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Analytical Methods

Study on infrared-assisted extraction coupled with high performance liquid chromatography (HPLC) for determination of catechin, epicatechin, and procyanidin B2 in grape seeds

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ABSTRACT

In this study, infrared-assisted extraction (IRAE) was developed and combined with HPLC for the determination of catechin, epicatechin, and procyanidin B2 in grape seeds. Three factors, extraction solvent, solid/liquid ratio, and illumination time, were investigated to optimise the extraction method of catechin, epicatechin, and procyanidin B2 from grape seeds. The chosen infrared-assisted extraction conditions were as follows: a 50% methanol solution as extraction solvent, solid/liquid ratio of 1:150 g/mL and illumination time of 30 min. The extraction efficiency of IRAE was compared with conventional microwave-assisted extraction (MAE), ultrasonic extraction (UE), and classical electrical heating (CEH) methods. An initial HPLC method was established to identify and quantitate catechin, epicatechin, and procyanidin B2 from grape seeds, simultaneously. The HPLC method was validated for parameters including linearity, analytic limit, reproducibility, accuracy and recovery. The results indicted that IRAE has a great potential for offering an alternative technique for extraction of active components from plants.

1. Introduction

Grape seeds are known to be rich in polyphenols, especially procyanidins (Gonzalez-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004; Cerpa-Calderon & Kennedy, 2008). The procyanidins identified in grape seeds can range in size from monomers to long-chain polymers, such as catechin, epicatechin, and procyanidin B2 (one of dimers). The dimers are the most widely distributed in grape seeds; dimer and trimer are the most active part of the procyanidins (Guo & Xu, 1996). As the number of isomer proanthocyanidins is numerous, it is currently very difficult to separate them by liquid chromatography (Prieura, Rigauda, Cheyniera, & Moutouneta, 1994). Grape procyanidins have significant anti-oxidation properties and their properties are excellent free radical scavengers. Radioprotective effects (Castillo et al., 2000), cataract prevention (Yamakoshi, Saito, Kataoka, & Tokutake, 2002), antihyperglycemic effects (Pinent et al., 2004), and anti-inflammatory effects (Terra et al., 2007) as well as anti-cancer efficacy (Veluri et al., 2006) have also been reported for these compounds. Therefore, the extraction of procyanidins from grape seeds for dietary supplement, pharmaceutical, and healthy foods has received great interest recently (Xu, Zhang, Wang, & Lu, 2010; Lafka, Sinanoglou, & Lazos, 2007; Shi et al., 2003).

It is well known that sample preparation is one of the most important and limiting steps for qualitative and quantitative analysis (Yu et al., 2009). The most commonly used extraction procedures for procyanidins from grape seeds are mechanical agitation, ultrasonic extraction, and the use of diverse extraction solvents (Changmou et al., 2010; Kallithraka, Garcia-Viguera, Bridle, & Bakker, 1995; Lafka et al., 2009; Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003; Pinelo, Del Fabbro, Manzocco, Nunez, & Nicoli, 2005; Yilmaz & Toledo, 2006). However, for the determination of target analytes, there has been an increasing demand for new extraction techniques that would be faster, consume less organic solvent, and be amenable to automation. Driven by these demands, several techniques, such as microwave-assisted extraction (MAE), supercritical fluid extraction and pressurised liquid extraction, have been developed (Yu et al., 2009). This study introduces an additional novel method: IRAE. IRAE, a process of using infrared lamp energy to heat solvent in contact with a sample in order to partition analytes from the sample matrix into the solvent, in most case, could improve the extraction efficacy of analytes compared to conventional techniques. In addition, compared to other modern extraction techniques as MAE, IRAE is easier to use, cheaper and free of irradiation.

The objective of this work was to explore the infrared-assisted extraction (IRAE) combined with HPLC for the determination of catechin, epicatechin, and procyanidin B2 from grape seeds, respectively. The extraction parameters including extraction solvent, solid/liquid ratio, illumination time were investigated. The extraction yield of catechin, epicatechin, and procyanidin B2 by IRAE was compared with those by CHE, MAE and UE under their investigated conditions.

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2. Materials and methods

2.1. Reagents and samples

Standards of epicatechin and catechin (purity > 99.0%) were purchased from The National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Procyanidin B2 (purity > 99.0%) was purchased from Tianjin Jianfeng Natural Product Co., Ltd. (Tianjin, China). HPLC-grade methanol was obtained from TEDIA company, Inc. (Ohio. USA). Potassium dihydrogen phosphate, analytical reagent grade, was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Water used for HPLC was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA). Grape seeds were obtained from the Shanghai First Class food Co., Ltd.

