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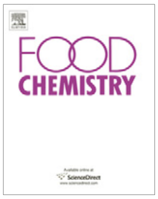

Analytical Methods

Matrix solid phase dispersion extraction of ginsenosides in the leaves of *Panax ginseng* C.M. Mey

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ABSTRACT

The matrix solid phase dispersion (MSPD) was applied to the extraction of eight kinds of ginsenosides from the leaves of *Panax ginseng* C.M. Mey. The experimental conditions were examined and optimised. The extracts were analysed by high performance liquid chromatography (HPLC). When the diatomaceous earth was used as dispersant and 75% methanol was used as elution solvent, the highest extraction yields of the eight kinds of ginsenosides were obtained. For comparison, the reflux extraction was applied. The extraction yields obtained by the proposed method were comparable with those obtained by the reflux extraction. The proposed method is convenient, time-saving, and organic solvent minimising.

1. Introduction

Panax ginseng C.M. Mey (*P. ginseng*) has been used as a traditional medicine in China for thousands of years (Chevallier, 2000). As we all know, the pharmaceutical properties of *P. ginseng* are mainly attributed to ginsenosides and up to now, more than 30 ginsenosides have been reported from *P. ginseng* (Fuzzati, 2004; Fuzzati, Gabetta, Jagaker, Pace, & Peterlongo, 1999). A lot of active constituents were found in the extracts of different species of ginseng (Attele, Wu, & Yuan, 1999). The scientists have demonstrated the extracts of ginseng have the anti-diabetic ability (Wang, 1965, 1980). Nowadays, ginseng is used mainly to enhance resistance to physical, chemical and biological stress and boost general vitality and frequently features in traditional medicine used by cancer patients (Chang, Seo, Gyllenhaal, & Block, 2003). However, the cost of roots of ginseng is too high. The stems and leaves of *P. ginseng* have been found containing the same ginsenosides with the pharmaceutical activity (Kiefer & Pantosu, 2003). So there were a lot of studies on ginsenoside contents in the leaves of *P. ginseng* (Shin, Kiyohara, Matsumoto, & Yamada, 1997).

As we all know, the most traditional and common method is reflux extraction. It is usually multi-step procedures, typically based on a large amount of organic solvents, and requires much manual handling of the extracts.

The simplification, miniaturisation of sample preparation and the minimisation of organic solvents are the modern trends in analytical chemistry, especially in the sample preparation. In order to reduce the analytical cost and ensure quality control, matrix solid phase dispersion (MSPD) have been successfully applied in some aspects, such as the isolation of a wide range of drugs (Martins Teixeira & Teixeira da Costa, 2005), pesticides (Abhilash, Singh, & Singh, 2009), naturally occurring constituents and other compounds from different complex plant and animal tissues (Barker, 2000). MSPD is based on a blending process between the samples and an abrasive dispersant. The mixture is then transferred into an empty glass column to form a packing column and finally the analytes are eluted by using the appropriate solvent. The major factors affecting MSPD are the dispersant material and the elution solvent.

MSPD was applied to the extraction of phenolic acids in *Melissa officinalis* (Žiaková, Brandšteterová, & Blahová, 2003). The method was also applied to the extraction of isoflavonoids (Xiao, Krucker, Albert, & Liang, 2004), and employed to extract and isolate isoflavone glucoside from leaves of leguminous plants (Eva de, Frans de, Freek, Udo, & Cees, 2004).

Some active compounds have been extracted from plants by MSPD. But as far as we know, this technique has never been applied to the extraction of ginsenosides from the leaves of ginseng.

In this work, MSPD was applied for the extraction of eight kinds of ginsenosides, including (20s)-3β-[2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyloxy]-20-[(6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranosyloxy] damma-24-ene-12β-ol (Rb_2), (20s)-3β-[2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosy loxy]-20-[6-*O*-(α-L-arabinofuranosyl)-β-D-glucopyranosyloxy]damma-24-ene-12β-ol (Rc), (20s)-3β-[2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyloxy]-20-(β-D-glucopyranosyloxy)damma-24-ene-12β-ol(Rd), (20S)-6-α–[2-*O*-(α-L-

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rhamnopyranosyl)-β-D-glucopyranosyloxy]-20-(β-D-glucopyranosyloxy)dam ma-24-ene-3β,12β-diol (Re), (20S)-6α-(β-D-glucopyranosyloxy)-20-(β-D-glucopyranosyloxy)damma-24-ene-3β,12β-diol (Rg$_1$), (20S)-20-(β-D-Glucopyranosyloxy)damma-24-ene-3β,6α,-12β-triol (F_1), (20S)-3β-(β-D-Glucopyranosyloxy)-20-(β-D-glucopyranosyloxy)damma-24-ene-12β-ol (F_2) and (20S)-20-[6-*O*-(α-L-arabinopyranosyl)-β-D-glucopyranosyloxy]-5α-damma-24-ene-3β,-6α,12β-triol (F_3), from the leaves of *P. ginseng* and compared with the reflux extraction.

