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Fast determination of chlorogenic acid in tobacco residues using microwave-assisted extraction and capillary zone electrophoresis technique

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ABSTRACT

In this work, for the first time, capillary zone electrophoresis (CZE) technique combined with microwave-assisted extraction (MAE) was developed for the fast quantification of chlorogenic acid (CA) in tobacco residues. CA in tobacco residue samples were extracted by MAE technique, and then analyzed by CZE. As a new sample preparation method for tobacco residues, the MAE procedure is optimized, validated and compared with conventional methods including ultrasonic extraction (USE) and reflux extraction (RE). It is found that MAE gives the best result due to the highest extraction efficiency within shortest extraction time (only 4.0 min). Here, CA is determined by CZE based on the calibration curve of its authentic standard. The method linearity, detection limit, precision and recovery are studied. The results show that the combined MAE and CZE method has a linearity (R^2 0.991, 0.003–0.5 mg ml^{-1}), a limit of detection (0.003 mg ml^{-1}), a limit of quantification (0.01 mg ml^{-1}), good precision (R.S.D. = 4.28%) and a finer recovery (89.0%). The proposed method was successfully applied to the analysis of CA in tobacco residue samples. The experiment results have demonstrated that the CZE combined with MAE is a convenient, fast, economical and reliable method for the determination of CA in tobacco residues.

1. Introduction

Tobacco, a non-food crop, is widely grown in the entire world. Previous studies have shown that China has produced the largest amount of tobacco in the world [1]. Tobacco leaves are the main source of cigarettes and cigars which are used for chewing and smoking [2]. As we all known, there are large numbers of tobacco wastes that are generated during processing and cigarettes making. It is necessary to deal with these wastes because tobacco residuals contain nicotine that is thought to be harmful to human health [3]. However, there are some significant potential and biologically active compounds present in tobacco residuals. The active compounds mainly include phenolic compounds, especially chlorogenic acid (CA) whose structure is shown in Fig. 1, a major family of phenolic compounds [4,5]. In the last few years, CA has been the subject of several investigations in view of its potentially positive biological effects on humans. Recently, increasing attention has also been directed to CA and its derivatives because they have antibacterial and antiviral properties and CA is a natural antioxidant and anticancer agent [6–10]. Some experiments have indicated that the concentration of CA is high in tobacco leaves as well as tobacco residues [11,12]. For people, extraction and reuse of CA from tobacco residuals are of thorough importance. As we know, the analysis of the content of CA in tobacco residuals should be done before industrial extraction and recycle. Therefore, it is quite necessary to develop a simple, fast and valid method for the determination of CA in tobacco wastes.

Prior to the analysis, the extraction of CA in tobacco residues is required. Conventional sample extraction techniques such as reflux extraction (RE) and ultrasonic extraction (USE) are widely used for routine herb drug extraction, but they are known as laborious, time-consuming and do not show satisfactory extraction efficiency [13–15]. Compared with traditional extraction techniques, microwave-assisted extraction (MAE) has received more and more attention as a potential extraction technique because it is fast, economical and high-efficiency [16–21]. In our group, MAE technique has successfully been applied to the analysis of traditional Chinese medicines (TCMs) [22–25].

Recently, some analytical methods have been developed to determine CA in Chinese herbs. High-performance liquid chromatography (HPLC) is currently the most useful approach for the analysis of non-volatile constituents in sample matrix [26–32]. Compared with HPLC, capillary zone electrophoresis (CZE) technique has the good separation efficiency and good sensitivity. Moreover, CZE needs short analysis time and little amount of sample. CZE technology has been widely applied to the rapid analysis

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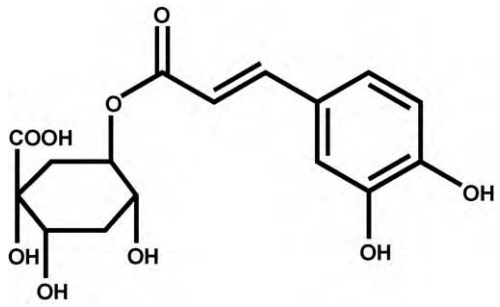

Fig. 1. The chemical structure of CA.

of active compounds in the traditional Chinese medicine [33–39]. However, to our best knowledge, no paper on using CZE technique to the analysis of CA in tobacco residues was reported. In this work, CZE technique combined with the sample technique of MAE was developed for the fast analysis of CA in tobacco wastes.

