Analytical Methods

Investigation of six bioactive anthraquinones in slimming tea by accelerated solvent extraction and high performance capillary electrophoresis with diode-array detection

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A rapid and effective method for effective separation and rapid simultaneous determination of six bioactive anthraquinones by capillary zone electrophoresis was developed. An accelerated solvent extraction procedure was used for the extraction of anthraquinones from slimming tea. Under the optimized conditions, the effective separation of six anthraquinones was achieved within 8 min. Good linearity was achieved, with a correlation coefficient (r) of >0.999. The limit of detection ranged from 0.33 to 1.40 µg mL⁻¹. The intra- and inter-day relative standard deviation (RSD) of the six analytes was in the range of 2.3–3.9% and 3.2–4.9%, respectively. The average recovery of the six analytes from real tea samples was in the range of 86.15–98.30% with the RSD of 1.04–4.99%. The developed and validated method has speediness, high sensitivity, recovery and precision, and can be applied for the quality control of slimming tea.

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1. Introduction

Slimming tea is a health product that makes use of the natural compounds found in tea to aid metabolic processes. By helping detoxify the body. It reduces the accumulation of subcutaneous fat. This product has no harmful side effect and is an efficacious aid to good dietary health. Most of slimming tea are made of pure leaves of plants which have multiple therapeutic actions related to the prevention of dementia and ischemia. More recently, tea and its extracts have been used in various industrial products such as cosmetics, foods, and beverages. The application of tea extracts in meat, oil/fat, dairy, and other foods as an antioxidant additive has been reviewed (Karaosmanoglu & Kilmartin, 2015). Modern pharmacological research has revealed tea having multiple functions, such as liver protection, cathartic function and immune system improvement, eyesight improvement, diuresis, anti-tumor activity, and antioxidation (Diao, Wang, Zhang, Chen, & Liu, 2013). The water extracts of unroasted semen cassiae might have a potential health activity in the chemoprevention of cancer and protective effects on DNA damage in human lymphocytes induced by 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole(Trp-P-1) (Wu & Yen, 2004). It contains a variety of bioactive anthraquinones including aurantio-obtusin, chryso-obtusin, obtusin, and others. The sprout of semen cassiae was suit to be selected in the healthy food. They are beneficial for human health, but they can also cause side effects such as obesity, irritable bowel syndrome and psoriasis. Chrysophanol and aurantio-obtusin were selected as the markers for semen cassiae in the Chinese Pharmacopoeia (State Pharmacopoeia Commission of China, 2010). However, as chrysophanol can be found in many other herbs, it is not a specific effective component of semen cassiae. Therefore, the determination of the active components including the markers in semen cassiae is required for the evaluation of its quality.

Of all the analytical methods for anthraquinones in traditional Chinese medicines, high performance liquid chromatography (HPLC) is still the most popular (Deng, West, Jarakae Jensen, Basr, & Westendorf, 2009; Gautam, Srivastava, & Jachak, 2011; He, Chen, Tian, & Yao, 2009; Huang, Zhong, Zhang, & Zhang, 2010; Koyama, Morita, & Kobayashi, 2007). However, since the content of some anthraquinones is too low, previous analytical methods only allow the quantification of a few anthraquinones or a hydrolysis step has to be included in the sample preparation. Some HPLC methods also suffer from low chromatographic resolution, large consumption of organic solvent, and long runtime. A HPLC-UV detection method was reported for the determination of five anthraquinones in Rhizoma Rhei, with long retention time (Zou, Xie, Jiang, Chen, & Yao, 2008). A HPLC-DAD method was reported for the determination of eight anthraquinones in semen cassiae,
with the retention time of 8.432–21.362 min (Xu et al., 2012). Recently, a HPLC-DAD/MS method for qualitative and quantitative analysis of anthraquinones in rhubarb was reported, but the retention time of the anthraquinones was too long, from about 54 min for rhein to about 98 min for emodin (Wei, Yao, Ji, Wei, & Peng, 2013). Therefore, it is required to develop a rapid and effective method of multi anthraquinones for the quality evaluation of semen cassiae and its products.

