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Effervescence and graphitized multi-walled carbon nanotubes assisted microextraction for natural antioxidants by ultra high performance liquid chromatography with electrochemical detection and quadrupole time-of-flight tandem mass spectrometry

Shu-Ling Wang, Xiao-Qing Pang, Jun Cao*, Wan Cao, Jing-Jing Xu, Qiong-Yao Zhu, Qian-Yun Zhang, Li-Qing Peng

College of Material Chemistry and Chemical Engineering, Hangzhou Normal University, Hangzhou 310036, China

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ABSTRACT

In this article, effervescence and graphitized multi-walled carbon nanotubes assisted microextraction was first developed for the extraction of antioxidants in hawthorn samples. The use of an effervescent tablet composed of sodium dihydrogen phosphate, sodium carbonate and micro-scale carboxyl graphitized multi-walled carbon nanotubes (extraction sorbent) was the core of the method. In this study, ultra high performance liquid chromatography coupled with electrochemical detection and quadrupole time-of-flight tandem mass spectrometry was performed for qualitative and quantitative analyses of target analytes in hawthorn foodstuffs. Several experimental factors, such as amount of effervescent salts, the sorbent, elution time and elution solvent, were systematically assessed. Under the optimized conditions, a good linearity with *R* values better than 0.9980 was obtained. The detection limits estimated at a signal-to-noise ratio of 3:1 were ranging from 0.01 to 0.18 ng/mL. These results suggested that the proposed method could be an alternative and promising sample preparation tool in future food analysis.

1. Introduction

Carbon nanotubes (CNTs) are novel and interesting carbonaceous nano-materials first discovered in 1991 by Iijima [1]. Owing to their extremely large surface area, unique structure, and remarkable physicochemical characteristics, CNTs have attracted more research interest than any other material and have been used in many analytical fields [2]. On the principle of carbon atom layers in the wall of nanotubes, CNTs were divided into two classifications: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) [3]. Among them, graphitized multi-walled carbon nanotubes (GMWCNTs) are produced by 2800 °C thermal treatment the relevant purified carbon nanotubes under inert gas for about 20 h, which have attracted special interest in interdisciplinary fields owing to their micro and macrostructural characteristics. In addition, they exhibit more highly ordered graphitic structure, higher electrochemical stability, more perfect crystal lattice and higher purity than pristine MWCNTs due to the lesser surface defects [4,5]. In recent years, numerous studies have reported the potential applications of GMWCNTs in adsorption removal of pollutants [4], durable cathode catalyst [5], and dispersive cleanup of acetonitrile extracts [6] due to their high surface area and inner volume, mechanical strength, stability and the possibility of establishing π–π interactions [7]. Microextraction techniques as common sample preparation tools are resulted from the evolution of classic sample treatment towards simplicity and miniaturization in the development of competitive analytical methodologies [8]. The large variety of techniques, including solid-phase microextraction and liquid-phase microextraction, covered a wide range of applications in food [9], pharmaceutical [10], environmental [11], and biological samples [12]. Among all the available techniques, effervescence-assisted microextraction (EAM), based on dispersive forces introduced by effervescent reactions, was developed by Lasarte-Aragonés et al. [13]. In the extraction process, the dispersion of the sorbent was produced by the carbon dioxide source when the tablet components were added into the aqueous

* Corresponding author. Tel.: +86 571 2886 7909; fax: +86 571 2886 7909.
E-mail address: caojun91@163.com (J. Cao).

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sample. This method was very rapid, effective and has been successfully applied to the extraction of trace-level compounds from plant, environmental and food samples [14,15]. However, up to now, no article has been published on the use of GMWCNTs as sorbent materials in EAM for the extraction of these target analytes in complicated real samples, especially for foodstuffs.

Recently, more attention has been paid to use high performance liquid chromatography (HPLC) coupled with electrochemical detection (ECD) to detect the analytes of interest. ECD, which has the advantages of high sensitivity and selectivity, could provide information on the physicochemical properties of a molecule and has been used for the determination of electroactive compounds in different samples [16]. Currently, columns with 3–5 μm particle size are widely used and provide good retention of the polar compounds. The main limitations of classical HPLC-ECD are the need for large sample volumes and the long analysis time [17]. Therefore, in order to increase the efficiency of chromatographic separation, an ultra-fast liquid chromatography-electrochemical detection system with column of sub-2 μm particle size has been developed and its properties were systematically studied in recent papers [18,19]. However, to the best of our knowledge, there have not been any reports of the measurements of foodstuffs using ultra high performance liquid chromatography-ECD (UHPLC-ECD) and microextraction. As a result, it is meaningful for us to develop a simple and accurate method based on the use of UHPLC-ECD for the analysis of electrochemical active analytes from food samples.

The hawthorn (*Crataegus pinnatifida* Bge.) is a member of the Rosaceae family, and has been widely used as food and medicinal material in Asia, Europe and North America [20]. Recent researches showed that phenolic compounds were the major group of active components in hawthorn, and they have been confirmed to possess remarkable antioxidative activities [21,22]. Therefore, extraction and quantitative determination of antioxidant phenolic compounds from hawthorn samples are important for physiological and pharmacological studies. In this study, a simple, rapid and eco-friendly analytical method, using effervescence and GMWCNTs assisted microextraction, was first proposed for the extraction of antioxidants from hawthorn samples by UHPLC-ECD. In this case, carbon dioxide release was used as dispersive force to produce the dispersion of carboxyl graphitized multi-walled carbon nanotubes (GMWCNTs-COOH) avoiding the usage of organic solvent or surfactant. The experimental variables, including amount of effervescent salts, elution time, elution solvent, amount of sorbent, and pH of sample solution, were optimized and discussed in detail. Finally, the proposal was used to the determination of antioxidants in complex hawthorn foodstuffs, and quadrupole time-of-flight tandem mass spectrometry (Q-TOF/MS) was applied to characterize and identify the target analytes in these samples.

