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Simultaneous determination of six triterpenic acids in some Chinese medicinal herbs using ultrasound-assisted dispersive liquid–liquid microextraction and high-performance liquid chromatography with fluorescence detection

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ABSTRACT

A novel analytical method was developed for simultaneous determination of six triterpenic acids using ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) follow by high-performance liquid chromatography (HPLC) with fluorescence detection. Six triterpenic acids (ursolic acid, oleanolic acid, betulinic acid, maslinic acid, betulonic acid and corosolic acid) were extracted by UA-DLLME using chloroform and acetone as the extraction and disperser solvents, respectively. After the extraction and nitrogen flushing, the extracts were rapidly derivatized with 2-(12,13-dihydro-7H-dibenzo[a,g]carbazol-7-yl)ethyl4-methylbenzenesulfonate. The main experimental parameters affecting extraction efficiency and derivatization yield were investigated and optimized by response surface methodology (RSM) combined with Box–Behnken design (BBD). The limits of detection (LODs) and the limits of quantification (LOQs) were in the range of 0.95–1.36 ng mL^{-1} and 3.17–4.55 ng mL^{-1}, respectively. Under the optimum conditions, the method has been successfully applied for the analysis of triterpenic acids in six different traditional Chinese medicinal herbs.

1. Introduction

Triterpenic acids are significant bioactive phytochemicals and ubiquitously present in nature in the form of free acids or aglycones for triterpenoid saponins [1]. Recent researches have demonstrated that triterpenic acids exert a number of multi-target properties, such as anti-inflammatory, antioxidant, antitumor [2], hepatoprotective effects, anti-HIV activity [3], antifungal, antihyperlipidemic [4,5], antiatherogenic, antidiabetic and enhancing the cellular immune system [6], and they are also described as important functional compounds with wide application in cosmetics and healthcare products [7]. Therefore, the development of sensitive and accurate method for rapid determination of triterpenic acids is of great importance and it could help control the quality of related herbs and functional food.

Regarding the determination of triterpenic acids, various methods have been proposed base on capillary electrophoresis (CE) [8,9], HPLC [10,11], gas chromatography (GC) [12,13], thin-layer chromatography (TLC) [7,14] and NMR [15,16]. Among these cited techniques, HPLC is frequently used for the separation and quantification of triterpenic acids. However, because of the lack of suitable chromophoric or fluorescent moieties in triterpenic acid molecules, employing post-column derivatization has been widely accepted to enhance the selectivity and sensitivity [17]. In this study, a new novel pre-column fluorescence labeling reagent 2-(12,13-dihydro-7H-dibenzo[a,g]carbazol-7-yl)ethyl4-methylbenzenesulfonate (DDCETS) has been synthesized and successfully applied to detect triterpenic acids. Compared with traditional derivatization reagents for labeling carboxyl moiety, such as 6-Oxy-(acetyl piperazine) fluorescein [18] and 9-anthryldiazomethane [19], DDCETS has much bigger conjugate structure, which could offer stronger photoluminescence property

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and improve the detection sensitivity. In addition, its derivatization procedure has the advantages of mild reaction conditions, simple procedure and short reaction min. To the best of our knowledge, DDCETS was first employed as the pre-column derivatization reagent for the determination of six triterpenic acids in real samples.

However, there are still many challenges remaining in the course of analyzing triterpenic acids. The target compounds in natural products are usually found at trace levels. Moreover, the complex matrix in samples not only interferes with accurate measurement of the analytes, but also contaminates the chromatographic column and shortens column lifetime, so a sample preparation prior to chromatographic analysis is apparently necessary and significant. Currently the most commonly used techniques for sample preparation were liquid–liquid extraction (LLE) [20] and solid phase extraction (SPE) [21,22], but various inevitable defects have also been found in their application. As a classical pretreatment method, LLE suffers from disadvantages such as being time-consuming and the consumption for large amounts of samples and toxic organic solvents [23]. Although SPE takes much less organic solvent and time than LLE, it is relatively expensive and still needs several complex steps prior to instrumental analysis. In addition, it may result in analytes losses and contamination [24]. Recently, dispersive liquid–liquid microextraction (DLLME) is a novel and emerging microextraction technique, which was proposed by Assadi and coworkers previously [25]. In comparison with the conventional extraction methods, DLLME could be a wiser choice because of its many distinct advantages such as high enrichment ability, simple operation, low organic solvent consumption, high recovery and low cost [26].

The aim of this work was to establish a sensitive and selective UA-DLLME/HPLC-FLD (ultrasound-assisted dispersive liquid–liquid microextraction/high-performance liquid chromatography with fluorescence detection) method for simultaneous determination of six triterpenic acids. The main experimental parameters that influence the derivatization yield and UA-DLLME efficiency were investigated and optimized by Box–Behnken design (BBD) of response surface methodology (RSM). Under the optimal conditions, the proposed method has been successfully applied to the analysis of six triterpenic acids in six traditional Chinese medicinal herbs, which possessed good sensitivity, satisfactory recoveries as well as repeatability.