High performance capillary electrophoresis (HPCE) has become a powerful tool in natural product analysis, because of its high resolution, short analysis time, and low solvent and sample consumption (Li, Li, & Wang, 2006; Zhao, Hu, Lao, Yang, & Li, 2014). The capillary electrophoresis was used for the analysis of three–five anthraquinones in herbs (Kuo & Sun, 2003; Li, Liu, Ji, & Li, 2000; Shang & Yuan, 2003; Sheu & Chen, 1995; Sun & Yeh, 2005; Wang et al., 2008). Several capillary zone electrophoresis (CZE) methods for the analysis of some anthraquinones in herbal medicines have been proposed. The contents of six anthraquinones in crude Rhei Rhizoma extract were determined within 39 min (Weng & Sheu, 2000). A CZE method with amperometric detection and 30 m borate solution (pH 9.5) as running buffer was used for the determination of aloe-emodin, emodin and rhein in Huangdan Yinchen Keli after the extraction with 15 mL ethanol for 24 h (Wang, Zhou, Wu, He, & Fang, 2004). Another CZE method modified by β-cyclodextrin was reported for the simultaneous determination of five anthraquinones in rheum and rheum-containing preparations after the extraction with 50 mL of chloroform after refluxing 2 h (Li, Cao, & Ding, 2004). A new flow injection-CZE method with ultraviolet detection at 254 nm was developed for the separation and determination of physcion, chrysophanol, aloe-emodin, and emodin in Rhubarb, Juemingzi, and Chinese herbal preparations (Liu, Fan, Chen, Chen, & Hu, 2005). A CZE-DAD method was developed for simultaneous determination of five anthraquinones including aloe-emodin, emodin, chrysophanol, physcion, and rhein in Rheum with a long runtime of 40 min (Gong, Li, Wang, Li, & Yang, 2005). A new capillary electrophoresis method with amperometry detection has been developed for the analysis of flavonoids and anthraquinones in chrysanthemum using a borate-phosphate running buffer (Zhang et al., 2010). In all of the previous research, the determination of aurantio-obtusin was not achieved using CE method, possibly due to its low solubility in water. Recently, we described a nonaqueous capillary electrophoresis method, but it could be used only for the separation and determination of aurantio-obtusin, emodin and rhein (Wang, Wu, Wu, Liang, & Sun, 2014). Therefore, a fast method is desirable to determine the content of aurantio-obtusin and other anthraquinones in semen cassiae.

Sample pretreatment is always a crucial step in deciding the levels of detection limits of the overall method. Especially when large number of samples are involved, rapid extraction becomes even more essential. Several extraction methods, such as ultrasonic extraction (He et al., 2009; Kuo & Sun, 2003; Wang et al., 2008) and refluxing extraction (Djozam & Assadi, 1995; Li et al., 2004; Peng et al., 2013) have been used for the extraction of anthraquinones, but most of them required large volumes of solvents and more time-consuming, in addition, the extracts were required to pass through multiple cleanup procedures including solid phase extraction (SPE) to remove potential molecular interference. However, additional clean up steps increase the time of analysis and volume of solvent/reagent. Accelerated solvent extraction (ASE) is an automated extraction technique, which uses conventional liquid solvents at high temperatures and high pressures to extract compounds from solid samples quickly, with the advantages of good recoveries, rapidity, and adequate precision as well as less solvent use (Gong et al., 2005; Sun, Ge, Lv, & Wang, 2012). In general, however, a followed clean up is required. Therefore, it is of critical importance to develop a simple, rapid and selective sample preparation procedure for effective separation and rapid simultaneous analysis of anthraquinones in commonly consumed tea and medicinal food.

The aim of this study was to develop such a rapid, highly sensitive and accurate analytical method for extracting six bioactive anthraquinones in semen cassiae and cassiae seed tea using a ASE procedure followed by determination on CZE.