2. Materials and methods

2.1. Chemicals and materials

Tobacco residues, such as Zhonghua I, Zhonghua II, Jinqiao, Nanjing, Yipinmei, Shuangxi I, Shuangxi II and Shuangxi III were collected in local tobacco firms (Shanghai, China). Standard CA was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Deionized water was purified by a Milli-Qsystem (Milford, MA, USA). Sodium tetraborate that was used for BGE preparation was made by Lingfeng chemical reagent company (Shanghai, China). Microwave oven (LWMC-205) was from Lingjiang Science and Technology Co. Ltd. (Nanjing, China).

2.2. CZE conditions

All CZE experiments were performed on a high-voltage dc power supplying with a voltage range from −30 to +30 kV (Shanghai Institute of Atomic Nuclear Science Research, Chinese Academy of Science, Shanghai, PR China) that was equipped with a UV-absorbance detector (Unimicro Technologies, TriSep-2010GV). Electrophoretic data were acquired and analyzed by N2000 software. Separations were performed in fused silica capillaries (Hebei Yongnian Optic Fiber Factory, Hebei, PR China) with dimensions of 75 μm (i.d.) × 68 cm (effective length). New capillaries were conditioned by rinsing with 0.1 M HCl for 20 min, and deionized water for 20 min, followed by 0.1 M NaOH for 20 min, then deionized water for 20 min again. The capillary was rinsed with 0.1 M HCl for 10 min, and deionized water for 10 min, followed by 0.1 M NaOH for 10 min, and deionized water for 10 min again, and then prerun with BGE for 15 min under 16 kV every morning to obtain the best reproducibility. Samples were injected into the capillary at the height of 10 cm for 10 s. Samples separation was conducted at 16 kV and 25 °C, and the wavelength of the UV detector was set at 254 nm.

2.3. Standard solutions

The standard of CA was weighted accurately then dissolved in deionized water and diluted to 0.5 mg ml^{-1}. Subsequently, the standard solution was determined by CZE. The standard solution of CA was stored in the dark under refrigeration at 4 °C, and it was found to be stable for two months. And the standard solution was diluted with deionized water to prepare a series of working standard solutions.

2.4. Optimization of mixed solvent extraction

2.4.1. Optimization of the proportion of mixed solvent

0.5 g of Zhonghua I residue powder was measured accurately, and then added into 20 ml of different proportions of acetone and water mixed solvent (acetone:water: 0:1, 3:7, 4:6, v/v) in a flask. The extraction in the contents of the flask was then carried out with microwave treatment (300 W) for a period of 1.0 min. After extraction, the flask was cooled to room temperature before opening. The extract was filtered, subsequently the filtrate was diluted with one-fold amount of acetone and water mixed solvent (according to the proportion above) and then was injected into the CZE.

2.4.2. Optimization of the amount of mixed solvent

A sample of 0.5 g of Zhonghua I residue powder was added into a series amount of 10, 15, 20, 25 and 30 ml of acetone and water mixed solvent (3:7, v/v) in a glass bottle. Then the operation was performed as the description above. Finally the filtrate was diluted with one-fold amount of acetone and water mixed solvent (3:7, v/v) and then was injected into the CZE.

2.5. Optimum conditions for MAE procedure

MAE parameters such as microwave power and time can affect the extraction efficiency [40], so, investigation of the MAE parameters was done. A tobacco residue sample, Zhonghua, was used for this study. At first, 0.5 g of Zhonghua I residue was placed in a 50-ml flask followed with the addition of 20 ml of acetone and water mixed solvent (3:7, v/v). The solution in the flask was shaken equably by hand, and then extracted at different microwave powers (200, 300, 400 and 700 W) and different irradiation times (1.0, 2.0, 4.0 and 6.0 min).

