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Analytical Methods

Hydrophilic interaction ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (HILIC-UPLC–TQ-MS/MS) in multiple-reaction monitoring (MRM) for the determination of nucleobases and nucleosides in ginkgo seeds

Guisheng Zhou [a], Hanqing Pang [a], Yuping Tang [a,*], Xin Yao [a], Yunhe Ding [a], Shaoqing Zhu [a], Sheng Guo [a], Dawei Qian [a], Juan Shen [a], Yefei Qian [a], Shulan Su [a], Li Zhang [a,b], Chun Jin [c], Yong Qin [c], Jin-Ao Duan [a,*]

[a] Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization and Jiangsu Key Laboratory for High Technology of TCM Formulae Research, Nanjing University of Chinese Medicine, Nanjing 210023, China
[b] Department of Analysis of Chinese Medicine, Nanjing University of Chinese Medicine, Nanjing 210023, China
[c] Jiangsu Shenlong Pharmaceutical Co., Ltd., Yancheng 224200, China

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ABSTRACT

In this study, a rapid, simple and sensitive analytical method was developed for the quantitative determination of 20 nucleosides and nucleobases in functional foods at trace levels using hydrophilic interaction ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (HILIC-UPLC–TQ-MS/MS) in multiple-reaction monitoring (MRM) mode. Under optimised chromatographic conditions, good separation of 20 target compounds was achieved using a Waters Acquity UPLC BEH Amide column and gradient elution in 11 min. The limits of detection (LODs) and quantification (LOQs) were between 0.02–42.54 ng/mL and 0.05–98.18 ng/mL for the 20 analytes, respectively. This is the first report about simultaneous analysis of nucleosides and nucleobases in functional foods using this method, which afforded good linearity, precision, repeatability and accuracy. The method developed was successfully applied to quantify target compounds in batches of ginkgo seeds. The method potentially could be used to determine polar and trace-level nucleosides and nucleobases in ginkgo seeds.

1. Introduction

Ginkgo biloba L. is the only remaining member of the Ginkgoaceae family (Major, 1967) and is widely distributed in extratropical, warm and subtropical zones especially in eastern China (Jiangsu, Shandong, Zhejiang, Anhui, and Guangxi provinces). Male and female flowers grow on different plants, and female plants bear a yellowish-green plum-like "fruit" (aril) (Choukchou-Braham, Asakawa, & Lepoittevin, 1994), which is the seed of ginkgo in gymnosperms. Ginkgo seed is rich in proteins, lipids, carbohydrates, vitamins, riboflavin, and many other nutrients including functional substance such as flavones and lactones (Yang et al., 2011). As a traditional food source, used for several thousand years, ginkgo seeds can be added to desserts, glazed fruit, beverages or alcoholic drinks (Deng et al., 2011). In addition to their food uses, ginkgo seeds have been used in many traditional medicines for treating cough, asthma, bronchitis and allergic inflammation (Singh et al., 2004). Ginkgo seeds may, therefore, be considered a so-called functional food, having nutritional as well as medicinal uses.

The nutritional and functional contents such as proteins, lipids, vitamins (Kobayashi, Yoshimura, Johno, Sasaki, & Wada, 2011), amino acids (Zhou et al., 2013) and carbohydrates from ginkgo seeds have attracted considerable attention (Singh, Kaur, Gopichand, Singh, & Ahuja, 2008). Recently, nucleobases and nucleosides have also been proven as important nutritional and functional contents related to multiple functions such as modulation of the immune response (Morimoto, Steinberg, Schlossman, & Borel, 1983), anti-tumor and anti-viral effects (Liu et al., 2012), and have also been selected as the quality control marker of several functional foods, such as *Ganoderma lucidumn* and *Cordyceps sinensis* (Liu, Li, Li, Wan, & Tang, 2011). Therefore, determination of nucleosides and nucleobases is beneficial for tapping potential values in ginkgo seeds as a food and convenient for quality control, which has not previously been reported.

* Corresponding authors. Address: Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization and Jiangsu Key Laboratory for High Technology of TCM Formulae Research, Nanjing University of Chinese Medicine, No. 138, Xianlin Road, Nanjing 210023, China. Tel./fax: +86 25 85811916.
E-mail addresses: yupingtang@njutcm.edu.cn, yupingtang9@126.com (Y. Tang), dja@njutcm.edu.cn (J.-A. Duan).

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Various analytical methods including high performance liquid chromatography (HPLC) (Li et al., 2004), UPLC (Yang, Guan, & Li, 2007), micellar electrokinetic chromatography (MEKC) (Li, Li, Dong, & Tsim, 2001) and capillary electrophoresis (CE) (Cheung, Ng, & Hood, 2001) have been used for the quantification of nucleosides in biological fluids and herbal materials. And these separation techniques are frequently coupled with mass spectrometric detection (Fan et al., 2006), which is highly sensitive and selective, and provides confirmation of structural to some degree. However, most of the above methods have the disadvantages of poor selectivity and/or limited identification for the nucleosides and nucleobases. Methods based on ion-pair reverse-phase (Yang, Li, Feng, Hu, & Li, 2010) and HILIC were developed subsequently to retain nucleobases and nucleosides, which have relatively high polarity (Chen, Bicker, Wu, Xie, & Lindner, 2012; Chen et al., 2011). However, equilibration times are prolonged and column lifetime reduced in ion-pair reverse-phase chromatography (Paglia et al., 2012), and analysis time prolonged in HILIC.