2. Materials and methods

2.1. Instrumentation

All the experiments were performed with an Agilent HP3D CE system with air-cooling and a diode-array detector (DAD: Agilent, Waldbronn, Germany). Data were collected with the Agilent Chemstation version A10.02 chromatographic data system. The extraction equipment was an APEL 2000 automatic accelerated solvent extraction apparatus (Beijing Titan Instruments Co., Ltd., China) equipped with 11 mL stainless-steel extraction cells. An ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Kunshan, China) working at 40 MHz with an output power of 40 W was employed as an ultrasonic source. A TGL-16 M centrifuge (Xiangyi Centrifuge Co., Hunan, China), RE-2000A rotary evaporator (Shanghai Yarong Biochemical Instrument Co.,), and PHS-3C pH meter (Shanghai Precision & Scientific Instrument Co., Shanghai, China) were used in sample treatment.

2.2. Chemicals and solutions

Anthraquinones (aurantio-obtusin, alo-e-emodin, rhein, emodin, chrysophanol, and physcion, purity: >99% for each one) in Fig. 1 were purchased from the National Institutes for Food and Drug Control (Beijing, China). Trisodium phosphate dodecahydrate (Na₃PO₄·12H₂O) disodium phosphate dodecahydrate (Na₂HPO₄·12H₂O) (analytical grade), methanol, acetonitrile, acetone and n-hexane (HPLC grade) were purchased from the Beijing Chemical Factory (Beijing, China). Ten

![Fig. 1. Chemical structure of six anthraquinones.](image-url)
mM Na₂HPO₄–6 mM Na₃PO₄ (pH = 11.8) was selected as a background electrolyte solution. Single stock standards of aurantio-obtusin, aloe-emodin, rhein, emodin, chrysophanol, and physcion were prepared by dissolving the compound in methanol, and their final concentrations were 224, 100, 180, 204, 104, and 100 µg mL⁻¹, respectively. The stock solutions were stored at 4 °C. Mixed standard working solutions were prepared by diluting the stock standard solution with methanol just before use.

2.3. Sample preparation and extraction

Semen cassiae and cassiae seed tea were taken from a supermarket. Samples were homogenized, sieved through 100 mesh and grinded. All samples were then dried at ambient temperature and kept in amber glass bottles at 4 °C prior to analysis.

The homogenized powder sample (0.2 g) and quartz sand (0.1 g) were mixed, and transferred into 11-mL accelerated solvent extraction cell. Acetonitrile was selected as an accelerated extraction solvent. The extraction conditions were as follows: oven temperature of 140 °C with 3 min heat-up time under a pressure of 10 MPa and two static cycles with a static time of 5 min. The flush volume was 40% of the extraction cell volume. The extract was purged from the sample cell using pressurized nitrogen for 90 s, and evaporated to dryness by the rotary evaporator under a stream of nitrogen at 45 °C. The obtained residue was dissolved with methanol to 10 mL. All sample solutions were filtered through a nylon microfilter (0.45 µm pore size) before CZE injections. All experiments were carried out in triplicate.

2.4. Electrophoresis conditions

A 48.5 cm (40 cm to the detector) 75 µm id uncoated fused silica capillary column (Yongan Optical Fabric Factory, Handan, China) was used, and was washed in turn with 1 M NaOH, 0.1 M NaOH, and water for 10 min. Prior to its use each day, the capillary column was washed consecutively with 0.1 M NaOH for 10 min, water for 10 min, and the buffer solution for 10 min at an electrophoretic pressure of 30 kV to improve the reproducibility. Between analysis the capillary was washed in turn with water and buffer solution for 1 min to maintain the repeatability of the analysis.

Sample introduction was made using 50 mbar pressure for 5 s. The high-voltage power supply was set at 30 kV. The 10 mM Na₂HPO₄ and 6 mM Na₃PO₄ (pH 11.8)–15% methanol (v/v) were used as background electrolyte (BGE) solution. The capillary temperature was kept at 20 °C. DAD was employed at a wavelength of 254 nm.

The resolution Rₛ was calculated by equation $Rₛ = 2Δtₚ/(W₁+W₂)$ used in conventional chromatography, where Δtₚ is the difference in migration times, W₁ and W₂ are the peak widths at the baseline.