2.6. Calibration curve, reproducibility and recovery

The eight-point calibration curve of CA was fabricated by plotting peak areas (y) of CA versus CA concentration (x), respectively. Concentration of CA in tobacco waste samples was calculated from each resulting peak area ratios and each regression equation of the calibration curve.

Three replicate injections of same standard sample of CA were analyzed by the CZE method to determine reproducibility in the conditions (system precision). The recovery of CA was studied with Zhonghua I residue with addition of 0.5 mg of CA powder. The mixture was processed under described sample preparation procedure and analyzed using CZE. Also, the detection limit (S/N = 3) of CA was calculated by the analysis of the standard solution with low concentration.

2.7. Comparison of MAE with USE and RE for extraction of CA in tobacco residue

USE is a conventional extraction technique, which is usually applied to extract and analyze CA in Chinese herbs [41]. RE is also applied to the CA extraction [42]. Here we make a comparison of MAE with USE and RE for the extraction of CA in tobacco wastes. 0.5 g of Zhonghua I residue powder was weighted accurately to a flask, and then 20 ml of acetone and water mixed solvent (3:7, v/v) was added to the flask. The powders were extracted in an ultrasonic bath for 30 min and RE for 4 h. At the same time, the same sample was extracted by MAE technique (400 W, 4.0 min). The three extracts were filtered and then the filtrates were diluted with one-fold amount of acetone and water mixed solvent (3:7, v/v) and injected into the CZE. The extraction efficiency was compared using the peak areas of the three analytes.

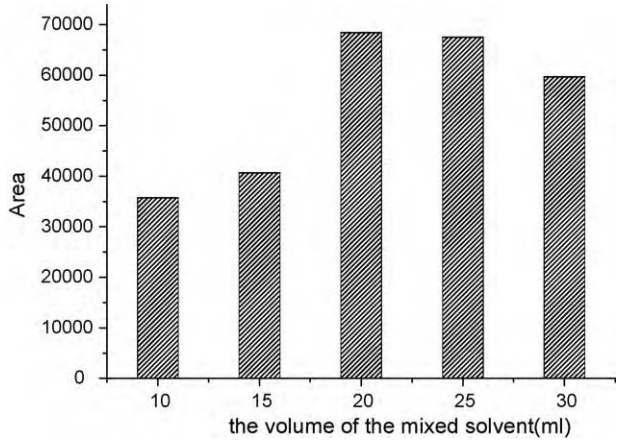

Fig. 2. The effect of solvent amount on the extraction efficiency of CA.

2.8. The fast analysis of CA in different tobacco residue samples using MAE and CZE technique

The proposed method with the optimal conditions was applied to the fast analysis of CA in different tobacco residue samples. 0.5 g of the tobacco residue sample was placed in a 50-ml flask followed with the addition of 20 ml of acetone and water mixed solvent (3:7, v/v). As above-mentioned procedure, the tobacco residue samples were extracted using MAE at 400 W and 4.0 min. The extract was filtered, subsequently the filtrate was diluted with one-fold amount of acetone and water mixed solvent (3:7, v/v) and then was injected into the CZE. The concentration of CA was calculated using its calibration curve.

3. Results and discussion

3.1. Optimization of mixed solvent extraction

3.1.1. Optimization of the proportion of mixed solvent

Acetone and water mixed solvent is often applied to the extraction of CA in Chinese herbs [12]. In order to obtain much higher extraction efficiency, different proportions of the mixed solvent were studied in our trials. As it was introduced in Section 2, Zhonghua I residue powder was extracted with mixed solvent in different proportions. Ultimately the proportion of 3:7 (v/v) has received the most satisfying result in our experiment. So we validate 3:7 (v/v) of acetone and water as the optimum condition.