So far, no report has described a rapid and sensitive method with good retention for the determination of nucleosides and nucleobases. Thus, a better method for identification and quantification of nucleosides and nucleobases needs to be developed and validated, which should include: (1) adequate retention of highly polar compounds using HILIC, which might be a promising alternative chromatographic column for these compositions (Fraser et al., 2012); (2) rapid and excellent separation using UPLC (Núñez, Gallart-Ayala, Martins, & Lucci, 2012); and (3) specific and sensitive ESI–MS/MS detection using MRM transitions, which excludes false-positive results.

HILIC is known to partly solve some issues that normal phase or polar-embedded columns pose for mass detectors, specifically the need to use ion-pairing agents combined with highly aqueous mobile phases, both of which affect MS sensitivity (Xing et al., 2012). With HILIC columns, the retention of polar analytes has been improved by increasing the percentage of organic solvents contained in the aqueous mobile phases (Jin et al., 2008). Progress in chromatographic techniques led to the development of UPLC using columns containing particles with a diameter of <2 μm, which operate at higher pressures, resulting in shorter analysis times and increased peak resolution, capacity, and sensitivity (Kang et al., 2011). MS has been used widely used for the identification and quantification of chemical components in medicinal plants and foods (Herrmann, Rosén, Jansson, & Hellenäs, 2012), especially for trace constituents, which occur at the limit of detection and require high selectivity (Chu, Gao, Yin, Krasner, & Templeton, 2012). It has also been used for the determination of nucleosides (Fan et al., 2006). Further development of different stationary phases led to increased application of this technique in analytical chemistry, e.g. in analysis of polar food compounds, amino acids, and polar metabolites in body fluids (Schettgen et al., 2007). HILIC–MS has proven to be an excellent analytical tool for polar and similar structural compounds.

In this paper, an efficient and sensitive method based on the above notion was developed and validated by using HILIC-UPLC–TQ-MS/MS for simultaneous identification and quantification of nucleosides and nucleobases in ginkgo seed extracts. It is the first time that HILIC-UPLC–TQ-MS/MS has been employed to analyse simultaneously 20 nucleosides and nucleobases in a functional food.

2. Experimental

2.1. Chemicals and reagents

Ammonium acetate and acetic acid were analytical grade (Sino pharm Chemical Reagent Co., Ltd., Shanghai, China). Twenty standards including adenosine, thymidine, guanosine, cytidine, uridine, inosine, 2′-Deoxyadenosine, 2′-deoxyguanosine, 2′-deoxycytidine, 2′-deoxyuridine, 2′-deoxyinosine, adenosine-3′,5′-cyclic monophosphate, 2′-deoxycytidine-5′-monophosphate, cytidine-5′-monophosphate, adenine, thymine, guanine, uracil, hypoxanthine, and xanthine are shown in Fig. 1. Uridine, adenosine-3′, 5′-cyclic monophosphate, adenine, guanine, uracil, and hypoxanthine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 2′-deoxyadenosine and 2′-deoxycytidine were obtained from Aladdin Chemical Co. (Nanjing, China). Other standards were from Sigma Chemical Co. (St. Louis, MO).

Individual standards (1 mg) were prepared by dissolving in 5 mL distilled water. A working standard mixture of 100 μg/mL was prepared by diluting the intermediate stock standard solution, from which the calibration standards within the range 10–100,000 ng/mL were prepared by serial dilution with acetonitrile/water (9:1, v/v). The standard solutions were filtered through a 0.22 μm cellulose membrane filter prior to injection.

2.2. Plant materials

Ginkgo seed samples (48), consisting of 15 samples of five different parts (episperm, mesosperm, endopleura, endosperm, and plumule), which are shown in Fig. 2,11 samples from trees of different ages (8, 10, 15, 20, 25, 30, 40, 50, 100, 300, and 600 years of age), and 22 samples from different habitats in China were analysed. Details of each sample are listed in Table 1. The botanical origins of the samples were identified as the seeds of *Ginkgo biloba* L. by Dr. Hui Yan (Department of Pharmacognosy, Nanjing University of Chinese Medicine, China), and the voucher specimens were deposited at the Herbarium in Jiangsu Key Laboratory for TCM Formulae Research, Nanjing University of Chinese Medicine, China. After collection, the samples were dried by air.

2.3. Sample preparation

1 g of each dry sample powder was accurately weighed into 50 mL conical flask, and 40 mL of distilled water was added to each conical flask, except for those of samples 3, 4, 5, 8, 9, 10, 13, 14, 15, into each of which 20 mL of water was added. All of the mixtures were precisely weighed and placed into an ultrasonic bath (40 kHz) for 60 min at room temperature, and water added to compensate for any lost during extraction. After centrifugation, the supernatants were stored at 4 °C and filtered through 0.22 μm cellulose membrane filters prior to injection.

2.4. HILIC-UPLC–TQ-MS/MS conditions

UPLC was performed using a Waters ACQUITY UPLC system (Waters, Milford, MA, USA). HILIC separation was performed on an ACQUITY UPLC BEH amide column (2.1 mm × 100 mm, 1.7 μm). The mobile phase consisted of A (0.8% acetic acid and 10 mmol/L ammonium acetate in aqueous solution) and B (0.1% acetic acid in acetonitrile). UPLC linear gradient conditions were: 0–6 min, 10% A; 6–9 min, 40% A; 9–11 min; 50% A. The injection volume was 1 μL, and the column temperature was maintained at 35 °C.

Mass spectrometry detection was performed by using a Xevo™ Triple Quadrupole MS (Waters Corp., Milford, MA) equipped with an electrospray ionisation (ESI) source operating in positive ionisation mode. The desolvation gas flow rate was set to 1000 L/h at a temperature of 550 °C, the cone gas flow rate was set at 50 L/h and the source temperature was set at 150 °C. The capillary voltage was set to 3000 V; the cone voltage was set