2. Materials and methods

2.1. Reagents and materials

Ginsenosides F_1 (98.9%), F_2 (99.0%), F_3 (97.5%), Re (98.1%), Rg$_1$ (98.0%), Rd (99.0%), Rc (98.2%) and Rb$_2$ (99.5%) were obtained by extraction, separation and purification from leaves of *P. ginseng* in our laboratory. The standard solutions were prepared by dissolving 4.16, 1.93, 3.49, 10.33, 6.01, 5.92, 3.27 and 3.22 mg of F_1, F_2, F_3, Re, Rg$_1$, Rd, Rc and Rb$_2$ into 10 mL of methanol, respectively, and stored at 4 °C. The leaves of *P. ginseng* were obtained from Jilin province in China. The leaves were dried, smashed and passed through a 80 mesh sieve.

Acetonitrile (Shandong Yuwang Industrial Co., Ltd., Chemical Branch) was of HPLC grade. Neutral alumina and alkaline alumina were obtained from Shanghai Ludu Chemical Reagent Factory (100–200 mesh). Silica gel was obtained from Qingdao Haiyang Chemical subsidiary factory. Diatomaceous earth was purchased from Tianjin Guangfu Fine Chemical Industry Research Institute, calcinated at 650 °C for 12 h and cooled at room temperature in desiccators before used. Dichloromethane, ethyl acetate and methanol were obtained from Beijing Chemical Works.

2.2. Chromatographic conditions

An Agilent 1200 series liquid chromatography was used. The analytes were separated on a Zobax SB-C$_{18}$ column (25 cm × 4.6 mm, particle size 5 μm). Chromatographic separation of compounds was achieved by using a gradient elution and the mobile phase consists of acetonitrile and water. From 0.00 to 26 min the volume ratio of acetonitrile to water was 20:80; from 27 to 42 min, the ratio was kept at 28.9:71.1; from 42 to 57 min, the ratio linearly changed to 40:60. The flow rate of mobile phase was 1 mL min^{-1}. The temperature of column was 25 °C. The absorption of compounds was detected at wavelength of 203 nm.

2.3. Scanning electron microscopy

Scanning electron microscope (SEM) images were taken with a JEOL FESEM 6700F electron microscope with primary electron energy of 3 kV. The samples were sputtered with a thin layer of Pt prior to imaging.

2.4. Extraction of ginsenosides

2.4.1. MSPD

About 0.25 g of sample, 0.5 mL of water and 1.0 g of dispersant were blended and ground with a pestle to obtain a homogenous mixture. Before the mixture transferred into the glass column, absorbent cotton was put on the bottom of the column. The mixture was transferred into the glass column and eluted with 20 mL of elution solvents. The flow rate of the elution solvent was 0.2 mL min^{-1}. The eluate was collected, evaporated to dryness under reduced pressure and then diluted to 25 mL with methanol. The obtained sample solution was filtered through a 0.45 μm filter membrane before HPLC analysis.

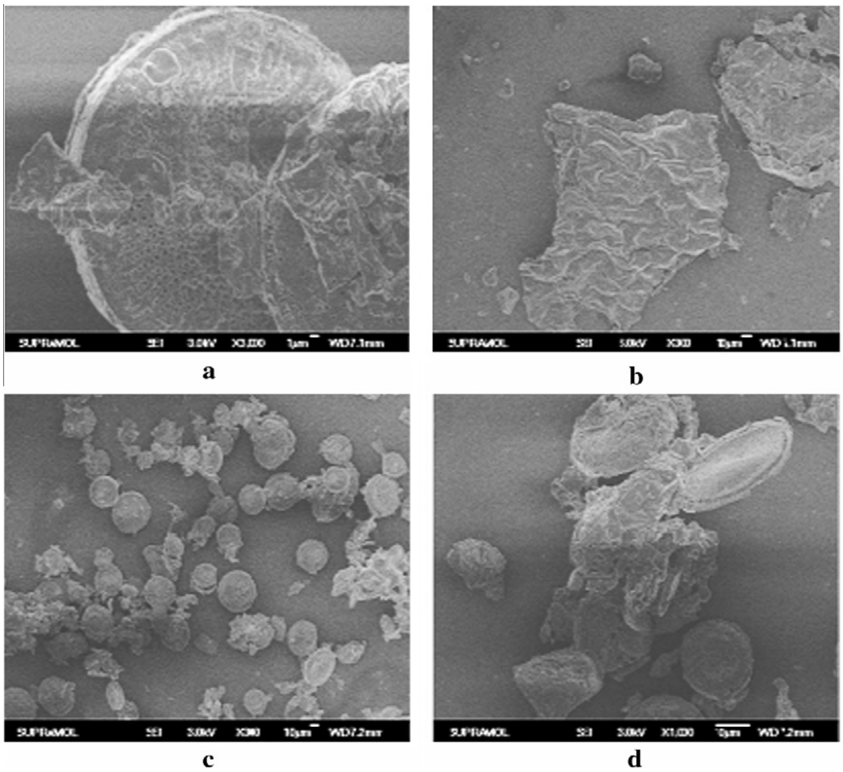

Fig. 1. The SEM images. (a) Diatomaceous earth; (b) powder of leaves of *P. ginseng*; (c and d) mixture of sample and diatomaceous earth.