3.1.2. Optimization of the amount of acetone and water mixed solvent

In an extraction process, it is important to maximize extraction yield, but also to minimize the consumption of solvent. In this study, the amount of mixed solvent was investigated. Zhonghua I residue powder was dissolved with different amounts of solvent of 10, 15, 20, 25 and 30 ml, but the extraction efficiency of CA was increasing at first and then decreasing when the solvent added to the Zhonghua I residue sample was more than 20 ml. As we can see in Fig. 2, the best amount of mixed solvent used to extract CA in Zhonghua I residue is 20 ml. In fact, in this process, if the solvent increases and the solid mass maintains constant, then the temperature of the mixture will change and it can influence the recovery [43].

3.2. Optimization of MAE parameters

The power and time of MAE are also important parameters that can affect the MAE efficiency. Here we investigated the microwave power of 200, 300, 400 and 700 W and the microwave extraction time of 1.0, 2.0, 4.0 and 6.0 min. The effects of microwave power and irradiation time on the extraction yields are represented in Fig. 3. The experimental results demonstrated that the extraction yield of CA increased with the enhancement of microwave power when it was in the range of 200–400 W and that the extraction yield did not significantly change from 400 to 700 W. So in this work, 400 W is the best condition. Meanwhile, the extraction efficiency of CA was increasing with the exposure time. However, the extraction efficiency decreased when the irradiation time was more than 4.0 min. This may be due to its decomposition at long irradiation time [44]. Therefore, the optimum conditions for the MAE are performed at a microwave power of 400 W and an irradiation time of 4.0 min.

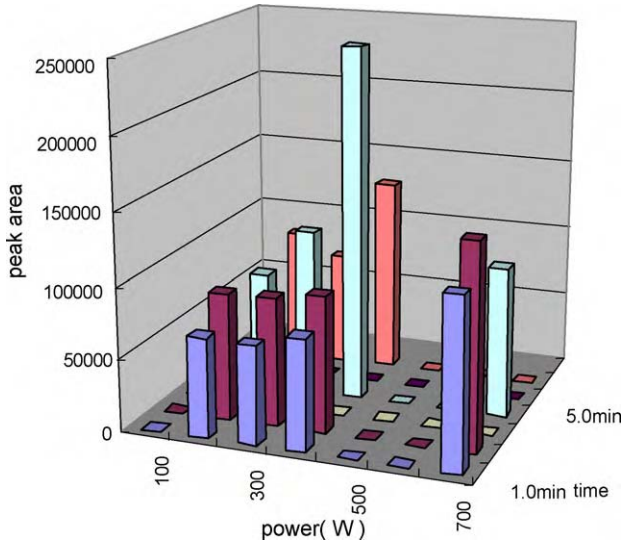

Fig. 3. The effect of microwave power and irradiation time on the extraction efficiency of CA in tobacco residues.

3.3. Method validations

In this work, external standard method is used since it is simple, fast and accurate for sample preparation. The calibration curve for CA is constructed by analyzing a series of CA samples in the concentration range from 0.003 to 0.5 mg ml^{-1} and by plotting concentration versus peak area (the simple plot is not shown). The calibration curve shows good linearity in 0.003–0.5 mg ml^{-1}. And the limit of detection (LOD) is 0.003 mg ml^{-1}, the limit of quantification (3.3-fold of LOD) is 0.01 mg ml^{-1}. The regression equation is $y = 5.36 \times 10^5 \times -6.09 \times 10^3$. Linear regression analysis of the data yields correlation coefficient $R^2 = 0.991$. A series of sample analysis are performed to validate the performance of the method. Reproducibility of the combined MAE and CZE method is assessed by the peak area of CA in three replicate analyses of the same extract. The percent of R.S.D. value is 4.28%. By spiking the standard sample powder of CA, the recovery of this component is measured. The amount of the spiked CA is calculated by subtracting the total amount of CA after spiking from the amount in the tobacco residue before spiking. The spiking experiments are repeated three times to get the mean amount of CA after spiking. The recovery is 89.0%.