2.2. Standard solutions

Stock solutions were obtained by dissolving of appropriate amounts of epicatechin, catechin, and Procyanidin B2 in 50% aqueous methanol and stored at 4 °C, respectively. Working solutions were prepared by diluting the stock solutions with 50% methanol solution. All solutions prepared for LC analysis were filtered through 0.45-μm nylon membranes before use.

2.3. Chromatographic conditions

HPLC analysis was performed on an Agilent 1100 LC system equipped with a G1310 A QuatPump, a G1322 A vacuum degasser, a G1316 A thermostated column compartment, a VWD variable wavelength UV/VIS detector, and an HP1100 series manual injector 20 μL fixed loop. The extracts were injected and separated on a Diamonsil C18 column (150 mm × 4.6 mm, 5 μm, Dikma, Beijing, China). The mobile phases were: (A) methanol and (B) 5 mmol/L Potassium dihydrogen phosphate buffer (20:80). The flow rate was 1.0 mL/min; the column temperature was 38 °C; the injection volume was 20 μL and the UV detector was operated at 280 nm. The method was validated for parameters including linearity, analytic limit, reproducibility, accuracy and recovery.

2.4. Extraction procedure

2.4.1. IRAE

An IR lamp system assembled with a 275 W Anndesum IR lamp purchased from Qiyi Lighting Company (Zhejiang, China), was employed for the extraction of catechin, epicatechin, and procyanidin B2 from grape seeds. The apparatus is shown in Fig. 1.

The ground, sun-dried grape seeds (0.1 g) accurately weighted and introduced into a 100 mL round-bottom flask with solvent. First, the flask was weighed before extraction. After weighing, the flask was fixed on the IR lamp system, and then the IR lamp was turned on to extract the contents. According to the experimental design, the extraction was performed under different conditions. The effects of extraction solvent (water, methanol, the different proportion mixture of water and methanol), ratio of solid/liquid (1:50, 1:100, 1:150, and 1:200), and illumination time (10, 20, 30, and 40 min) were investigated.

2.4.2. MAE

For traditional MAE, a 0.1 g sample of ground, sun-dried grape seeds sample was put into a 100 mL round-bottom flask with 50% methanol. The effects of extraction ratio of solid/liquid (1:20, 1:50, 1:100, and 1:150), the power of microwave (200, 400, and 600 W), and illumination time (5, 7, 9, and 11 min) were investigated.

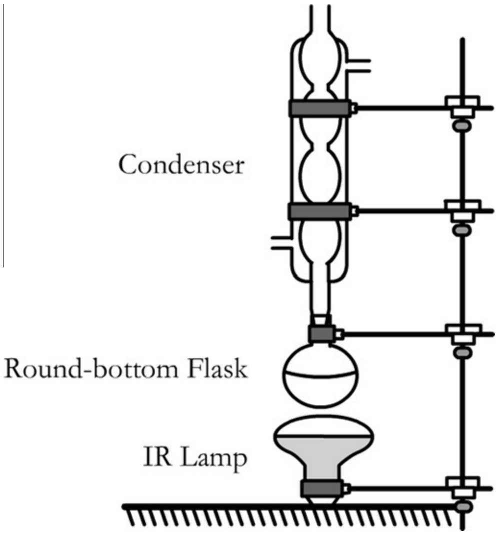

Fig. 1. The apparatus of IR lamp system.

2.4.3. UE

For conventional UE, a 0.1 g sample of ground, sun-dried grape seeds sample was put into a 100 mL round-bottom flask with 50% methanol. The effects of extraction ratio of solid/liquid (1:20, 1:50, 1:100, and 1:150), and illumination time (30, 60, 90 and 120 min) were investigated.

2.4.4. CEH

For conventional UE, 0.1 g sample of ground, sun-dried grape seeds sample was put into a 100 mL round-bottom flask with 50% methanol. The effects of extraction were investigated.

2.5. Method validation

To demonstrate that the method would be useful in laboratories as a routine analysis procedure, performance criteria were examined for parameters including linearity, detection limit, repeatability, accuracy and recovery.

2.6. Analysis of catechin, epicatechin, and procyanidin B2 in grape seeds

After the extraction, the flasks were cooled at room temperature for a while and the solvent was added to the flask to maintain the concentration of the sample. Then the extracts obtained from IRAE procedure were centrifuged at the speed of 8000 rpm for 10 min. The supernatant was filtered through a 0.45-μm filter and 20 μL was injected into the HPLC system. Peak area calibration curves were used for the quantitative analysis of catechin, epicatechin, and procyanidin B2. The linearity, LOD, precision and recovery were investigated for the validation of the method. The results of IRAE were compared to the MAE and UE methods.

3. Results and discussion

3.1. Investigation of IRAE conditions

Effects of extraction solvents, ratio of solid/liquid, and irradiation time on the extraction efficiency were studied. Extraction yield of catechin, epicatechin, and procyanidin B2 were used as a marker for evaluation.