3. Results and discussion

3.1. Optimization of CE conditions

3.1.1. Choice of BGE

The selection of suitable electrolytes is very important for CE. The correct selection of BGE can improve peak shape and separation effect. Several electrolytes were used as a buffer component for the separation of 3–5 anthraquinones (no aurantio-obtusin) by capillary zone electrophoresis, such as, potassium tetraborate (K₂B₄O₇) + NaH₂PO₄ (Liu et al., 2005; Shang & Yuan, 2003) and K₂B₄O₇ + Na₃PO₄ (Zhang et al., 2010). The initial experiments were conducted using disodium phosphate, trisodium phosphate, sodium bicarbonate, sodium carbonate, and sodium borate as electrolyte, respectively (Fig. 2).

When NaH₂PO₄ + Na₃PO₄ solution (pH 7.5)–10% methanol or borate buffer solution (pH 9.2)–15% methanol was used a buffer system, the peak of physcion, aloe-emodin and chrysophanol did not appeared, while using NaHCO₃–Na₂CO₃ aqueous solution (pH 10)–5% methanol, the three analytes could not be separated. Using Na₂HPO₄ aqueous solution (pH = 10.8)–10% methanol can separate the six analytes, but an off current appeared sometimes when a separation voltages over 23 kV. Using 10 mM Na₂HPO₄–6 mM Na₃PO₄ buffer solution (pH 11.8)–15% methanol achieved the separation of the six analytes.

3.1.2. Effect of buffer pH and electrolyte concentration

The apparent pH of the electrolyte is a governing factor in separation. The pKa value of the analytes was in the range of 8.25–9.47 (Li, Ding, & Cao, 2007), it suggests the difficulty of their complete separation by CZE, especially for physcion, aloe-emodin, and chrysophanol. Some buffer solutions (pH 8.2–10.1) were used for the separation of these anthraquinones (Liu et al., 2005; Shang & Yuan, 2003; Wang et al., 2004; Weng & Sheu, 2000; Zhang et al., 2010). The apparent pH value of electrolyte solution should be controlled to pKₐ + 2 in order to achieve complete ionization of these weak acidic anthraquinones. The effect of apparent pH (11.24–12.51) of the electrolyte solution on migration behavior was investigated. The result showed that migration time decreased with an increase of the apparent pH. It is because that using high pH buffer can increase ionization degree of these weak acidic anthraquinones. Otherwise, the buffer [H⁺] influences the effective mobility (jₑfᵣ) of a target component. There was a linear relation...
between the count backwards value \((1/\mu_{\text{eff}})\) of the target component's mobility and the buffer \([\text{H}^+]\) (Wang, Yang, & Song, 2001). So that increasing pH can increase the effective mobility, resulting in shorter migration time of the target analytes.

It is known that the acidity of free anthraquinones is depended on type and number of group, the order (High to low) of which is as follows: \(-\text{COOH} \rightarrow \geq 2 \beta-\text{OH} \rightarrow 1 \beta-\text{OH} \rightarrow \geq 2 \alpha-\text{OH} \rightarrow 1\alpha-\text{OH} \). Based on structure analysis, it is seen that the order of acidity may be suggested as follows: physcion < aloe-emodin < chrysophanol < emodin < aurantio-obtusin < rhein. Their migration order is related to the order (low to high) of acidity, but the reason needs to be examined further. When the buffer pH = 11.8, the six analytes could be completely separated, and resolution \(R_{1-2} = 3.88\) between physcion (1) and aloe-emodin (2) and resolution \(R_{2-3} = 3.00\) between aloe-emodin (2) and chrysophanol (3) were achieved.

The electrophoretic mobility and the EOF are related to the viscosity, which is a function of electrolyte concentration. The effects of electrolyte concentrations in the buffer system (pH 11.8) on the CE separation were investigated. The result showed that the migration time of the six analytes increased with an increase of electrolyte concentration. This is because the thickness of the electric double layer between the capillary walls and the buffer solution decreased with the increase of electrolyte concentration, otherwise an increased viscosity \((\eta)\) results in the decrease of mobility. The results indicated that with the increase of the concentration of the buffer, the peak currents of the analytes increased, and the peak shapes changed from flat to sharp, improving peak resolution. The migration time increased too. This is because the ionic strength of the buffer increases, which will result in the decrease in electroosmotic flow in the capillary. So the migration time was prolonged. The electric current in the capillary also increased with the increase of the concentration of the buffer, which causes the Joule heating (Chen & Zhang, 2000). Finally, 10 mM Na2HPO4–6 mM Na3PO4 buffer (pH 11.8) was used as electrolyte solution for the separation of the six analytes.