3.4. Comparison of microwave-assisted extraction with ultrasonic extraction and reflux extraction

To demonstrate the MAE, the conventional sample extraction technique of USE and RE were also used to the extraction of Zhonghua I residue sample. Comparison of extraction efficiency for CA by MAE, USE and RE is shown in Fig. 4, which shows that

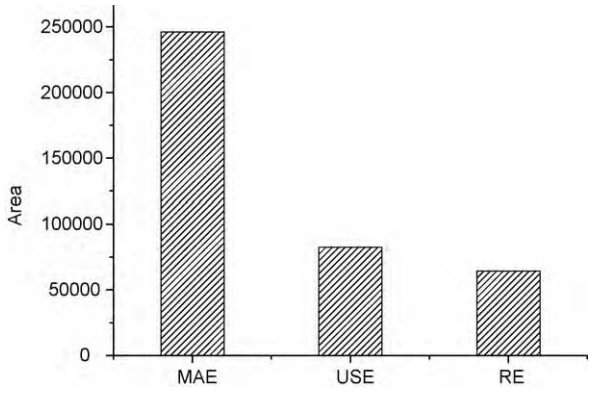

Fig. 4. Comparison of extraction efficiency of MAE with USE and RE.

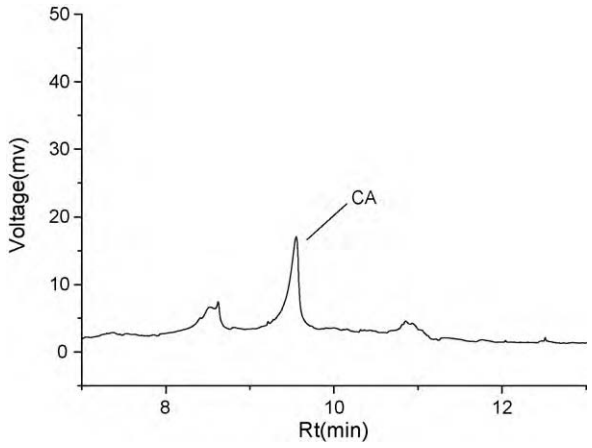

Fig. 5. The electropherogram of CA in tobacco residuals by CZE combined with MAE.

MAE obviously has a higher efficiency than USE and RE. Moreover, USE and RE are very time-consuming, so MAE is a fast, convenient, and efficient technique for the extraction of CA in tobacco residues.

3.5. Determination of CA in tobacco residue samples by the proposed method

This proposed method based on CZE combined with MAE is applied to the fast determination of CA in several kinds of tobacco residues, such as Zhonghua I, Zhonghua II, Jinqiao, Nanjing, Yipinmei, Shuangxi I, Shuangxi II, and Shuangxi III. Take Zhonghua I, for example, the experimental process was as above detailed and the filtrate was diluted with one-fold amount of acetone and water mixed solvent (3:7, v/v), then detected by CZE. As a result, we can determine the concentration of CA from each peak area in Fig. 5. The concentration of CA is calculated according to its calibration curve, and the results are shown in Table 1. The content of CA in different kinds of tobacco residue samples is various. The CA concentration in the six samples is from 7.82 to 12.88 mg g^{-1}. It demonstrates that we can quantify CA in tobacco residues using this method.

Table 1
The content of CA in different tobacco residues.

Tobacco residues	Peak area	Content of CA (mg g^{-1})
Zhonghua I	80,249	12.88
Zhonghua II	65,907	10.74
Jinqiao	46,315	7.82
Nanjing	63,427	10.37
Yipinmei	69,751	11.32
Shuangxi I	75,473	12.17
Shuangxi II	52,941	8.81
Shuangxi III	59,145	9.73

4. Concluding remarks

The extraction of chlorogenic acid from tobacco residues with the method of acetone and water (3:7, v/v) mixed solvent extraction, using microwave as an extraction aid, was effectively achieved. MAE required little solvent and little time (4.0 min) for the extraction of CA, and CZE was simple and fast analysis (10 min). The CZE technique combined with optimized MAE method dramatically reduced the extraction time and is proved to be a simple, time-saving, convenient and reliable method for the qualitative and quantitative assessments of chlorogenic acid in tobacco residues.

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