 1. berberine; 2. palmatine; 3. jatrorrhizine.

**Fig. 4** Effect of detection temperature on the CL intensity. Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; other conditions as in Fig. 1. 1. berberine; 2. palmatine; 3. jatrorrhizine.

**Fig. 5** Effect of potassium permanganate concentration on the CL intensity. Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; other conditions as in Fig. 1.
**Fig. 6** Effect of sulfuric acid concentration on the CL intensity. Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; other conditions as in Fig. 1.

**Fig. 7** Electropherogram of the standard mixture solution of alkaloids (1), the 100 times diluted extract of *Rhizoma Coptidis* (2), and the extract of *Rhizoma Coptidis* (3). Running buffer: 60mM PBS (pH= 8.0)-50% (v/v) methanol; other conditions as in Fig. 1. 1. berberine; 2. palmatine; 3. jatrorrhizine.
Table 1 The precision of the CE-CL method \(^a\)

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Concentration (µg/mL)</th>
<th>RSD% (intra-day, n=5)</th>
<th>RSD% (inter-day, n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Migration time</td>
<td>Peak height</td>
</tr>
<tr>
<td>Berberine</td>
<td>15.0</td>
<td>2.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Palmatine</td>
<td>15.0</td>
<td>2.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Jatrorrhizine</td>
<td>25.0</td>
<td>2.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

\(^a\) Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; Other conditions as in Fig. 1.
Table 2 The analytical performance of the CE-CL method

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>Linear range (µg/mL)</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>$Y = 10.0X+98.2$</td>
<td>0.9965</td>
<td>3.00-25.0</td>
<td>0.90</td>
<td>3.00</td>
</tr>
<tr>
<td>Palmatine</td>
<td>$Y = 11.1X+46.2$</td>
<td>0.9981</td>
<td>3.00-25.0</td>
<td>0.81</td>
<td>2.70</td>
</tr>
<tr>
<td>Jatrorrhizine</td>
<td>$Y = 2.2X+43.1$</td>
<td>0.9985</td>
<td>5.00-50.0</td>
<td>4.11</td>
<td>13.6</td>
</tr>
</tbody>
</table>

\(^a\) Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; Other conditions as in Fig.1.

\(^b\) Here, $Y$ and $X$ are CL intensity and concentration of alkaloids (µg/mL), respectively.

\(^c\) The limits of detection (LOD) corresponding to concentrations giving signal-to-noise ratio of 3.

\(^d\) The limits of quantity (LOQ) corresponding to concentrations giving signal-to-noise ratio of 10.
<table>
<thead>
<tr>
<th>Method</th>
<th>Linearity</th>
<th>LOD (µg/mL)</th>
<th>Berberine content (mg/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNMR</td>
<td>30</td>
<td>12.97</td>
<td></td>
<td>[6]</td>
</tr>
<tr>
<td>HNMR</td>
<td>2040-19320</td>
<td>40</td>
<td>30.27-90.40</td>
<td>[8]</td>
</tr>
<tr>
<td>UPLC-UV</td>
<td>36-840</td>
<td>0.29</td>
<td>79.8-84.3</td>
<td>[10]</td>
</tr>
<tr>
<td>UPLC-UV/MS</td>
<td>0.322-96.6(UV)</td>
<td>0.06(UV)</td>
<td>36.2-72.7</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE-UV</td>
<td>27-4388</td>
<td>56</td>
<td></td>
<td>[17]</td>
</tr>
<tr>
<td>NACE-UV</td>
<td>40.6-405.5</td>
<td>6.15</td>
<td>53.3-78.6</td>
<td>[18]</td>
</tr>
<tr>
<td>CE-CL</td>
<td>3.0-25.0</td>
<td>0.90</td>
<td>14.3</td>
<td>This present method</td>
</tr>
</tbody>
</table>

*Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; Other conditions as in Fig.1.*
Table 4 Determination results of the recovery for this method ($n=3$)

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Added amount (µg/mL)</th>
<th>Determined amount (µg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>2.50</td>
<td>2.09</td>
<td>83.6</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>5.37</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.06</td>
<td>90.6</td>
</tr>
<tr>
<td>Palmatine</td>
<td>2.50</td>
<td>1.98</td>
<td>79.2</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>5.13</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>11.0</td>
<td>110</td>
</tr>
<tr>
<td>Jatrorrhizine</td>
<td>2.50</td>
<td>1.78</td>
<td>71.2</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.52</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.5</td>
<td>105</td>
</tr>
</tbody>
</table>

Running buffer: 60mM PBS (pH= 8.0)-50% (v/v) methanol; Other conditions as in Fig.1.
Fig. 1 Effect of pH on the separation of alkaloids. CE conditions: fused silica capillary: 60 cm×75 µm i.d.; separation voltage: 18 kV; injection time: 8 s (at 18 kV). Running buffer: 60 mM PBS-50% (v/v) methanol. CL conditions: 3.0×10^{-4} M potassium permanganate; 3.0 M sulfuric acid; the flow rate of CL solution: 6.0 mL/h; detection temperature: 30°C.

1. berberine; 2. palmatine; 3. jatrorrhizine.
Fig. 2 Effect of PBS concentration on the separation of alkaloids. Running buffer: PBS (pH=8.0)-50% (v/v) methanol, other conditions as in Fig.1. 1.berberine; 2.palmatine; 3.jatrorrhizine.
Fig. 3 Effect of methanol concentration on the separation of alkaloids. Running buffer: 60mM PBS (pH=8.0)-methanol; other conditions as in Fig.1. 1.berberine; 2.palmatine; 3.jatrorrhizine.
Fig. 4 Effect of detection temperature on the CL intensity. Running buffer: 60mM PBS (pH=8.0)-50%(v/v) methanol; other conditions as in Fig.1. 1.berberine; 2.palmatine; 3.jatrorrhizine.
Fig. 5 Effect of potassium permanganate concentration on the CL intensity.

Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; other conditions as in Fig. 1.
**Fig. 6** Effect of sulfuric acid concentration on the CL intensity. Running buffer: 60mM PBS (pH=8.0)-50% (v/v) methanol; other conditions as in Fig. 1.
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