### 3.1.3. Effect of methanol

The solvent has an important role in modulating electroosmotic flow (EOF). In order to increase the solubility of less polar compounds and improve the selectivity, organic solvents are usually added as additives in CE analysis. It is known that when organic modifier is added in the 10 mM Na2HPO4–6 mM Na3PO4 buffer (pH 11.8), the dielectric constant and viscosity of the solution will be changed. In this work, methanol was used to improve the selectivity and resolution of CZE.

Using the buffer without methanol, the peaks of only three analytes were observed, and using the buffer with methanol, peaks of the six analytes were observed and baseline separated. This is possibly because the higher dissolving capacity and lower viscosity \((\eta = 0.55 \text{ mPa s})\) as well as higher dielectric constant \((\varepsilon = 33.64)\) of methanol improved both the sample ion mobility and the EOF level. In addition, organic solvents generate low electric current and Joule heat because of their low conductivity. With increasing methanol dosage, peak shape improved, both resolution and migration time increased. Using 10 mM Na2HPO4–6 mM Na3PO4 buffer (pH 11.8)–15% methanol can achieve complete separation of the six analytes.

### 3.1.4. Effect of voltage and temperature on separation

Electrophoretic velocity is directly proportional to the field strength. With the increase of separation voltage, the migration time decreased. However, the high voltage led to Joule heating and affected the separation of the analytes. The effect of voltage from 15 to 35 kV on the separation of the six analytes was investigated with 10 mM Na2HPO4–6 mM Na3PO4 buffer (pH 11.8) at 20 °C. By increasing the separation voltage, the migration times of the analytes decreased due to the increase of mobility of the analytes. However, the higher voltage caused the decrease in peak areas of the analytes and would cause higher current and lead to more Joule heating, which produces radial temperature gradient and decreases separation efficiency. When the voltage is at 30 kV, the peaks of the six analytes could be baseline separated within 8 min. Therefore 30 kV voltages were selected in this work, with a higher sensitivity and a shorter analytical time. The effect of the capillary temperature on separation was also investigated in the range from 15 to 30 °C under 30 kV voltages. The results showed that the migration time and resolution decreased with the increase of capillary temperature. Therefore, using a voltage of 30 kV and a column temperature of 20 °C can separate effectively the six analytes within 8 min.

### 3.2. Optimization of ASE conditions

#### 3.2.1. Effect of extraction solvent

Solvent is an important factor in ASE process. The results obtained using acetonitrile, methanol, acetone, and n-hexane as solvent showed that methanol caused interference from an impurity peak to the aurantio-obtusin and chrysophanol, while using acetone as extraction solvent very low response for four analytes was observed, and n-hexane could only extract physcion. Use of acetonitrile can simultaneously extract the six analytes with a better extraction effect (Fig. 3A).

#### 3.2.2. Effect of temperature and extraction time

Temperature and extraction time are also important factors contributing to increase recoveries. The effects of different
temperatures of 80, 110, 140 and 160 °C, and different static times of 3, 5 and 10 min on the analyte’s yield under 10.3 MPa were investigated (Fig. 3B). The extraction yield of the six analytes increased with increasing temperature from 100 to 140 °C, while over 140 °C their extraction yield did not increase. Use of temperature 140 °C obtains higher extraction yield for the six analytes with the RSD (n = 3) of 0.7–2.4%. Therefore, 140 °C is accepted as the optimum extraction temperature. Under a temperature of 140 °C and the static extraction for 5 min and 2 cycles the higher extraction yield for the six analytes was achieved.

### 3.3. Performance of the method

#### 3.3.1. Selectivity

Under the optimized conditions, selectivity was determined for each analyte in the assay. The electropherograms obtained using the standards, semen cassiae, and cassia seed tea samples are shown in Fig. 4.