2. Experimental

2.1. Chemicals and reagents

Hydroxyl graphitized multi-walled carbon nanotubes (GMWNTs-OH), carboxyl multi-walled carbon nanotubes (MWCNTs-COOH), GMWNTs-COOH and GMWNTs with o.d. of 8–15 nm and lengths of 50 μm were obtained from Nanjing Jicang Nano Technology (Nanjing, China). MWCNTs of 1–3 nm inner diameter (i.d.) × 3–20-nm outer diameters (o.d.) with lengths of 0.1–10 μm were provided by Alfa Aesar China (Tianjin, China). Chromatographic grade sodium dihydrogen phosphate and phosphoric acid were purchased from Sigma–Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). Methanol, acetonitrile and isopropanol (HPLC grade) were supplied by Tedia Company Inc. (Fairfield, US). Analytically pure ethanol, ethyl acetate, sodium hydroxide, sodium dihydrogen phosphate dehydrate, anhydrous sodium carbonate and acetone were obtained from Hangzhou Chemical Reagent Co., Ltd. (Hangzhou, China). Purified water (Wahaha Group Ltd., Hangzhou, China) was employed throughout the experiment. The 0.2 μm disposable nylon membranes with a diameter of 50 mm were purchased from Jinteng Laboratory Equipment Co., Ltd. (Tianjin, China). The tested standards including catechin, chlorogenic acid, caffeic acid, epicatechin, protocatechuic acid, ferulic acid, hyperoside and isoquercitrin were collected from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China) with purity higher than 98%. The standard solutions were prepared by dissolving each compound in chromatographic grade methanol at a concentration of 500 μg/mL and stored at 4 °C in darkness. Hawthorn fruit was purchased from local drugstore (Hangzhou, China), and the hawthorn foodstuffs (Sweetend roll, Haw flakes, Sandwich hawthorn, Alpha hawthorn beverage, Yixin hawthorn beverage, Weizhiwang hawthorn beverage) were supplied by local supermarket (Hangzhou, China).

2.2. Instrumentation and chromatographic conditions

An Agilent 1290 liquid chromatography system (Agilent Technologies, Santa Clara, CA), equipped with a binary solvent delivery pump, an auto-sampler, a thermostated column compartment, and a Antec SDC ECD(Antec, Netherlands) was used. Chromatographic separation was carried out at a flow rate of 0.5 mL/min using an Agilent SB-C_{18} column (1.8 μm, 4.6 mm i.d. × 50 mm) maintained at 35 °C. The mobile phase consisted of 25 mM sodium dihydrogen phosphate buffer with 5% (v/v) methanol (pH 3.5) as eluent A and 25 mM sodium dihydrogen phosphate buffer with 80% (v/v) methanol as eluent B. The pH of the mobile phase was adjusted by phosphoric acid. The following gradient program was used: 0–8.5 min, 20–20%B; 8.5–9.0 min, 20–40%B; 9.0–15.0 min, 40–40%B; 15.0–17.0 min, 40–100%B. The injection volume was 1 μL. ECD, equipped with a superior Advanced Digital Filtering and one flow cell, were performed at the cell potential of E_{cell} = +0.70 V. The detector was set at 500 nA range versus the Ag/AgCl reference electrode.

This UHPLC system was connected to a 6530 Q-TOF mass spectrometer (Agilent, Technologies, Santa Clara, CA) equipped with a dual ESI source working in negative ion mode. High-purity Nitrogen (N_2) was used as nebulizing gas. The optimized parameters were as follows: drying gas temperature, 350 °C; nebulizer gas pressure, 45 psi; capillary voltage, 3500 V; fragmentor, 175 V; octapole RF, 750 V; skimmer voltage, 65 V; drying gas flow, 12 L/min and collision energy, 15–25 V. For qualitative analysis, the salt in the mobile phase was changed to the volatile ammonium acetate, and the mass spectrometer was operated in targeted MS/MS scan mode. Data processing was carried out using Mass Hunter software (version B 05.00 Qualitative Analysis), and the mass range was recorded in the range *m/z* 100–700.

2.3. Preparation of hawthorn samples

The KQ-100B ultrasonic bath (Kunshan Ultrasonic Instruments Co., Ltd., China) was applied for the extraction of hawthorn samples (Frequency 40 kHz, power 100 W). Hawthorn liquid samples (beverages) were directly used in this study. Because hawthorn solid sample contains hydrophobic and hydrophilic compounds, 70% methanol solution is suitable to extract target analytes by the optimization of extraction solvents. The solid samples were prepared as follows: two grams hawthorn fruit powder or hawthorn foodstuffs (after grinding) was transferred into a 50 mL erlenmeyer flask and dissolved with 15 mL of 70% methanol. The flask was covered and then placed in an ultrasound water bath for 60 min. The temperature of the water was set at 60 °C. Next, the samples were

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