2. Materials and methods

2.1. Chemicals

2-(12,13-Dihydro-7H-dibenzo[a,g]carbazol-7-yl)ethyl4-methylbenzenesulfonate (DDCETS) was synthesized in our laboratory. Ursolic acid (UA) and oleanolic acid (OA) were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Maslinic acid (MA), betulinic acid (BIA), corosolic acid (CA) and betulonic acid (BOA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade), ethanol, chloroform and acetone were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Other reagents were of analytical grade from Jining Chemical Reagent (Jining, Shandong Province, China). Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

An Agilent 1100 Series HPLC/mass spectrometry system was used for all analysis. The mass spectrometer (MSD Trap SL, model G2445D) from Bruker Daltonik (Bremen, Germany) was equipped with an atmospheric pressure chemical ionization (APCI) source (model G1947A). Ion source conditions: APCI in positive ion detection mode; nebulizer pressure 60 psi; dry gas temperature, 350 °C; dry gas flow, 5 L/min. APCI Vap temperature 350 °C; corona current 4000 nA; capillary voltage 3500 V. An ultrasonic cleaner (KQ3200E, Kunshan Ultrasonic Instrument, Jiangsu, China) set at 40 kHz (equivalent to the wavelength of 37.5 mm) was used to emulsify the solutions. A XH-100A microwave-oven (Xianghu Science and Technology Development Co., Ltd, Beijing, China) was used for microwave assisted extraction, which equipped with a monitor of temperature, microprocessor programmer software and a microwave power of 1000 W.

2.3. Chromatographic parameters

Chromatographic separation was performed on a Hypersil C18 (4.6 mm × 200 mm, 5 μm) column with a gradient elution. The mobile phase was water containing 5% acetonitrile (A) and 100% acetonitrile (B) with a flow rate of 1 mL min^{-1}, and the column temperature was set at 30 °C. The elution program was as follows: 0–5 min, 65–90% B; 5–15 min, 90–92% B; 15–35 min, 92–92.5% B; 35–38 min, 92.5–100% B. The injection volume was 10 μL for each analysis and the detection wavelength was set at $\lambda_{ex}/\lambda_{em}$ = 292/402 nm.

2.4. Synthesis of derivatization reagent (DDCETS)

2.4.1. Syntheses of 12, 13-dihydro-7H-dibenzo [a,g] carbazole

According to the previously reported method [27], DDCETS was synthesized and shown in Fig. S1. Concentrated hydrochloric acid (90 mL), water (250 mL) and naphthylhydrazine (19 g) were well mixed and rapidly heated to reflux with stirring. Next, 3.4-dihydro-1(2H)-naphthalenone (1 mL) was added dropwise within 1 h. After refluxing for 1 h, the mixture was cooled. Then the precipitated solid was recovered by filtration, washed with water and 75% ethanol, and dried at room temperature for 48 h. The crude product was recrystallized three times to afford a rufous crystal, yield 80%. m.p. 193.7–194.2 °C. Found (%): C 89.12%, H 5.63%, N 5.28%; calculated (%): C 89.22%, H 5.57%, N 5.20%. IR (KBr): 3403.56 (N–H), 3048.03, 2952.89, 2882.32, 1503.86 (Ar), 1392.13 (C–N), 802.21, 765.83; MS: m/z $(M+H)^+$: 270.1.

2.4.2. Synthesis of 2-(12, 13-dihydro-7H-dibenzo [a,g]carbazol-7-yl) ethanol

12,13-Dihydro-7H-dibenzo[a,g] carbazole (25 g), KOH (20 g), and 2-butanone (80 mL) were well dissolved at 40 °C in a 500-mL round-bottomed flask and rapidly cooled to 0 °C with ice-water by vigorous stirring. A freezing mixture of oxane (7.5 g) in 50 mL 2-butanone solution was added dropwise within 1 h. After keeping the temperature constant for 2 h with stirring, the solution was heated to 55 °C for 2 h and concentrated by rotary evaporation. After cooling, the residue was transferred into 200 mL ice-water with vigorous stirring for 0.5 h. The precipitated solid was recovered by filtration, washed with water and 75% ethanol and dried at room temperature for 48 h. The crude product was recrystallized three times to afford white crystals, yield 78%. m.p. 131.4–132.2 °C. Found (%): C 84.28%, H 6.12%, N 4.50%; calculated (%): C 84.35%, H 6.07%, N 4.47%. IR (KBr): 3368.54 (O–H), 3050.03 (Ar), 1137.21 (C–O), 816.51, 725.83; MS: m/z $(M+H)^+$: 313.5.

2.4.3. Synthesis of 2-(12,13-dihydro-7H-dibenzo[a,g]carbazol-7-yl)ethyl 4-methylbenzenesulfonate (DDCETS)

A mixture of 2-(12,13-dihydro-7H-dibenzo[a,g]carbazol-7-yl) ethanol (5 g) in 50 mL of pyridine solution was added dropwise