The migration times of 3.23, 3.33, 4.43, 5.02, 5.15, and 7.34 min were obtained for physcion, aloe-emodin, chrysophanol, emodin, aurantio-obtusin, and rhein standards, respectively, and the good resolution of \( R_{Y_{12}} = 3.62 \) between physcion (1) and aloe-emodin (2) and \( R_{Y_{23}} = 3.59 \) between aloe-emodin (2) and chrysophanol (3) was achieved. The same migration times were observed for semen cassiae and cassia seed tea samples. Otherwise, it was shown that baseline separation of the peaks of the six analytes could be achieved. Some unknown peaks appearing did not interfere with the six analytes. There was no interference peak in real samples. The results show that good consistency of migration time and effective baseline separation of the peaks were achieved.

#### 3.3.2. Linearity and detection limit

The linearity of the method was evaluated by determining the analytes at the six concentration points in triplicate. The matrix-calibration curves of the peak area towards the analyte concentration were constructed using a least-squares linear regression. The equations of the calibration curves obtained based on three parallel measurements are listed in Table 1. It can be seen that the linearity is satisfactory with correlation coefficient (\( r \)) higher 0.999.

The LOD was considered to be the minimum analyte concentration yielding a signal to noise ratio (S/N) of 3, and the limit of quantification (LOQ) was considered to be the minimum analyte concentration yielding an S/N of 10. The LOD and LOQ values for the six analytes were determined, and their values are also listed in Table 1. The LOD values for the six analytes varied from 0.33 to 1.40 µg mL\(^{-1}\).

#### 3.3.3. Precision

The precision of the method was investigated at room temperature by determining the mixed standard solution of physcion (30 µg mL\(^{-1}\)), aloe-emodin (16 µg mL\(^{-1}\)), chrysophanol (12.5 µg mL\(^{-1}\)), emodin (16.3 µg mL\(^{-1}\)), aurantio-obtusin (22.4 µg mL\(^{-1}\)), and rhein (18.0 µg mL\(^{-1}\)). Under the optimized conditions the intra-day variability (RSD) of peak area for 5 determinations and inter-day RSD of peak area for 3 determinations for each day within 3 days were investigated. For physcion, aloe-emodin, chrysophanol, emodin, aurantio-obtusin, and rhein, the intra-day RSD was 3.4%, 2.6%, 2.7%, 2.7%, 3.9%, and 2.3%, and inter-day RSD was 4.9%, 4.5%, 3.2%, 3.6%, 4.0%, and 3.4%, respectively. It shows that this method has satisfactory repeatability and reproducibility.

These results indicate that the proposed method provides the selectivity, sensitivity, linearity and precision necessary for selective and simultaneous analysis of the tested anthraquinones.

### 3.4. Application

To examine the method accuracy, recovery of the six analytes was determined in triplicate by adding known amounts of the anthraquinones to the samples. The data in Table 2 shows that the average recovery of physcion, aloe-emodin, chrysophanol, emodin, aurantio-obtusin, and rhein was in the range of 86.15–98.30% with the RSD of 1.04–4.99%. A high recovery for both semen cassiae and cassia seed tea samples shows that the method can be used for accurate determination of the studied anthraquinones.

The optimized conditions were applied to the separation and determination of the six analytes in real tea. All the six analytes

<table>
<thead>
<tr>
<th>Table 1 Performance of the method.</th>
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<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linear range (µg mL(^{-1}))</th>
<th>Linear equation</th>
<th>Correlation coefficient (( r ))</th>
<th>LOD (µg mL(^{-1}))</th>
<th>LOQ (µg mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physcion</td>
<td>3.13–100.00</td>
<td>( Y = 0.60594 + 0.4131X )</td>
<td>0.99919</td>
<td>0.94</td>
<td>3.13</td>
</tr>
<tr>
<td>Aloe-emodin</td>
<td>1.39–22.24</td>
<td>( Y = -1.8729 + 1.8420X )</td>
<td>0.99946</td>
<td>0.42</td>
<td>1.39</td>
</tr>
<tr>
<td>Chrysophanol</td>
<td>1.11–17.78</td>
<td>( Y = -1.27006 + 2.3931X )</td>
<td>0.99910</td>
<td>0.33</td>
<td>1.11</td>
</tr>
<tr>
<td>Emodin</td>
<td>1.42–45.33</td>
<td>( Y = 1.04542 + 2.3302X )</td>
<td>0.99914</td>
<td>0.43</td>
<td>1.42</td>
</tr>
<tr>
<td>Aurantio-obtusin</td>
<td>4.67–149.33</td>
<td>( Y = -1.18918 + 0.91498X )</td>
<td>0.99969</td>
<td>1.40</td>
<td>4.67</td>
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<tr>
<td>Rhein</td>
<td>3.44–110.21</td>
<td>( Y = -1.76027 + 1.74317X )</td>
<td>0.99937</td>
<td>1.03</td>
<td>3.44</td>
</tr>
</tbody>
</table>
were found in semen cassiae and cassia seed tea (Table 2). Among cassia seed tea samples, the content of physcion, aloe-emodin, chrysophanol, and aurantio-obtusin for one sample was found to be 716.199.209. and 537. Chrysophanol, and aurantio-obtusin for one sample was found to be 94.9 and rhein were found. Emodin content for cassia seed tea was 2188.3 and 2833.8 respectively.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Content (μg g⁻¹)</th>
<th>Added (μg g⁻¹)</th>
<th>Found (μg g⁻¹)</th>
<th>Recovery (%)</th>
<th>Average recovery (%)</th>
<th>RSD (%)</th>
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<tbody>
<tr>
<td>Semen cassiae</td>
<td>Physcion</td>
<td>919.2</td>
<td>442.0</td>
<td>1176.5</td>
<td>103.5</td>
<td>98.30</td>
<td>4.74</td>
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<td></td>
<td>Aloe-emodin</td>
<td>102.4</td>
<td>49.1</td>
<td>197.0</td>
<td>95.17</td>
<td>92.53</td>
<td>2.75</td>
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<td>Chrysophanol</td>
<td>168.6</td>
<td>73.7</td>
<td>234.6</td>
<td>89.55</td>
<td>88.40</td>
<td>2.38</td>
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<td>157.8</td>
<td>73.7</td>
<td>227.1</td>
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<td>94.92</td>
<td>1.04</td>
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<td>600.5</td>
<td>294.7</td>
<td>865.6</td>
<td>89.96</td>
<td>91.66</td>
<td>2.03</td>
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<td>Rhein</td>
<td>239.8</td>
<td>122.8</td>
<td>347.2</td>
<td>87.46</td>
<td>87.01</td>
<td>2.96</td>
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<tr>
<td>Cassia seed tea</td>
<td>Physcion</td>
<td>1561.8</td>
<td>744.4</td>
<td>2234.5</td>
<td>90.37</td>
<td>88.42</td>
<td>4.02</td>
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<td></td>
<td>Aloe-emodin</td>
<td>422.5</td>
<td>200.3</td>
<td>608.6</td>
<td>92.91</td>
<td>90.63</td>
<td>2.99</td>
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<td>Chrysophanol</td>
<td>188.2</td>
<td>99.5</td>
<td>274.8</td>
<td>87.04</td>
<td>91.04</td>
<td>4.99</td>
</tr>
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<td></td>
<td>Emodin</td>
<td>94.9</td>
<td>49.2</td>
<td>138.4</td>
<td>88.41</td>
<td>89.58</td>
<td>3.70</td>
</tr>
<tr>
<td></td>
<td>Aurantio-obtusin</td>
<td>259.9</td>
<td>124.1</td>
<td>376.5</td>
<td>94.04</td>
<td>96.29</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>Rhein</td>
<td>306.5</td>
<td>148.0</td>
<td>434.8</td>
<td>86.69</td>
<td>86.15</td>
<td>2.38</td>
</tr>
</tbody>
</table>

### 4. Conclusions

Using the accelerated solvent extraction procedure can extract rapidly and effectively physcion, aloe-emodin, chrysophanol, emodin, aurantio-obtusin and rhein from tea sample, no needing cleanup step. A novel capillary zone electrophoresis method for simultaneous determination of the six anthraquinones was developed. The present method offers the advantages of being speedy, simple, sensitive and accurate as well as a low consumption of reagents. The method can be used for effective separation and simultaneous determination of the studied anthraquinones with running time of 8 min for quality control of tea.

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